Evaluation of florpyrauxifen-benzyl for use in pastures and hayfields

Wykle C. Greene

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Plant Pathology, Physiology, and Weed Science

Michael L. Flessner, Chair
E. Scott Hagood
A. Ozzie Abaye
Benjamin F. Tracy

September 10, 2021
Blacksburg, VA

Keywords: herbicides, forage production, legume inclusion

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Academic Abstract

Weed control is a critical component in pastures and hayfields in order to ensure maximum forage yields. Typically, broadleaf weed control in pastures and hayfields is achieved through the use of synthetic auxin. However, these herbicides also control desirable broadleaf species such as forage legumes, including white clover. Use of herbicides can lead to severe injury and often complete elimination of white clover, making it difficult for producers to maintain legumes in mixed grass-legume swards while controlling weeds. It is often desirable to have legumes present in the sward due to their high nutritive forage value and ability to fix nitrogen compared to grass only swards. Florpyrauxifen-benzyl + 2,4-D is a new herbicide which is reported to control broadleaf weed species, while preserving white clover. Little published research exists on this herbicide, particularly for use in pastures and hayfields. Research evaluating sward composition indicates that florpyrauxifen-benzyl + 2,4-D is effective in controlling broadleaf weed species while also preserving greater amounts of white clover than any other herbicide treatments. Florpyrauxifen-benzyl + 2,4-D also resulted in significantly more forage grass production than the nontreated control. Florpyrauxifen-benzyl + 2,4-D was less effective than other herbicides when applied via fertilizer impregnation. Additional research assessing the spectrum of broadleaf weed control found that florpyrauxifen-benzyl + 2,4-D is a viable herbicide for the control of several broadleaf weed species including bulbous buttercup, Canada thistle, broadleaf plantain, plumeless thistle, and common ragweed. However, florpyrauxifen-benzyl + 2,4-D was less effective than other herbicides for controlling certain weeds, such as horsenettle. White clover was injured from florpyrauxifen-benzyl + 2,4-D, but was able to fully recover in 90 to 120 days. There were no differences in white clover response between the four varieties tested. When evaluating establishment of forage species, florpyrauxifen-benzyl + 2,4-D did not injure or reduce biomass of tall fescue or orchardgrass plantings, indicating a high level of safety. Florpyrauxifen-benzyl + 2,4-D was also safe to both drilled and frost seeded clover when applied prior to and at planting. Greenhouse trials revealed that flowering white clover is more sensitive to herbicides compared to vegetative white clover, and that safety of white clover to florpyrauxifen-benzyl + 2,4-D is dependent upon use rate. Considerations such as weed species present, and the amount of white clover injury that is considered acceptable will dictate the decision to utilize florpyrauxifen-benzyl + 2,4-D in pastures and hayfields. This research demonstrates the effectiveness and overall utility of florpyrauxifen-benzyl + 2,4-D for use in pastures and hayfields due to the effectiveness of weed species as well as the level of safety to white clover.

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General Audience Abstract

Pastures and hayfields are a critical component in livestock production. Grazing livestock perform best on highly nutritious forages. Legumes such as white clover are highly nutritious in forage systems and offer other benefits such as the ability to fixate nitrogen. Conversely, weed species negatively impact forage production by competing for resources with desirable forage species. Additionally, many species of broadleaf weeds are toxic to livestock.

Because grasses are the backbone of forage systems, the majority of weed control efforts are aimed at controlling broadleaf weed species. However, beneficial forage legumes such as white clover are susceptible to broadleaf herbicides commonly used. This creates a management dilemma for producers who wish to control troublesome weeds, but also have white clover present in their pastures and hayfields.

Florpyrauxifen-benzyl + 2,4-D is a herbicide combination which is new for pastures and hayfields. This herbicide is reported to control broadleaf weeds while also preserving white clover. Research trials were conducted in order to determine if florpyrauxifen-benzyl + 2,4-D could be used in forage systems to control weeds, without killing white clover. Several research trials were established to evaluate florpyrauxifen-benzyl + 2,4-D for broadleaf weed control and white clover safety.

Research trials were established to determine the effect of florpyrauxifen-benzyl + 2,4-D on the number and overall amount of forage produced and the proportion of weeds and desirable forages as affected by herbicide treatment. Florpyrauxifen-benzyl + 2,4-D resulted in a 140% increase in forage grass production, and more legume production than any other herbicide treatment, while also decreasing the quantity and amount of broadleaf weed species.

Because there is little existing research on what weed species florpyrauxifen-benzyl + 2,4-D controls, research trials were established to determine the spectrum of weed species that florpyrauxifen-benzyl + 2,4-D controls. Greenhouse trials were also established to evaluate the effect of white clover variety on injury from herbicide. Results showed that florpyrauxifen-benzyl + 2,4-D is effective in controlling several weeds such as bulbous buttercup, Canada thistle, broadleaf plantain, plumeless thistle, and common ragweed. Greenhouse trials showed that white clover variety did not influence the level of injury from herbicide applications.

Seedling forages are more vulnerable to weed competition and therefore weed control around the time of planting is critical. However, seedlings are typically very sensitive to herbicides, compared to mature plants. Research trials were established to determine the effect of florpyrauxifen-benzyl + 2,4-D on the establishment of forage grasses tall fescue and orchardgrass, as well as white clover. White clover was established using two commonly used methods: drilling and frost-seeding. Results from the field show that florpyrauxifen-benzyl + 2,4-D is safe use around the time of tall fescue and orchardgrass establishment, as well as white clover planting with either

method. Greenhouse trials were also established to determine if white clover's growth stage at the time of herbicide application influences the response. Results show that white clover is more sensitive to herbicides applied to flowering white clover compared to vegetative growth and the level of injury is dependent upon herbicide rate.

Overall, our results demonstrate the utility of florpyrauxifen-benzyl + 2,4-D for forage production by controlling weed species and being safer to white clover than commonly used herbicides.

Acknowledgements

There's a long list of people who I could thank for leading me to where I am. I'd like to take this opportunity to show my sincere thanks for their support. First and foremost, I'd like to think my advisor Dr. Michael Flessner. Thank you for cultivating an environment in our lab that encourages teamwork, curiosity, and a work-life balance that is often absent in graduate school. Without your guidance and input, I would not have developed into the scientist I am today.

Thank you to my committee including Dr. Scott Hagood, Dr. ('Miss' Ozzie) Abaye and Dr. Ben Tracy. I have been extremely fortunate to have such a group of well-respected experts to turn to for advice and encouragement throughout my studies.

Many, many thanks go to Kevin Bamber. I speak for myself and the rest of our lab group when I say that without you, our lab would fall apart. Thank you for the countless hours of assistance, whether it be in the form of driving across the Commonwealth, forage harvesting, counting weeds, and simply being a source of constant encouragement with your endless repertoire of impersonations and musical knowledge.

Thank you to all my lab mates, current and former, including Lucas Rector, Eric Scruggs, Kara Pittman, Matthew Spoth, Cynthia Sias, and Eli Russell for the countless help throughout the years. Thank you as well to the entire crew at Glade Road for the great comradery.

I would be remiss if I didn't give my deepest thanks to my better half Alexandra Lovrinic. I can't imagine completing this process without your constant encouragement. Thank you for being there to listen to me think out loud on numerous occasions and thank you for the tough love that you gave when I needed to hear it most. I love you.

Thank you to my family, especially my mother, Karinne, and my brother, Jay, for always believing in me and encouraging me to pursue what I am passionate about.

Lastly, and perhaps most importantly, I would like to thank Abita, Nalu, and Molly. You three are a constant, unrivaled source of joy in my life and I couldn't imagine making it through these years without you.

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Literature Review

Benefits of Clover in Pastures and Hayfields

White clover (*Trifolium repens* L.), is a perennial cool-season legume species. White clover has a rhizomatous, prostrate growth habit that is capable of filling in bare spaces in pastures and hayfields (Gibson and Cope 1985), which can help to maximize forage production and reduce weed species present (Bunton et al. 2020; Tracy et al. 2004; Tracy and Sanderson 2004).

Being a legume, white clover is able to fix atmospheric nitrogen into mineral nitrogen, which is available to neighboring plants (Brophy et al. 1987). Although the amount of nitrogen fixed by can vary greatly due to many environmental factors and management practices, white clover can produce 146 kg N ha^{-1} $year^{-1}$ (Smith and Valenzuela 2002) up to 650 kg N ha^{-1} $year^{-1}$ (Sears et al. 1965). The nitrogen fixed by white clover can be taken up by neighboring plant species, such as desirable grasses. Gylfadóttir et al. (2007) showed that 50% of the total nitrogen in grass species was derived from neighboring white clover plants. One of the determining factors in the amount of nitrogen that grass plants derive from white clover is the distance between the two plants, as well as the clover/grass ratio. Higher clover/grass ratios and closer distance between the two species lead to increased nitrogen transfer (Brophy et al. 1987). Legumes can transfer nitrogen up to 20 cm (Brophy et al. 1987). Another factor influencing the transfer of nitrogen from clover to grass species is season. Grasses benefit greater during the summer months (Ledgard 1991). The main route of nitrogen transfer between clovers and grasses is decomposition of clover roots and nodules rather than direct transfer from root nodules. Legume decomposition can contribute up to 70 kg N ha^{-}

^{1} year^{-1} below ground, whereas direct transfer only accounts for 7 to 11 kg N ha^{-1} year^{-1} (Brophy et al. 1987; Ledgard 1991; Russelle et al. 1994). Because most of the nitrogen transfer comes from decomposing roots, a process known as rhizodeposition (Whipps and Lynch 1985), the rate at which nitrogen is transferred greatly depends on the rate of root turnover in the soil (Rasmussen et al. 2007). In addition to below ground nitrogen deposition, above ground processes such as the decomposition of leaves and stems as well as deposition from grazing animals also supply plant available nitrogen (Belsky and Gelbard 2000; Rasmussen et al. 2007).

The practical effects of nitrogen accumulation, and transfer, from legumes to grass species has been extensively studied. Tall fescue (*Lolium arundinaceum* Schreb.)/clover mixtures produce as much forage as pure tall fescue stands that are fertilized with 179 kg N ha^{-1} (Vines et al. 2006). Orchardgrass (*Dactylis glomerata* L.) can produce up to two and a half times the amount of forage when grown with white clover, compared to a monoculture (Dobson and Beaty 1977). Varying ratios of white clover/tall fescue mixtures can be more productive in terms of overall forage production than their respective monocultures (Tekelİ and Ates 2005). Other studies have also found that grass monocultures would have to be heavily fertilized in order to produce the same amount of biomass as grass-legume mixtures (Carter and Scholl 1964).

In addition to increased overall forage production, adding legumes such as white clover to grass swards can enhance livestock performance. Total digestible nutrients is a metric used to measure the feed energy of a particular forage. Legumes have more total digestible nutrients compared to grasses (Ellis and Lippke 1976). More specifically, legumes have more protein and vitamins, and less fiber (Frame 2005). Ulyatt (1970), suggested that overall forage quality is determine by the level of forage intake by the animal and the nutritive value of the forage, and legume forages generally result in greater levels of intake than grass species. This increase in intake is likely due to the morphological characteristics of legume species (Penning et al. 1995). In dairy systems, several studies have shown that mixed swards of grass and clover result in greater overall milk production, milk fat yields, and milk protein yields (Harris et al. 1998; Phillips et al. 2000; Phillips and James 1998; Ribeiro Filho et al. 2003). Weight gain in beef steers and lambs has also been greater when grazing mixed grass-legume swards, compared to grass monocultures (Blaser et al. 1956; Golding et al. 2008; Schaefer et al. 2014). Other than during flowering, white clover maintains greater quality throughout the season, in contrast with grasses whose digestibility typically decreases with maturity (Frame and Newbould 1986; Moeller and Statens Planteavlsforsoeg n.d.; Ulyatt 1981).

In addition to nitrogen fixation and the positive effects on livestock performance, the addition of legumes into grass pastures and hayfields also contributes to the overall forage production. Legume-grass mixtures create considerably more biomass per area than grass monocultures (Aberg and Wilsie 1943; Bélanger et al. 2014; Evers 1985; Giambalvo et al. 2011; Roberts and Olson 1942; Saia et al. 2016; Sanderson et al. 2013; Taylor and Allinson 1983). While overall forage production is crucial to forage systems, another aspect that must be considered is the seasonal distribution of available forage. Cool-season grasses, such as tall fescue and orchardgrass, often begin dormancy as summer temperatures rise and precipitation declines. This period of growth is commonly referred to as the “summer slump” (Riesterer et al. 2000), and is one of the greatest concerns with regard to forage grass monocultures (Cooper 1973; Lauriault et al. 2005).

Researchers have suggested that the inclusion of forage legumes can help to fill in gaps of limited forage production (Lauriault et al. 2003; Sleugh et al. 2000; Vines et al. 2006). Additionally, legumes inclusion into grass swards can lead to greater stand longevity (Droslom and Smith 1976). The addition of legumes has also been shown to increase forage yields in warm-season perennial grass systems and increases seasonal forage distribution to a greater extent than when included in cool-season forage systems (Bartholomew and Williams 2010; Evers 1985).

Tall fescue is the predominant forage grass grown throughout Virginia (Smith et al. 2009). While tall fescue is a high quality forage, especially during periods of fall and spring growth (Beck et al. 2006), the majority of tall fescue acreage is infected with the fungal endophyte *Acremonium coenophialum* (Ball et al. 2015). The endophyte causes the production of alkaloids which can be very detrimental to grazing animals, resulting in decreased appetite, reduced conception rates, hyperthermia, reduced milk production, and lower weight gains compared to animals grazing noninfected tall fescue (Gay et al. 1988; Schmidt and Osborn 1993). Although the toxic endophyte has negative impacts on grazing animals, endophyte infected tall fescue performs better than newer, non-infected varieties of tall fescue (Ball et al. 2015). The increased performance of toxic endophyte-infected tall fescue is due to the symbiotic relationship that the fungus has with the plant, allowing it to handle various stressors such as overgrazing, drought and predation much better than endophyte-free tall fescue (Arachevaleta et al. 1989; Bacon 1993; Latch 1993; Malinowski and Belesky 2000; West et al. 1993). Due to the lack of stress tolerance in endophyte-free tall fescue, there is greater potential for reduced stand longevity (Ball et al. 2015). There are strains of the endophyte that have been bred into tall fescue so that the plant still produces the compounds which aid in stress tolerance, these varieties are known as novel-endophyte tall fescue. However, converting a pasture from infected tall fescue to novel-endophyte tall fescue is very costly, and producers are often hesitant to do so (Ball et al. 2015).

Other than converting toxic-endophyte tall fescue to endophyte-free or novel-endophyte varieties, one of the tactics that producers can do is to dilute the effect of toxic tall fescue through the addition of other forages (Aiken and Strickland 2013; Ball 1997; Roberts and Andrae 2004). The addition of white clover to endophyte infected tall fescue pastures has shown to increase livestock weight gain up to 0.15 pounds per day (Thompson et al. 1993) and up to 0.64 pounds per day (McMurphy et al. 1990).

Impacts of Weeds on Forage Production

Like any crop production system, weeds can negatively impact growth and decrease yields anywhere from 17% to 71% (Lym and Messersmith 1985) by competing for resources such as light, soil, and water (Bradbury and Aldrich 1956; Gorrell et al. 1981; Masters and Mitchell 1985; Smith and Calvert 1980). Tolson et al. (2012) showed increases in tall fescue production up to 1,760 kg ha^{-1} when weeds were effectively controlled. In the rangelands of the western United States it has been reported that weeds are the source of more economic loss than all other types of pests combined, accounting for over $2 billion dollars per year (Bovey 1987; Quimby et al. 1991). Certain weed species have been shown to reduce the grazing capacity of an area by more than 50% (Olson 1999). In addition to the direct negative impact that weeds have on desirable forage species, weeds can also negatively impact natural plant communities by reducing species diversity, species richness, and overall landscape productivity (Belcher and Wilson 1989; MacMahon and Parmenter 1983; Rickard and Cline 1980; Tracy et al. 2004; Wallace et al. 1993). Furthermore, many problematic weeds in forage production systems are perennials with deep taproots which can negatively impact water and nutrient availability throughout growing seasons and also contribute less to organic matter in the upper soil region (Olson 1999).

There are many species of plants that are harmful to grazing livestock either through mechanical injury or the presence of poisonous compounds (Kingsbury 1964). Livestock losses due to poisonous plants has been documented as far back as 1850 (Dwyer 1978). The most common cause of livestock injury from plants is through the ingestion of poisonous compounds in the plant. Common toxic compounds include alkaloids, oxalates, hydrocyanic acid, and various others (James et al. 2019). Even though the ingestion of poisonous plants may not always lead directly to livestock death, ingestion will likely cause decreased performance and overall health (Cook et al. 2009). Another indirect impact of poisonous plants is the disruption to the reproductive cycle. Certain poisonous plants can cause females to abort pregnancies or even be infertile (James et al. 1992, 2019; Panter et al. 2002). Additionally, grazing livestock can be injured from plant parts themselves, such as knotroot foxtail (*Setaria parviflora* Poir.), which contains seedheads that commonly cause ulcers in the mouths of horses fed contaminated hay (Israel et al. 2013).

One aspect of weeds in forage crops, specifically pastures, is the effect that weeds have on grazing animals. Weeds such as musk thistle (*Carduus nutans* L.) and horsenettle (*Solanum carolinense* L.) contain thorns and spines which may prevent or deter livestock from grazing adjacent, desirable forage (Belsky and Gelbard 2000; Bruzzese and Lane 1996). Research has shown that undesirable plant species that livestock do not graze can create a microsite in which other species can be protected from grazing (Callaway et al. 2000). Ecologists have characterized these species interactions as plant defense "guilds", or mechanisms as to how one species can impact the consumption of another species indirectly (Atsatt and O'Dowd 1976; McNaughton 1978). When palatable plant species are surrounded by, or even grown near, unpalatable species, herbivores such as livestock will be deterred from feeding on the palatable species (Brown and Ewel 1987; Holmes and Jepson-Innes 1989; Rausher 1981).

Weed Control in Forages

There are numerous approaches and tactics for controlling weeds in forage crops. Factors such as weed species, forage type, harvest method, and location are all important to consider when deciding on a management technique. Weed control methods can be grouped into several categories: mechanical, cultural, chemical, and biological (McWhorter and Shaw 1981).

Mechanical weed control typically consists of methods such as tillage, hoeing, hand-pulling, mowing, and others (Vincent et al. 2001). Of all the mechanical weed control methods typically employed in forage crops, mowing is the most employed management strategy (Benefield et al. 1999; Trumble and Kok 1982). Mowing controls or suppresses weeds by depleting underground energy reserves of the plant, as well as removing the central growing point. Because the growing point on grasses is the basal meristem, mowing is typically more effective on broadleaf species. Additionally, perennial species often require multiple mowings in order to sufficiently decrease the energy reserves within the plant (Tredaway and Colvin 2000). Mowing can be a viable control measure against numerous weed species common to pastures and hayfields such as Canada thistle (*Cirsium arvense* L.) (Amor and Harris 1977), yellow star-thistle (*Centaurea solstitialis* L.) (Benefield et al. 1999; Thomsen et al. 1996), musk thistle (McCarty and Hatting 1975), dogfennel (*Eupatorium capillifolium* Lam.) (Macdonald et al. 1994), western ironweed (*Vernonia baldwinii* Torr.), gray goldenrod (*Solidago nemoralis* Ait.) (Peters and Lowance 1978), tarweed (*Holocarpha virgata*) (Perrier et al. 1981), wild blackberry (*Rubus armeniacus* Focke) (Ingham 2014), and spotted knapweed (*Centaurea maculosa* Lam.) (Rinella et al. 2001). Several factors are important with regard to mowing and subsequent weed control. Benefield et al. (1999) showed that yellow starthistle control by mowing is highly dependent on the timing of mowing, therefore each weed species has a critical timing where mowing may be more effective. Although less common than mowing, tillage has been shown to control certain weed species such as Canada thistle (Wilson and Kachman 1999), however producers are less likely to perform tillage operations due to the destruction of desirable forage species.

Cultural weed control is a method of weed control in which the environment around the desirable crop is managed in such a way that it gives the crop a competitive advantage. Thus, any measure that increases the health and vigor of the crop may be considered a cultural control method. Typically, cultural methods are often part of a standard forage maintenance program such as fertilization. Often cultural methods such as reseeding of desirable forage are combined with other control measures, such as herbicides, are successful in controlling certain weeds (Hubbard 1975; Wilson and

Kachman 1999). Fertilization increases desirable forage growth enough to give it a competitive advantage over numerous weed species (Ang et al. 1994; Cipriotti et al. 2011; Derscheid et al. 1961; Hay and Ouellette 1959; Jacobs and Sheley 1999; Sheley et al. 1984; Thrasher et al. 1963; Wilson and Kachman 1999). Hay and Ouellette (1959) found that nitrogen fertilizer, in combination with 2,4-D, increased desirable forage grasses in a sward up to 390 kg dry matter ha $^{-1}$. Canada thistle density decreased when tall fescue pastures were fertilized with increasing levels of nitrogen (Thrasher et al. 1963). Sheley and Jacobs, (1997) found that combining fertilizer applications and herbicides resulted in a synergistic effect, increasing total forage grass yields.

Biological weed control uses biological agents such as pathogens, parasites, herbivores, or other plants to induce stress on the target weed and reduce its competitive advantage in the landscape (Wilson and McCaffrey 1999). More than 200 biological control agents have been released worldwide over the last century, and 165 released in the United States, with the vast majority of control agents being arthropods (Blossey et al. 1994; Goeden et al. 1993; Julien 1989). Although there has been considerable effort devoted to investigating biological weed control agents, there has been very limited success. Complete or acceptable weed control from biological agents has only been successful on 29% of the targeted species (De Loach 1991). Some of the more successful attempts to control weeds through the use of biological control agents include the control of tansy ragwort (*Jacobaea vulgaris* Gaertn.) due to the ragwort flea beetle (*Longitarsus jacobaeae*) (McEvoy et al. 1991). In some instances, researchers suggest that even though biological control agents may be unsuccessful, they may help to reduce seed production which could eventually decrease their abundance (Balciunas and Villegas

1999; DiTomaso 2000; Sheley et al. 1998). Grazing livestock could also be considered a biological weed control agent if the livestock are feeding on the target weed. However, it has been shown that animals grazing areas with high levels of undesirable species can exhibit decreased performance (Byenkya 2004). The effectiveness of control is dependent on various factors such as: the ability to limit livestock to specific areas, stocking rate, stocking density, weeds of interest, and type of grazing livestock (Popay and Field 1996). Grazing has shown to be effective in controlling or suppressing weed species such as blackberry (*Rubus* spp.) (Crouchley 1983; Dellow et al. 1987), buttercups (*Ranunculus* spp.) (Betteridge et al. 1994), leafy spurge (*Euphorbia esula* L.) (Landgraf et al. 1984; Lym and Kirby 1987; Walker et al. 1992), Canada thistle (Hartley et al. 1984; Mitchell and Abernathy 1993), and bull thistle (Hartley 1981; Rolston et al. 1981). Rolston et al. (1981) showed that Scotch thistle (*Onopordum acanthium* L.) control was nearly 100% when goats were allowed to graze for up to six months. Lym et al. (1997) found that grazing alone was able to significantly reduce leafy spurge density over the course of two years.

Chemical weed control, through the use of herbicides, is an important method of weed control for forage crops such as pastures and hayfields as herbicides can offer a quick, effective, and often economical method of controlling weeds (DiTomaso 2000). Historically, auxin, or growth regulating herbicides, have been the most important group of herbicides in pasture and hayfield weed control due to their activity on broadleaf weed species and safety on grasses (DiTomaso 2000). Common herbicides in this group include: 2,4-D, dicamba, aminopyralid, triclopyr, picloram, and others. Unlike most commercial row crops, herbicides used in forage crops may be applied in various ways such as broadcast, spot spraying, and wiper applications. The timing of herbicide application has a tremendous effect on the achieved level of control. In general winter annual weeds are best controlled by herbicide application in late fall and early spring, whereas summer annuals are best controlled by spring – summer applications. However, many of the most common pasture and hayfield weeds are perennials, and the efficacy of herbicide application can vary greatly from one weed to another based on timing. Dogfennel, for example, is best controlled by applications made in spring, whereas horsenettle is best controlled by applications made in late summer (Lingenfelter et al. 2019).

One of the biggest limitations to herbicide use in pastures and hayfields is the lack of safety to desirable legumes from commonly used herbicides (Renz 2010). There are very few effective, registered herbicides that are safe to clover species in grass-clover mixtures while controlling a broad range of weed species. Therefore, achieving effective broadleaf weed control often comes at the expense of eliminating or severely injuring clovers (Almquist and Lym 2010; Enloe et al. 2014; Griffin et al. 1984; Malik and Waddington 1989). Because of the lack of effective herbicide options, effective weed control must be made prior to legume establishment, or by means other than herbicidal control. If herbicides are used prior to legume establishment, care must be taken to assure that the plant back interval has passed so that the herbicide is no longer persistent in the soil at quantities harmful to germinating legumes (Beeler et al. 2004; Marshall et al. 2006; Renz 2010). Because of the risk of legume injury, growers are often hesitant to treat pastures and hayfields with herbicides which may lead to fields that are heavily infested with weeds.

Florpyrauxifen-benzyl

Florpyrauxifen-benzyl is a newer herbicide that was originally developed for postemergence weed control in rice (Perry and Ellis 2015). Florpyrauxifen-benzyl is a synthetic auxin herbicide that has the same site of action, TIR1 auxin receptor, as the other commonly used herbicides such as clopyralid, triclopyr, and quinclorac, therefore it is primarily active on broadleaf weed species. However, florpyrauxifen-benzyl is unique in that it belongs to a new class of synthetic auxin herbicides in the arylpicolinate family (Weimer et al. 2015). Florpyrauxifen-benzyl has been shown to have little to no residual activity (Miller and Norsworthy 2018; Miller et al. 2018; Teló et al. 2019).

Preliminary data (not shown) have shown that white clover has a level of tolerance to POST applications of florpyrauxifen-benzyl. If florpyrauxifen-benzyl is effective in controlling common broadleaf weed species found in pastures and hayfields, while maintaining safety to white clover, it could be a tremendous weed management tool for forage producers. The overall objective of this research is to evaluate the efficacy of florpyrauxifen-benzyl on common weeds found in pastures and hayfields, the tolerance of white clover to florpyrauxifen-benzyl, and the effect of florpyrauxifen-benzyl on the establishment of desirable forage grasses and forage legumes.

As a result of these studies, we hope to provide producers with as comprehensive recommendations as possible for the use of florpyrauxifen-benzyl in cool season pastures and hayfields. We also hope to determine, with reasonable certainty, the level of safety of cool-season grasses and clovers to florpyrauxifen-benzyl.

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Forage sward response to florpyrauxifen-benzyl containing herbicides and other herbicides commonly used in hayfields

Abstract. Effective weed control in pastures and hayfields is critical for maximizing overall forage production. Although herbicides can be extremely effective in managing weeds, producers are often hesitant to employ them over the fear of eliminating desirable forage legumes. Currently, there very few effective, registered herbicides that are safe to clover species in grass-clover mixtures while controlling a broad range of weed species. Florpyrauxifen-benzyl + 2,4-D is reported to preserve established white clover. Herbicides are typically broadcast spray applied, but the process of applying herbicides by means of impregnated fertilizer is gaining popularity due to the ease of application and reported efficacy. Field trials were conducted in order to determine the effect of these new herbicide combinations, as well as other commonly used pasture herbicides, when sprayed and applied via impregnated fertilizer on sward composition in separate experiments. Herbicides tested included combinations of florpyrauxifen-benzyl + 2,4-D and florpyrauxifen-benzyl + aminopyralid at different rates, and other commonly used hayfield herbicides. Trials were conducted in cool-season grass pastures containing white clover and assorted weed species. Biomass and species composition were measured throughout the growing season following herbicide application and were grouped into: (1) forage grasses, (2) forage legumes, (3) weedy grasses, and (4) broadleaf weeds. For the sprayed application study there were no differences in early season forage grass production between any treatment. All herbicides resulted in greater late-season forage grass biomass compared to the nontreated control. Florpyrauxifen-benzyl + 2,4-D resulted in the greatest forage legume biomass of any herbicide but reduced biomass relative to the nontreated. With the exception of metsulfuron, broadleaf weed species were reduced with all herbicide treatments. For the fertilizer impregnation study, florpyrauxifen-benzyl + 2,4-D treatments did not increase forage grass biomass or reduce broadleaf weed biomass. However, florpyrauxifen-benzyl + aminopyralid resulted in increased forage production and decreased broadleaf weeds. This research suggests that florpyrauxifen-benzyl + 2,4-D is a viable weed control option when spray applied for broadleaf weed species, while also preserving greater amounts of white clover compared to any other herbicide. Aminopyralid containing herbicides were most effective when applied via fertilizer impregnation.

Nomenclature: florpyrauxifen-benzyl; 2,4-D; white clover, *Trifolium repens* L.

Keywords: broadleaf weed control; hayfields; pasture

Introduction. Cool-season perennial grasses such as tall fescue (*Lolium arundinaceum* Schreb.) and orchardgrass (*Dactylis glomerata* L.) are the predominant forage species within their vast range of adaptation throughout the United States (Burns and Bagley 1996; van Santen and Sleper 1996; Sleper and West 1996). Mixed swards of grass species, such as tall fescue and orchardgrass with legumes such as white clover (*Trifolium repens* L.) have numerous benefits compared to grass monocultures. Such benefits include: (1) nitrogen fixation by the legume can be transferred to grass species (Brophy et al. 1987; Gylfadóttir et al. 2007), (2) enhanced livestock performance due to the increase in total digestible nutrients (Ellis and Lippke 1976), (3) greater overall forage yields (Aberg and Wilsie 1943; Evers 1985; Tekelİ and Ates 2005; Payne et al. 2010; Bélanger et al. 2017), and (4) greater seasonal distribution of forage (Sleugh et al. 2000; Lauriault et al. 2005; Vines et al. 2006). More diverse species mixtures are also more likely to resist weed invasions compared to less diverse plant communities (Elton 1958; Tracy et al. 2004; Benjamin F Tracy and Sanderson 2004). Additionally, an ecological benefit of the inclusion of legumes into forage production systems is the effect on pollinator habitat. Compared to grass monocultures, forage-legume mixtures can increase floral resources, and therefore may lead to an increase in the quality of pollinator habitat (Woodcock et al. 2014) In order to maximize forage yield, weed species must be controlled throughout the season (Thrasher et al. 1963; Smith et al. 1977; Gorrell et al. 1981) as overall forage production can be reduced up to 70% when weeds are allowed to persist (Kivuva et al. 2014). Weeds can impact forage yield and forage quality, and in some instances, weed species may be toxic to livestock when grazed or fed in hay (Gunning 1949; Williams and James 1978; Welsh et al. 2007; Cook et al. 2009).

Several herbicides are available for weed control in pastures and hayfields including 2,4-D, dicamba, metsulfuron, aminopyralid, clopyralid, triclopyr, and fluroxypyr (DiTomaso 2000; Sellers et al. 2009; Payne et al. 2010). However, one of the main issues with using herbicides for weed control in forage crops is the lack of safety to forage legumes such as white clover. Currently, there are very limited registered herbicides that offer selective broadleaf weed control in mixed forage grass-white clover swards while controlling a range of weeds (Flessner and Taylor 2021).

Florpyrauxifen-benzyl is a synthetic auxin herbicide (Weed Science Society of America [WSSA] Group 4) commercially released in 2018. Florpyrauxifen-benzyl has been shown to be effective in controlling several weed species common in rice production (Miller and Norsworthy 2018). Currently, florpyrauxifen-benzyl is labeled in rice as a stand-alone product and in combination with the herbicide aminopyralid in forage crops. However, aminopyralid is extremely active against broadleaf species, including forage legumes such as white clover (DuraCor herbicide; Corteva AgriSciences, LLC, 9330 Zionsville Rd., Indianapolis, IN 46268). Florpyrauxifen-benzyl combined with 2,4-D is reported to preserve established white clover while controlling broadleaf weed species (Sleugh et al. 2020), and 2,4-D applied alone has been shown to not completely eliminate white clover (MacRae et al. 2005; Enloe et al. 2014). If this herbicide does indeed preserve white clover while controlling broadleaf weeds in forage systems, it can be a valuable tool for forage producers.

While herbicides are most commonly applied to forages with broadcast spray applications, impregnating herbicides on fertilizer is becoming increasingly common. Commercial applicators often favor this method as it allows them to treat a larger number of acres than with spray applications. Growers may also realize a cost savings compared to applying fertilizer and herbicides separately. Research exists showing that herbicide applied via fertilizer impregnation can control weeds (Braverman 1995; Koscelny and Peeper 1996). Some research also exists on the effect of several auxin-mimicking herbicides, which are commonly used in forages, on weed control when applied via fertilizer (Loughner and Nolting 2010). However, most of this prior research was conducted in row crops and turf and there are limited data on the efficacy of common pasture and hayfield herbicides when impregnated onto fertilizer.

The following research was conducted in order to determine the effect of florpyrauxifen-benzyl plus 2,4-D, as well as other commonly used forage herbicides, on hayfield yield and sward composition, including the effect on forage legumes and broadleaf weed species, from both foliar broadcast and fertilizer impregnation application methods.

Materials and Methods

Foliar Broadcast Study.

Study Sites. Two field trials were established in 2020. Locations included Blacksburg (37.23, -80.46) and Raphine (37.93, -79.21), VA, USA (Table 1.1). Both sites contained naturalized weed populations in a mixed stand of well-established tall fescue, orchardgrass, white clover, and red clover (*Trifolium pratense* L.). Natural weed populations were present at both locations. Weed species present as well as category are shown in Table 1.S1.

Experimental Design. The experiment was designed as a randomized complete block with four replications. Plot size was 4.6 m by 7.6 m. Herbicide applications were made using a 3 m-wide handheld backpack sprayer with 6 TeeJet (Spraying Systems Co.; Wheaton, IL) 11002XR nozzles calibrated to deliver 140 L ha^{-1} at 207 kPa. Treatments are presented in Table 1.2 in addition to a nontreated control.

Data Collection and Analysis. Following herbicide application, visible weed control and white clover injury data were taken at 30-day intervals throughout the growing season. Species composition and biomass data were taken at the boot stage of forage grass growth, correlating to a typical hay cutting. Dates of data collection are listed in Table 1.3. Visible weed control and white clover injury were rated on a scale of 0 – 100% with 0 being no observable injury and 100 being complete plant necrosis relative to the nontreated check as described by Frans et al. (1986).

To determine species composition, two transect lines were established within plots at 2 and 2.6 m across the width of the plot, and 0.9 m from the plot edges. Species were noted every 0.6 m along the transect line for a total of 10 points per transect. Individual plant species were grouped into the same four species groups as biomass data. Above-ground forage biomass was determined by harvesting all plant material above 10 cm, simulating hay cutting height in a random 0.5 m^2 quadrant, and then separating plants into the following species groups: (1) forage grasses, (2) forage legumes, (3) broadleaf weeds, and (4) grass weeds. Following separation plants were bagged and dried at 50C for 72 hours and then weighed. Following data collection, plots were mowed to a height of approximately 10cm and the forage was removed in order to simulate a hay cutting. Following typical production practices for the region there were 2 hay cuttings at both locations (Table 1.3).

Data were subject to ANOVA and subsequent means separation using Fisher's Protected LSD test ($P \leq 0.05$) to compare across treatments using JMP Pro 15 (SAS Institute, Inc; Cary, NC). Fixed effects consisted of herbicide treatment. Location and replication were considered random effects to allow inferences to be made over a range of environments and conditions (Blouin et al. 2011). For weeds not present at both locations, visible control of weed species data were analyzed by location.

Fertilizer Impregnation Study.

Study Sites. Field trials were established in 2018 in Blacksburg, VA, USA (37.19, -80.58) and Garden City, MO, USA (38.55, -94.16) (Table 1.1). Both sites were mixed stands of forages including grasses and legumes. Natural weed populations were present at both locations (Table 1.S1).

Experimental Design. The experiment was designed as a randomized complete block design with four replications. Plot size was 4 m by 14 m. Herbicides used are presented in Table 1.2 in addition to a fertilizer-only (no herbicide) control. All herbicides were impregnated onto dry fertilizer through the use of a cement mixer and handheld spray boom. The fertilizer carrier consisted of a mixture of 121.8 kg ha^{-1} urea, 33.2 kg ha^{-1} triple super phosphate, and 124.4 kg ha^{-1} sulfate of potash. A drying agent of diatomaceous earth at 8 g kg^{-1} fertilizer was added to the mixture.

Data Collection and Analysis. Following herbicide application, forage biomass and species data were taken throughout the season at times that correlated to a typical hay cutting. Above-ground forage biomass was determined by harvesting 2, 1 m^2 quadrants per plot and then separating plants into the following groups 1) forage grasses, (2) forage legumes, (3) broadleaf weeds and (4) grass weeds. Three samplings were taken at both the Blacksburg and Garden City location in 2018. In 2019, treatments were not reapplied, but two samplings were taken at the Blacksburg location. Data were combined in order to evaluate cumulative season coverage by species group. Following separation plants were bagged and dried at 50 C for 72 hours and then weighed. In addition to biomass, three transect lines with permanent points were established across each plot, running the length of the plot, starting 1m into the plot and continuing to 12 m. At the time of sampling, the species present at each point along the transects were noted and separated into the four categories listed above. Following data collection, plots were mowed to a height of approximately 10 cm and the forage was removed in order to simulate a hay cutting. This impregnation protocol was in accordance with herbicide manufacturer's directions.

All data were subject to ANOVA and subsequent means separation using Fisher's Protected LSD test ($P \leq 0.05$) to compare across treatments using JMP Pro 15 (SAS Institute, Inc; Cary, NC). Fixed effects consisted of herbicide treatment. Location, year and replication were considered random effects to allow inferences to be made over a range of environments and conditions (Blouin et al. 2011).

Results

Foliar Broadcast Study.

Visible weed control and clover injury. (Table 1.4)**.**

Red clover was only present at one rating timing. All treatments caused significant injury (>72%) to red clover 30 days after application (DAA). Aminopyralid + 2,4-D and aminopyralid + florpyrauxifen-benzyl resulted in the greatest levels of injury, 100%.

All treatments except for florpyrauxifen-benzyl + 2,4-D resulted in greater than 41% injury to white clover 30 DAA. By 60 DAA, all treatments resulted in 95% injury or greater, with the exception of florpyrauxifen-benzyl + 2,4-D which resulted in 28% injury. At 90 and 120 DAA, all herbicide treatments, with the exception of florpyrauxifen-benzyl + 2,4-D caused nearly 100% white clover injury, while florpyrauxifen-benzyl + 2,4-D injury was 4 and 6%, respectively.

Horsenettle control was greatest across all evaluation timings with aminopyralid + 2,4-D and aminopyralid + florpyrauxifen-benzyl, with control at 84 and 95%, respectively, 90 DAA. Florpyrauxifen-benzyl + 2,4-D failed to control horsenettle, resulting in 45% control 90 DAA.

There were no differences in wild carrot control across treatments, as all treatments effectively controlled (>88%) wild carrot 30 DAA.

All treatments, except for metsulfuron, resulted in 86% or greater control in plumeless thistle 30 DAA. At 60 DAA, florpyrauxifen-benzyl + 2,4-D (99%), aminopyralid + 2,4-D (100%), and aminopyralid + florpyrauxifen-benzyl (100%) resulted in the greatest control, followed by 2,4-D + dicamba (85%) and metsulfuron (48%). Except for metsulfuron (21%), all treatments resulted in greater than 89% control of plumeless thistle 90 DAA. At 120 DAA, all treatments except metsulfuron (23%) resulted in 98% or greater control of plumeless thistle.

Chicory (*Cichorium intybus* L.) and white heath aster (*Symphyotrichum pilosum* Willd.) were only present to evaluate 60 DAA. Metsulfuron resulted in the least control (87 and 77% respectively), while all other treatments resulted in 100% control of both weeds.

No treatment offered considerable control of yellow foxtail at 90 and 120 DAA.

Species composition. (Table 1.5)

Transect data 30 DAA showed that aminopyralid + 2,4-D and aminopyralid + florpyrauxifen-benzyl resulted in the greatest composition of forage grasses, 95 and 91%, respectively. The other herbicide treatments, florpyrauxifen-benzyl + 2,4-D, 2,4-D + dicamba, and metsulfuron resulted in 78, 82, and 72% forage grasses, respectively. Early season (30 DAA) legume amount was greatest in the nontreated control (23%). There were no differences in weedy grass amount between treatments. Broadleaf weed amount was greatest in the nontreated control (34%). There was no difference between florpyrauxifen-benzyl + 2,4-D, aminopyralid + florpyrauxifen-benzyl, aminopyralid + 2,4-D, and 2,4-D + dicamba in broadleaf weed amount and all resulted in 2-12%. Metsulfuron resulted in 18% broadleaf weeds.

At 90 DAA, forage grass coverage was greatest with aminopyralid + florpyrauxifen-benzyl (88%). There were no differences between florpyrauxifen-benzyl + 2,4-D (74%), aminopyralid + 2,4-D (76%), and 2,4-D + dicamba (70%). Metsulfuron resulted in the least coverage of forage grasses of all treatments (51%). The only treatment with greater than 0% legume ground cover was florpyrauxifen-benzyl + 2,4-D (15%), while the nontreated control resulted in 49% legume cover. Metsulfuron had the most cover of weedy grasses (31%), followed by 2,4-D + dicamba (30%). Florpyrauxifen-benzyl + 2,4-D, aminopyralid + florpyrauxifen-benzyl, and the nontreated control had the least coverage of grassy weeds with 10%, 13%, and 4%, respectively. All treatments except for metsulfuron (18%) resulted in 1% or less broadleaf weed coverage. The nontreated control had 26% broadleaf weed coverage.

When species composition data were combined across evaluation timings for a cumulative estimate of sward composition, aminopyralid + florpyrauxifen-benzyl resulted in the greatest amount of forage grasses (90%), followed by 2,4-D + aminopyralid (89%) and 2,4-D + dicamba (78%). Other than the nontreated control (31%), florpyrauxifen-benzyl + 2,4-D resulted in the greatest amount of forage legumes (12%). All other herbicide treatments resulted in 1% or less legumes. There were no differences in weedy grass between treatments. All treatments significantly reduced cumulative coverage of broadleaf weeds. Aminopyralid + florpyrauxifen-benzyl (0%) had the least broadleaf weed coverage, followed by aminopyralid + 2,4-D (1%) and florpyrauxifen-benzyl + 2,4-D (7%).

Forage and weed biomass. (Table 1.6)

Above ground biomass data 30 DAA showed no differences in forage grass production across all treatments, including the nontreated control. Forage legume biomass was greatest in the nontreated control (421 kg ha^{-1}), followed by florpyrauxifen-benzyl + 2,4-D (149 kg ha^{-1}). All other treatments resulted in less than 11 kg ha^{-1} of forage legumes. Weedy grass biomass was greatest with aminopyralid + florpyrauxifen-benzyl (642 kg ha^{-1}). There were no differences in early season broadleaf weed biomass across all treatments, while the nontreated control resulted in 562 kg ha^{-1}.

Approximately 75 DAA, all treatments resulted in greater forage grass biomass compared to the nontreated control (1374 kg ha^{-1}). Florpyrauxifen-benzyl + 2,4-D resulted in the greatest late season forage grass biomass (3292 kg ha^{-1}). Among treatments, florpyrauxifen-benzyl + 2,4-D resulted in the greatest amount of late season forage legume biomass (118 kg ha^{-1}). All other treatments completely eliminated forage legumes. There were no differences in late season weedy grass biomass across all treatments. Metsulfuron (432 kg ha^{-1}) did not reduce late season broadleaf weed biomass. All other treatments resulted in decreased broadleaf weed biomass.

All treatments resulted in greater season-long cumulative forage grass biomass than the nontreated control, producing 77% to 122% more forage grass relative to the nontreated control, however there were no differences between treatments. All treatments resulted in less cumulative forage legume biomass compared to the nontreated control (863 kg ha^{-1}), however florpyrauxifen-benzyl + 2,4-D resulted in 100% more forage legumes compared to any other treatment (259 kg ha^{-1}). There were no differences in cumulative weedy grass biomass across all treatments, including the nontreated control. All treatments reduced cumulative broadleaf weed biomass compared to the nontreated control. Florpyrauxifen-benzyl + 2,4-D, aminopyralid + florpyrauxifen-benzyl, aminopyralid + 2,4-D, and 2,4-D + dicamba resulted in the least cumulative broadleaf weed biomass. Metsulfuron resulted in the greatest cumulative broadleaf weed biomass (637 kg ha^{-1}).

Fertilizer Impregnation Study. (Table 1.7)

Species Composition.

Aminopyralid + florpyrauxifen-benzyl treatments had the greatest composition of forage grasses (73% and 70%, respectively). Florpyrauxifen-benzyl + 2,4-D applied at the higher rate resulted in the lowest amount of forage grasses (46%), not different from the nontreated control (46%). Forage legumes were most abundant with both the low (13%) and high (10%) rate of florpyrauxifen-benzyl + 2,4-D, compared to all other herbicide treatments, which resulted in 1% or less forage legumes. Weedy grass amount was greatest with aminopyralid + metsulfuron (27%), while there were few differences between other treatments. Broadleaf weeds were most abundant in the fertilizer-only control (23%), followed by both the low (22%) and high (21%) rates of florpyrauxifen-benzyl + 2,4-D. Aminopyralid +2,4-D (14%), aminopyralid + metsulfuron (13%), and the high rate of aminopyralid + florpyrauxifen-benzyl (13%), resulted in the least broadleaf weeds.

Forage and weed biomass.

Cumulative forage grass biomass was greatest with aminopyralid + florpyrauxifen-benzyl at the lower rate (3327 kg ha^{-1}), a 44% increase compared to the nontreated control, followed by aminopyralid + 2,4-D (2994 kg ha^{-1}), and aminopyralid + florpyrauxifen-benzyl at the higher rate (2851 kg ha^{-1}). Aminopyralid + metsulfuron (2415 kg ha^{-1}) and florpyrauxifen-benzyl + 2,4-D (2340 kg ha^{-1}) resulted in the least forage grass biomass among herbicide treatments. All herbicides except for florpyrauxifen-benzyl + 2,4-D at both the high (276 kg ha^{-1}) and low (187 kg ha^{-1}) rate nearly eliminated forage legumes, reducing biomass by 87 to 96%. Aminopyralid + metsulfuron (1281 kg ha^{-1}) resulted in the greatest weedy grass biomass among herbicide treatments, followed by florpyrauxifen-benzyl + 2,4-D at the lower rate (738 kg ha^{-1}). Both the low and high rate of florpyrauxifen-benzyl + 2,4-D resulted in the greatest broadleaf weed biomass among herbicide treatments, 840 kg ha^{-1} and 813 kg ha^{-1}, respectively. Aminopyralid + 2,4-D resulted in the lowest broadleaf weed biomass (283 kg ha^{-1}), a 71% reduction compared to the fertilizer-only control.

Discussion

Our results are similar to others who have found that commonly used herbicides decrease and even completely eliminate forage legume presence as well as biomass when used in pastures and hayfields (Payne et al. 2010; Mikkelson et al. 2013; Enloe et al. 2014; Harrington et al. 2014; Miller et al. 2015). Even 2,4-D, which at lower rates is less injurious than most herbicides to white clover (Enloe et al. 2014), can eliminate stands when used at rates that are normally required to achieve satisfactory weed control (Payne et al. 2010). Metsulfuron has also been shown to severely injury and even completely eliminate white clover (James et al. 1999).

Overall comparison of foliar broadcast to impregnated fertilizer applications show that similar results can be achieved through both methods for aminopyralid-containing treatments. Treatments that did not contain soil-residual herbicides, such as florpyrauxifen-benzyl + 2,4-D, did not result in commercially acceptable weed control when applied via impregnated fertilizer. Regardless of application method, weed control will ultimately depend on the weed species targeted. Our research findings are similar to other who found that herbicide impregnated onto dry fertilizer offers postemergence broadleaf control (Brosnan and Breeden 2019).

Our findings suggest that acceptable control of certain broadleaf weeds can be achieved, while simultaneously preserving white clover through the use of florpyrauxifen-benzyl + 2,4-D. This research mimicked haying operations, but it may be that in a pasture system where animals can selectively graze, white clover may not be reduced to the extent observed in this study, due to reduced competition from grazed grasses. This should be examined in further research. Where white clover can be preserved while controlling weeds, the benefits of mixed swards of legumes and grasses such as: nitrogen fixation (Smith and Valenzuela 2002), enhanced livestock performance(Phillips and James 1998; Golding et al. 2008), improved seasonal distribution of forages (Sleugh et al. 2000), greater overall forage yields (Vines et al. 2006), and perhaps an enhanced habitat for pollinator species may be realized. Preserving white clover in the sward may have the additional benefit of enhanced pollinator habitat compared to grass-only forages (Woodcock et al. 2014), which should be the subject of future research.

Conclusion.

Florpyrauxifen-benzyl containing herbicides are effective in decreasing the number, as well as the overall biomass production of certain broadleaf weed species. Aminopyralid + florpyrauxifen-benzyl was more effective than florpyrauxifen-benzyl + 2,4-D in controlling weed species, regardless of the rates and application method. However, aminopyralid + florpyrauxifen-benzyl severely diminished, or even eliminated desirable forage legumes, while florpyrauxifen-benzyl + 2,4-D provided weed control of certain species while preserving forage legumes. Weed control from herbicides resulted in increased forage grass yields. Yields approximately doubled when broadcast applied and increased up to 44% when applied via fertilizer impregnation compared to fertilizer-alone.

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Tables

Table 1.1 Field site data for sward response following herbicide application by foliar spray and impregnated fertilizer in 2018-2020.

Study	Site	GPS coordinates	Soil series	Application Date(s)
Foliar Broadcast	Blacksburg, VA	37.2363, -80.4690	Berks-Groseclose silt loam	July 28, 2020
	Raphine, VA	37.9335, -79.2109	Bookwood silt loam	June 4, 2020
Fertilizer Impregnation	Blacksburg, VA	37.1937, -80.5818	Guernsey silt loam	May 3, 2018
	Garden City, MO	38.5516, -94.1684	Mandeville silt loam	May 11, 2018

Table 1.2 Herbicide treatments and sources for sward response following herbicide application through foliar broadcast spray and fertilizer impregnation in Virginia and Missouri in 2018-2020.

Herbicide(s)	Rate(s)	Trade Name(s)	Manufacturer(s)	Location(s)
	g ai/ae ha^{-1}			
Foliar Broadcast				
florpyrauxifen-benzyl + 2,4-D [a]	9 + 1,120	ProClova®	Corteva Agriscience	Wilmington, DE
2,4-D + aminopyralid [b]	933 + 115	GrazonNext HL®	Corteva Agriscience	Wilmington, DE
2,4-D + dicamba [b]	1,120 + 560	Shredder® Amine 4 + Clarity®	Winfield Solutions; BASF Corporation	St Paul, MN; Research Triangle Park, NC
metsulfuron-methyl [b]	7	Cimarron® Max	Bayer CropScience	St. Louis, MO
florpyrauxifen-benzyl + aminopyralid [a]	9 + 665	DuraCor™	Corteva Agriscience	Wilmington, DE
Impregnated Fertilizer				
florpyrauxifen-benzyl + 2,4-D[c]	12 + 747	ProClova®	Corteva Agriscience	Wilmington, DE
florpyrauxifen-benzyl + 2,4-D[c]	18 + 1,120	ProClova®		
florpyrauxifen-benzyl + aminopyralid[c]	9 + 94	DuraCor™		
florpyrauxifen-benzyl + aminopyralid[c]	12 + 117	DuraCor™		
2,4-D + aminopyralid[c]	933 + 115	GrazonNext HL®		
aminopyralid + metsulfuron[c]	121 + 22	Chaparral™		

[a] Included methylated seed oil (1% v v^{-1}); MSO® Concentrate, Loveland Products, Loveland, CO.

[b] Included nonionic surfactant (0.25% v v^{-1}); Scanner®, Loveland Products.

[c] Included methylated seed oil impregnated onto fertilizer herbicide mixture at 1% w/v; MSO® Concentrate.

Table 1.3 Dates for data collection for sward response following herbicide application through foliar broadcast spray and fertilizer impregnation in Virginia and Missouri in 2018-2020.

	Foliar Broadcast		Fertilizer Impregnation		
Sampling	Raphine, VA	Blacksburg, VA	Blacksburg, VA		Garden City, MO
	2020		2018	2019	2018
Biomass 1	July 8	August 25	June 12	June 11	June 10
Biomass 2	September 30	October 7	July 2	October 7	July 17
Biomass 3	═══	═══	August 8	═══	September 13
Biomass 4	═══	═══	September 10	═══	═══
Transect 1	July 8	August 24	June 1	June 10	June 10
Transect 2	September 30	October 7	July 2	October 7	July 16
Transect 3	═══	═══	August 2	═══	September 12

Table 1.4 Visible weed control in hayfields following foliar broadcast postemergence herbicides in hayfields in Raphine and Blacksburg, Virginia in 2020.

		30 DAT[a]					60 DAT					90 DAT				120 DAT		
		White clover[b]	Red clover	Horsenettle	Wild carrot	Plumeless thistle	White clover	Plumeless thistle	Chicory	Horsenettle	White heath aster	White clover	Plumeless thistle	Yellow foxtail	Horsenettle	White clover	Plumeless thistle	Yellow foxtail
Treatment	Rate	%																
	g ai/ae ha^{-1}																	
florpyrauxifen-benzyl + 2,4-D	9 + 1,120	41 c	74 b	56 b	95 ab	99 a	28 b	99 a	100 a	71 b	100 a	4 b	100 a	10 b	46 b	6 b	98 a	23 NS
2,4-D + aminopyralid	933 + 115	100 a	100 a	87 a	98 ab	98 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	46 a	85 a	100 a	100 a	40 NS
2,4-D + dicamba	1,120 + 560	73 b	73 b	56 b	96 ab	86 a	100 a	85 b	100 a	81 b	100 a	100 a	90 b	5 b	63 b	100 a	100 a	28 NS
metsulfuron	7	79 b	85 b	36 c	88 b	65 b	95 a	48 c	87 b	55 c	77 b	100 a	21 c	0 b	9 c	98 a	23 b	10 NS
florpyrauxifen-benzyl + aminopyralid	9 + 665	100 a	100 a	93 a	100 ab	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	44 a	94 a	100 a	100 a	35 NS

[a]Abbreviation: DAT: days after treatment.

[b]Means followed by the same letter are not significantly different according to Fisher's LSD ($p \leq 0.05$), within column.

Table 1.5 Hayfield sward species composition following foliar broadcast postemergence herbicides in Raphine and Blacksburg, Virginia in 2020.

Treatment	Forage Grass			Forage Legume			Weedy Grass			Broadleaf Weed		
	Early[a]	Late	Cumulative	Early	Late	Cumulative	Early	Late	Cumulative	Early	Late	Cumulative
	---	---	---	---	---	---	%	---	---	---	---	---
nontreated	38 C	21 D	34 D	23 A	49 A	30 A	6 NS	4 D	4 NS	34 A	26 A	32 A
florpyrauxifen-benzyl + 2,4-D	78 AB	74 AB	78 BC	10 B	15 B	11 B	3 NS	10 CD	4 NS	10 BC	1 C	8 C
florpyrauxifen-benzyl + aminopyralid	91 A	88 A	91 A	0 B	0 C	0 C	9 NS	13 BCD	9 NS	0 C	0 C	1 C
2,4-D + aminopyralid	95 A	76 AB	90 AB	1 B	0 C	0 C	2 NS	24 ABC	8 NS	2 C	0 C	2 C
2,4-D + dicamba	82 AB	70 B	79 ABC	1 B	0 C	0 C	5 NS	30 AB	12 NS	12 BC	0 C	9 C
metsulfuron	72 B	51 C	66 C	0 B	0 C	0 C	10 NS	31 A	16 NS	18 B	18 B	18 B

[a]Means followed by the same letter are not significantly different according to Fisher's LSD ($p \leq 0.05$), within column.

Table 1.6 Hayfield sward above ground biomass following foliar broadcast postemergence herbicides in Raphine and Blacksburg, Virginia in 2020.

Treatment	Forage Grass			Forage Legume			Weedy Grass			Broadleaf Weed		
	Early[a]	Late	Cumulative	Early	Late	Cumulative	Early	Late	Cumulative	Early	Late	Cumulative
	-kg ha^{-1}-											
nontreated	1376 NS	1374 C	2188 B	421 A	218 A	855 A	4 B	123 NS	127 NS	562 A	572 A	1759 A
florpyrauxifen-benzyl + 2,4-D	1594 NS	3292 A	4362 A	149 B	118 B	259 B	149 AB	110 NS	269 NS	60 B	15 B	75 C
florpyrauxifen-benzyl + aminopyralid	1906 NS	2861 AB	4309 A	0 C	0 C	0 C	642 A	309 NS	951 NS	150 B	0 B	150 C
2,4-D + aminopyralid	1458 NS	2671 AB	4353 A	1 C	0 C	0 C	84 B	233 NS	317 NS	43 B	77 B	120 C
2,4-D + dicamba	1744 NS	2984 AB	4861 A	4 C	0 C	3 C	134 AB	109 NS	243 NS	123 B	10 B	133 C
metsulfuron	1680 NS	2367 B	3881 A	10 C	0 C	11 C	369 AB	279 NS	648 NS	142 B	432 A	637 B

[a]Means followed by the same letter are not significantly different according to Fisher's LSD (p≤0.05), within column.

Table 1.7 Hayfield sward above ground biomass and species composition following herbicide impregnated application in Virginia and Missouri in 2018.

Treatment	Rate	Forage Grass[a]		Forage Legume		Weedy Grass		Broadleaf Weed	
	g ai/ae ha^{-1}	kg ha^{-1}	-%-	kg ha^{-1}	-%-	kg ha^{-1}	-%-	kg ha^{-1}	-%-
nontreated		2310 C	46 C	470 A	25 A	308 C	8 BC	990 A	23 A
florpyrauxifen-benzyl + 2,4-D	12 + 747	2340 BC	52 BC	187 B	13 B	738 AB	17 AB	840 AB	22 A
florpyrauxifen-benzyl + 2,4-D	18 + 1,120	2340 C	46 C	276 B	10 B	520 BC	16 ABC	813 AB	21 AB
florpyrauxifen-benzyl + aminopyralid	9 + 94	3327 A	73 A	0 C	0 C	460 BC	19 AB	425 BC	16 BC
florpyrauxifen-benzyl + aminopyralid	12 + 117	2851 B	70 A	0 C	0 C	429 BC	17 AB	370 BC	13 C
2,4-D + aminopyralid	933 + 115	2994 AB	62 AB	1 C	0 C	436 BC	15 ABC	283 C	14 C
aminopyralid + metsulfuron	121 + 22	2415 BC	59 AB	0 C	1 C	1281 A	27 AB	460 BC	13 C

[a]Means followed by the same letter are not significantly different according to Fisher's LSD ($p \leq 0.05$), within column.

Table 1.S1 Percentage cover of species present at time of treatment for sward response studies by foliar broadcast and fertilizer impregnation studies in Virginia and Missouri 2018-2020.

Species	Species Group	Location(s) Present			
		Foliar Blacksburg	Foliar Raphine	Fertilizer Blacksburg	Fertilizer Garden City
		----------%	----------	----------	----------
tall fescue	forage grass	33	40	27	47
orchardgrass	forage grass	40	30	37	3
timothy	forage grass	2	4	——	——
white clover	forage legume	10	9	8	6
red clover	forage legume	1	0.2	0.4	0.7
annual lespedeza	forage legume	——	——	——	0.6
horsenettle	broadleaf weed	15	2.7	——	——
common chickweed	broadleaf weed	——	——	0.2	——
mouseear chickweed	broadleaf weed	——	0.8	——	0.2
hairy bittercress	broadleaf weed	——	——	0.1	——
common lambsquarters	broadleaf weed	——	——	0.2	——
buckhorn plantain	broadleaf weed	——	0.4	7	——
common ragweed	broadleaf weed	——	——	8	8
common milkweed	broadleaf weed	——	0.2	——	0.4
yellow woodsorrel	broadleaf weed	——	——	——	——
annual fleabane	broadleaf weed	——	——	0.1	1
plumeless thistle	broadleaf weed	——	5	——	——
common dandelion	broadleaf weed	——	0.4	0.1	——
broadleaf dock	broadleaf weed	——	——	1.2	——
multiflora rose	broadleaf weed	——	——	0.1	——
prostrate knotweed	broadleaf weed	——	——	4	——
Persian speedwell	broadleaf weed	——	——	0.4	——
shepherd's-purse	broadleaf weed	——	——	0.7	——
Italian ryegrass	weedy grass	0.2	——	——	——
broomsedge	weedy grass	——	——	——	——
barnyardgrass	weedy grass	0.6	——	——	——
annual bluegrass	weedy grass	0.2	7	4	28
giant foxtail	weedy grass	2	——	——	——
broomsedge	weedy grass	——	——	0.1	0.2

Broadleaf weed control and white clover response to florpyrauxifen-benzyl + 2,4-D and common pasture herbicides

Abstract. Florpyrauxifen-benzyl is a newer group 4, synthetic auxin herbicide in the arylpicolinate herbicide family. The combination of florpyrauxifen-benzyl + 2,4-D is a new herbicide product, ProClova, for use in pastures and hayfields in the United States. Unlike many other pasture herbicides, florpyrauxifen-benzyl + 2,4-D is reported to preserve white clover. However, there is limited research on the efficacy of florpyrauxifen-benzyl + 2,4-D on common weed species, as well as the level of tolerance of white clover to postemergence applications of florpyrauxifen-benzyl + 2,4-D. Field trials were conducted across Virginia in 2018-20 to evaluate control of various broadleaf weeds with florpyrauxifen-benzyl + 2,4-D as well as field and greenhouse studies assessing white clover tolerance across varieties to florpyrauxifen-benzyl containing herbicides. Weed species evaluated included: bulbous buttercup, Canada thistle, horsenettle, and broadleaf plantain. Bulbous buttercup control was evaluated following fall and spring applications in order to determine the effect of application timing. Florpyrauxifen-benzyl + 2,4-D provided 75 to 100% control of all weeds except for horsenettle, while resulting in the least white clover injury. Overall, spring herbicide applications resulted in greater bulbous buttercup control compared to fall applications, but florpyrauxifen-benzyl + 2,4-D resulted in greater than 81% control for both application timings. There were no differences in above ground biomass between white clover varieties, however all herbicides reduced white clover biomass compared to the control. This research suggests that florpyrauxifen-benzyl + 2,4-D is an effective herbicide for several broadleaf weed species, as well as safer to white clover than several other commonly used herbicides. Florpyrauxifen-benzyl + 2,4- D can offer tremendous value by controlling broadleaf weeds in mixed grass-legume stands while preserving white clover.

Nomenclature: florpyrauxifen-benzyl; 2,4-D; white clover, *Trifolium repens* L.; bulbous buttercup (*Ranunculus bulbosus* L.); Canada thistle (*Cirsium arvense* L.); horsenettle (*Solanum carolinense* L.); broadleaf plantain (*Plantago major* L.)

Keywords: hayfields; application timing

Introduction. Broadleaf weed species are one of the biggest factors limiting forage production (Grekul and Bork 2004; Seefeldt et al. 2005; Eagle et al. 2007). A survey conducted by the Weed Science Society of America reported that of the six most troublesome weed species in pasture, rangeland, and hay, five were broadleaf weed species. Because of their ability to infest pastures, as well as typically having low palatability by livestock, broadleaf weed species can reduce forage yield, decrease forage quality, and contaminate forage with toxic weed species (Gunning 1949; Cook et al. 2009; Welsh et al. 2007), and ultimately reduce livestock weight gain (Marten et al. 1987). Hartley (1983) showed that when musk thistle, (Carduus nutans L.), was present at a density of one plant per m2, sheep weight gain could be reduced by 20%. Additionally, even the presence of certain broadleaf weed species can deter grazing of nearby desirable forage (Tiley 2010), therefore reducing forage utilization.

Because of the perennial nature of pasture systems, a different spectrum of weed species can impact production throughout the year, complicating management efforts. Certain weed species are more susceptible to herbicides at certain times in the growing season. For example, perennial weed species such as horsenettle (*Solanum carolinense* L.), are best controlled by herbicides applied at the bloom stage, where as warm-season annuals such as common ragweed (*Ambrosia artemisiifolia* L.) are best controlled by spring and early summer applications (Flessner and Taylor 2021). Additionally, cool season weed species that emerge in the fall are often targeted with herbicide applications in the following spring. Some research suggests that fall herbicide applications can be effective in controlling warm-season perennials (Marshall et al. 2006), however little research exists on the efficacy of fall versus spring-applied herbicides for cool-season perennial weeds. Because many weed species affect pasture productivity, and these weed species are rarely present at the same time, producers must decide which weeds are the most detrimental to forage production and target those species in a single application, as it is rarely economical to make multiple herbicide applications per year in pastures (Gylling et al. 2009).

Another management concern when using herbicides is desirable forage legumes such as white clover (*Trifolium repens* L.). There are many common and widely available herbicides that are frequently used to control broadleaf weeds in pastures and hayfields. However, the majority of these herbicides kill desirable forage legumes such as white clover (Beeler et al. 2003; Payne et al. 2010; Miller et al. 2020). Forage legumes including white clover have a plethora of benefits when included in pastures such as increased forage quality (Posler et al. 1993), which can ultimately lead to increases in livestock performance (Burns et al. 1973). Compared to grass monocultures, grass-legume mixtures have a longer grazing season (Gibson and Cope 1985) and lead to greater grass yield through the transfer of nitrogen, fixed through the legumes, to grasses (Wagner 1954; Sleugh et al. 2000; Sanderson et al. 2005).

A potential solution to the management tradeoff between weed control and keeping legumes is florpyrauxifen-benzyl + 2,4-D (ProClova™) is expected to be commercially available in 2022 and is reported to preserve white clover (Sleugh et al. 2020). However, the weed control spectrum, optimal application timing, and potential varietal response of white clover need further evaluation in order to make well informed management decisions regarding applications to pastures and hayfields.

The overarching objective of this research is to determine the utility of florpyrauxifen-benzyl + 2,4-D for pasture and hayfield weed management by evaluating its weed control spectrum and white clover response. To do so, four objectives were identified: (1) determine the efficacy of florpyrauxifen-benzyl + 2,4-D on common broadleaf weeds found in pastures and hayfields, (2) compare the efficacy of fall versus spring-applied herbicides for weed control, (3) evaluate white clover response to determine the level of tolerance to florpyrauxifen-benzyl + 2,4-D, and (4) determine if white clover variety has an effect on injury following florpyrauxifen-benzyl + 2,4-D application

Materials & Methods.

Single Application Studies.

Study Sites. Numerous field trials were established across Virginia in 2019 and 2020. All sites contained naturalized weed populations as well as mixed stands of cool-season grasses such as tall fescue (*Lolium arundinaceum* (Schreb.)) and orchardgrass (*Dactylis glomerata* L.). Treatments were applied at the recommended time based on the Virginia Field Crops Pest Management Guide (Flessner and Taylor 2021) for the weed species being targeted at each location. In general, applications were made in April for warm season annual weeds, July for warm season perennials and November and April for cool season perennials. Application dates as well as locations and weed species present are presented in Table 2.1.

Experimental Design. All sites were designed as a randomized complete block design with four replications. Herbicide applications were made using a 3 m-wide handheld backpack sprayer with 6 TeeJet (Spraying Systems Co.; Wheaton, IL) 11002XR nozzles calibrated to deliver 140 L ha^{-1} at 207 kPa. Treatments are presented in Table 2.2 in addition to a nontreated control.

Data Collection and Analysis. Following herbicide application, visible weed control and white clover injury were evaluated throughout the growing season on a scale of 0 to 100 %, with 0 being no observable injury and 100 being complete plant necrosis relative to the nontreated check as described by Frans et al. (1986). Depending on the study site, trials were either managed for hay production, or fenced off for 30 days, and then grazed by cattle.

Data were subject to ANOVA and subsequent means separation using Fisher's Protected LSD test ($P \leq 0.05$) to compare across treatments using JMP Pro 15 (SAS Institute, Inc; Cary, NC). Fixed effects consisted of herbicide treatment. Year, location, and replication nested within year were considered random effects to allow inferences to be made over a range of environments and conditions (Blouin et al. 2011). Visible control of weed species data were analyzed by location for weeds not present at only certain locations.

Fall Versus Spring Application Timing Study.

Study Sites. Field trials were established in Amelia Court House (37.29, -77.86) and Blacksburg (37.27, -80.36), Virginia in the fall of 2018, and in Blacksburg, Virginia in the fall of 2019. All sites contained naturalized weed populations, consisting primarily of bulbous buttercup (*Ranunculus bulbosus* L.) as well as mixed stands of cool season grasses such as tall fescue and orchardgrass. Late October / early November was targeted for the fall application, and late March / early April was targeted for the spring application. Exact application dates, as well as trial locations are listed in Table 2.1. All trials were fenced off for 30 days following both applications, and then allowed to be grazed by cattle.

Experimental Design. All sites were designed as a factorial with factor A being timing and factor B being herbicide. Treatments were arranged in a randomized complete block design. The Amelia County site and the Blacksburg site in 2019 had four replications, while the Blacksburg site in 2018 had three replications. Plot size was 4m by 9m in 2018 and 5m by 7m in 2019. Herbicides and sources are presented in Table 2.2 in addition to a nontreated control.

Data Collection and Analysis. Following herbicide application, visible weed control data were taken on 30-day intervals following the fall application, up until the spring application. Following the spring application, visible weed control data were also taken on 30-day intervals for 120 days.

All data were subject to ANOVA and subsequent means separation using Fisher's Protected LSD test ($P \leq 0.05$) to compare across treatments using JMP Pro 15. Fixed effects consisted of herbicide treatment. Location and replication, nested within location, were considered random effects. Following spring herbicide application, data were analyzed as a factorial, with herbicide and application timing as fixed effects in order to determine the effect of herbicide timing.

White clover response.

Established white clover response.

Study Sites. Field trials were established in 2020 in Raphine (37.93, -79.21) and Blacksburg (37.23, -80.36), Virginia. Both locations were seeded with 'Ladino' white clover in the years prior. Dates of herbicide application are listed in Table 2.1.

Experimental Design. All sites were designed as a randomized complete block design with four replications. Plot size was 3m by 6m. Herbicide applications were made using a 1.8 m-wide handheld backpack sprayer with four TeeJet (Spraying Systems Co.; Wheaton, IL) 11002XR nozzles calibrated to deliver 140 L ha^{-1} at 207 kPa. Herbicide treatments included: (1) florpyrauxifen-benzyl + 2,4-D at 9 + 560 g ai/ae ha^{-1}, respectively, (2) florpyrauxifen-benzyl + 2,4-D at 18 + 1,120 g ai/ae ha^{-1}, (3) florpyrauxifen-benzyl at 9 g ai ha^{-1}, (4) florpyrauxifen-benzyl at 18 g ai ha^{-1}, (5) 2,4-D at 560 g ae ha^{-1}, (6) 2,4-D at 1,120 g ae ha^{-1}, (7) dicamba + 2,4-D at 560 + 1,120 g ai/ae ha^{-1}, and (8) a mowing treatment at a 13 cm height in order to mimic the common practice of mowing for pasture weed control.

Data Collection and Analysis. Following herbicide application, a 0.5 m^2 section of above ground biomass was collected every 2 weeks for 6 weeks from a different area within the treated plot. Additionally, visible injury ratings were taken on a scale of 0 to 100% for the 4 weeks following herbicide application.

All data were subject to ANOVA and subsequent means separation using Fisher's Protected LSD test ($P \leq 0.05$) to compare across treatments using JMP Pro 15. Fixed effects consisted of herbicide treatment. Location and replication, nested within location, were considered random effects. The nontreated control was excluded from visible injury ratings.

Greenhouse white clover varietal response.

Study Site and Experimental Design. Greenhouse trials were established in Blacksburg (37.23, -80.43), Virginia in 2020 and 2021. Four different varieties of white clover were seeding into 15 cm diameter pots at a seeding rate of 5.6 kg ha^{-1}. Varieties included: (1) ‘Ladino’, (2) ‘Durana’ (Pennington Seed, Inc.; Madison, GA), (3) ‘Alice’ (Barenbrug USA.; Tangent, OR), and (4) ‘Patriot’ (Pennington Seed, Inc.; Madison, GA). Following seeding, clover was allowed to grow until flowering, and then all plants were trimmed to approximately 10 cm in height. Plants were then allowed to regrow for two weeks before treatments were applied. Herbicide applications were made using a 1.8 m-wide handheld backpack sprayer with four TeeJet (Spraying Systems Co.; Wheaton, IL) 11002XR nozzles calibrated to deliver 140 L ha^{-1} at 2017 kPa. Treatments are listed in Table 2.7 and were arranged in a randomized complete block design with five replications. The trial was replicated thrice in time.

Data Collection and Analysis. Following herbicide application plants were allowed to grow for 6 weeks. Above-ground biomass was then collected from each pot, dried at 52C for 72 hours, and weighed.

All data were subject to ANOVA and subsequent means separation using Fisher’s Protected LSD test ($P \leq 0.05$) to compare across treatments using JMP Pro 15. Data were analyzed as a factorial with herbicide being factor A and variety as factor B. Trial run and replication, nested within run, were considered random effects.

Results

Single Application Study. (Table 2.3)

White clover injury.

White clover injury was greater than 83% with all herbicides, except for 2,4-D (19%) and florpyrauxifen-benzyl + 2,4-D (30%) 30 days after application (DAA). At 60 DAA, injury from florpyrauxifen-benzyl + 2,4-D had decreased to 16%, while injury from 2,4-D decreased to 18%. All other herbicide treatments resulted in 91% or greater white clover injury. Injury from florpyrauxifen-benzyl + 2,4-D and 2,4-D had decreased to 3% and 9%, respectively 90 DAA. Aminopyralid + 2,4-D, 2,4-D + dicamba, metsulfuron, and triclopyr + fluroxypyr all resulted in 98% white clover injury or greater 90 DAA. Injury from triclopyr + 2,4-D was less than other treatments, but still significant (85%).

Broadleaf weed control.

Canada thistle. Initially, several herbicides provided good control of Canada thistle 30 DAA. Florpyrauxifen-benzyl + 2,4-D and aminopyralid + 2,4-D resulted in the greatest control 30 DAA, 89% and 94%, respectively. However, control by all herbicides dropped throughout the growing season. Aminopyralid + 2,4-D provided the greatest control (89%) 90 DAA followed by florpyrauxifen-benzyl + 2,4-D (75%) and 2,4-D + dicamba (73%). By 120 DAA, aminopyralid + 2,4-D still provided the greatest control (79%), followed by triclopyr + 2,4-D (75%), florpyrauxifen-benzyl + 2,4-D (69%), triclopyr + fluroxypyr (68%), 2,4-D + dicamba (63%), 2,4-D (44%), and metsulfuron (38%).

Broadleaf plantain. With the exception of triclopyr + fluroxypyr (75%), all herbicides provided greater than or equal to 85% control of broadleaf plantain 30 DAA. Control levels were similar 60 DAA. By 90 DAA, broadleaf plantain control was greatest with florpyrauxifen-benzyl + 2,4-D (98%), followed by aminopyralid + 2,4-D (93%), 2,4-D + dicamba (90%), triclopyr + 2,4-D and metsulfuron (83%), 2,4-D (80%), and triclopyr + fluroxypyr (68%).

Horsenettle. Horsenettle control was greatest following aminopyralid + 2,4-D (88%) and triclopyr + fluroxypyr (85%) 30 DAA. No other herbicide resulted in greater than 75% horsenettle control 30 DAA. Aminopyralid + 2,4-D (95%), 2,4-D + dicamba (95%), triclopyr + 2,4-D (93%), and triclopyr + fluroxypyr (91%) resulted in the greatest control 60 DAA followed by florpyrauxifen-benzyl + 2,4-D (71%), 2,4-D (59%) and metsulfuron (38%). Aminopyralid + 2,4-D, 2,4-D + dicamba, triclopyr + 2,4-D, and triclopyr + fluroxypyr resulted in the greatest control 90 DAA with 89%, 81%, 75%, and 74%, respectively. Horsenettle control from florpyrauxifen-benzyl + 2,4-D (56%) declined 90 DAA, as did control from 2,4-D (48%) and metsulfuron (26%).

Additional weeds. (Table 2.4)

Plumeless thistle control was greatest with florpyrauxifen-benzyl + 2,4-D (99%), aminopyralid + 2,4-D (98%), and 2,4-D + dicamba (86%). At both 60 and 90 DAA, control was greatest with florpyrauxifen-benzyl + 2,4-D and aminopyralid + 2,4-D followed by 2,4-D + dicamba. By 120 DAA, all herbicides other than metsulfuron (27%), resulted in at least 98% control or greater.

There were no differences in wild carrot control at 30 and 60 DAA. Florpyrauxifen-benzyl + 2,4-D, aminopyralid + 2,4-D, and triclopyr + fluroxypyr all resulted in the greatest wild carrot control 90 DAA, with 100%, 90%, and 93% control, respectively. Metsulfuron (49%) offered the least control 90 DAA.

With the exception of metsulfuron, all herbicides resulted in 100% control of common ragweed at 30, 60 and 90 DAA, whereas metsulfuron resulted in 43%, 21%, and 8% control, respectively.

Fall versus spring application timing study. (Table 2.5)

White clover injury. Following fall herbicide applications, florpyrauxifen-benzyl + 2,4-D resulted in the least white clover injury 30 days after fall application (DAF) (15%), followed by 2,4-D (33%), and triclopyr + fluroxypyr (35%). Aminopyralid + 2,4-D (65%), 2,4-D + dicamba (63%), and triclopyr + 2,4-D (55%), resulted in the greatest white clover injury 30 DAF. Other than florpyrauxifen-benzyl + 2,4-D (12%), white clover injury from all herbicide treatments increased by 60 DAF. 2,4-D resulted in 65% injury, while all other herbicide treatments caused 90% injury or greater. At 120 DAF, florpyrauxifen-benzyl + 2,4-D and 2,4-D resulted in 1%, and 4% injury, respectively. All other herbicides resulted in 98% injury or greater.

Following spring application, data were analyzed as a factorial in order to determine the effect of application timing. There was a significant interaction between application timing and herbicide treatment ($p=0.012$), therefore data were not pooled across timing or herbicides. Herbicides which caused the least white clover injury 90 days following the spring application (90 DAS) include florpyrauxifen-benzyl + 2,4-D applied in the fall and spring (0%), and 2,4-D applied in the fall (0%). 2,4-D + dicamba applied in the fall (20%), followed by 2,4-D applied in the spring (29%) and triclopyr + 2,4-D applied in the fall (36%). All other herbicides, across timings, resulting in 80% injury or greater to white clover 90 DAS.

Bulbous buttercup. Following fall herbicide applications, bulbous buttercup control was poor to fair for all herbicide treatments 30 DAF, with the greatest control being 54% (aminopyralid + 2,4-D). For most herbicide treatments, control gradually improved throughout the winter and early spring. At 90 DAF, control was greatest from aminopyralid + 2,4-D (89%), metsulfuron (82%), and florpyrauxifen-benzyl + 2,4-D (81%). The final rating prior to spring applications, 120 DAF, showed that 2,4-D resulted in the least buttercup control (39%), and there were no differences between any of the herbicide treatments, where control ranged from 65 to 81%.

Following spring application, data were analyzed as a factorial in order to determine the effect of application timing. There was a significant interaction between application timing and herbicide treatment (p=0.011), therefore data were not pooled across timing or herbicide. In general, bulbous buttercup control was better from spring rather than fall application, 30 DAS. However, the herbicides that resulted in significantly less control from their fall application compared to their spring application 30 DAS were dicamba + 2,4-D (81% vs 95%), 2,4-D (60% vs 73%), triclopyr + 2,4-D (70% vs 97%), and triclopyr + fluroxypyr (59% vs 95%). The same general trend persisted 60 DAS. Spring application resulted in greater control compared to fall application for all herbicides with the exception of florpyrauxifen-benzyl + 2,4-D, aminopyralid + 2,4-D, and metsulfuron. At 90 DAS, all herbicide treatments except for aminopyralid + 2,4-D showed greater buttercup control from spring compared to fall applications. Certain herbicides however, showed a greater disparity in control between fall and spring applications. The difference in control between fall and spring applications was greatest with triclopyr + 2,4-D (60% vs 98%), 2,4-D (38% vs 70%), and triclopyr + fluroxypyr (44% vs 94%).

Comparing all herbicide treatments, across timing, demonstrated that no herbicide resulted in greater control when applied in the fall compared to the spring, but there were instances where certain herbicides offered greater control regardless of timing. Aminopyralid + 2,4-D (90%), metsulfuron (84%), and florpyrauxifen-benzyl + 2,4-D (82%) resulted in greater buttercup control when applied in the fall compared to 2,4-D applied in the spring (70%).

Established white clover tolerance. (Table 2.6)

Florpyrauxifen-benzyl + 2,4-D applied at 18 g ai ha^{-1} and 1,120 g ae ha^{-1}, and dicamba + 2,4-D resulted in the greatest levels of visible injury following treatment. Visible injury was characterized by lodging and epinasty for both treatments, consistent with auxin herbicide symptomology. For florpyrauxifen-benzyl + 2,4-D at 18 g ai ha^{-1} and 1,120 g ae ha^{-1}, visible injury was greatest 1 week after treatment (WAT) (63%) and declined to 56% and 46% at 2 and 3 WAT, respectively. Dicamba + 2,4-D injury was 65% 1 WAT, then increased to 84% and 89%, 2 and 3 WAT and remained ~90% until above ground biomass was taken.

Above-ground biomass results 2 WAT showed that only dicamba + 2,4-D (135 kg ha^{-1}) and florpyrauxifen-benzyl at 9 g ai ha^{-1} (312 kg ha^{-1}) resulted in significantly lower biomass than the nontreated control (647 kg ha^{-1}). At 4 WAT, florpyrauxifen-benzyl + 2,4-D at 9 g ai ha^{-1} + 560 g ai ha^{-1} (823 kg ha^{-1}), florpyrauxifen-benzyl at 18 g ai ha^{-1} (748 kg ha^{-1}), and dicamba + 2,4-D (172 kg ha^{-1}) decreased biomass compared to the nontreated control (1736 kg ha^{-1}). At 6 WAT all herbicide treatments reduced white clover biomass compared to the nontreated control (2102 kg ha^{-1}), while the mowing treatment (1583 kg ha^{-1}) did not significantly reduce clover biomass. With the exception of dicamba + 2,4-D, which completely eliminated all white clover, all other herbicides reduced white clover biomass, however there were no differences between herbicide treatments.

Greenhouse white clover varietal tolerance. (Table 2.7)

Results from the greenhouse showed that herbicide treatment was significant, but not variety, and there was no interaction between the two factors. Therefore, results were pooled across variety. All herbicide treatments reduced white clover biomass compared to the nontreated control (3892 kg ha^{-1}). 2,4-D applied at 560 g ae ha^{-1} resulted in the greatest biomass across herbicide treatments (2543 kg ha^{-1}), while 2,4-D applied at 1120 g ae ha^{-1} resulted in the second greatest biomass across herbicide treatments (1940 kg ha^{-1}). Both rates of florpyrauxifen-benzyl + 2,4-D reduced white clover biomass, however the lower rate resulted in significantly greater biomass (1315 kg ha^{-1}) compared to the higher rate which resulted in 599 kg ha^{-1}, which was not significantly different from the dicamba + 2,4-D treatment (539 kg ha^{-1}).

Discussion

Although there is little data on the efficacy of florpyrauxifen-benzyl + 2,4-D on broadleaf weed species, our findings are similar to others such as (Perry et al. 2015) who found that florpyrauxifen-benzyl did provide control of broadleaf weed species. Additionally, our findings are similar to those who found that commonly used pasture herbicides can result in high levels of desirable forage legume injury, and even death as seen with aminopyralid (Beeler et al. 2003; Harrington et al. 2014; Mikkelson et al. 2013; Miller et al. 2020), aminopyralid + 2,4-D (Enloe et al. 2014; Payne et al. 2010), 2,4-D (Payne et al. 2010), 2,4-D + dicamba (Payne et al. 2010), and metsulfuron (Payne et al. 2010.)

Although little research exists on the efficacy of fall versus spring applied herbicides for bulbous buttercup control, our findings support the general recommendation (Enloe et al. 2014; Flessner and Taylor 2021) that spring herbicides are most effective in bulbous buttercup control. However, our data suggest that certain fall applied herbicides can result in equal, or even greater control of bulbous buttercup than spring-applied herbicides. These data suggest that producers need to consider not only the weed species present when determining application timing, but also the specific herbicide to be used.

Although florpyrauxifen-benzyl containing herbicides did significantly injure established white clover, the clover was not completely eliminated and did experience some recovery during the trial period. While the higher rate of florpyrauxifen-benzyl + 2,4-D did result in greater visible injury and lodging than the lower rate, there were no differences in clover biomass. Mowing remained the safest weed management option if the primary objective is to maintain the highest levels of white clover, while employing a weed control tactic. The only herbicide that completely eliminated all white clover present was dicamba + 2,4-D.

Our findings suggest that white clover variety is not a significant factor in plant response to florpyrauxifen-benzyl, alone or in combination with 2,4-D with clover grown in the greenhouse. All white clover varieties tested responded similarly to each treatment. Our research findings are similar to those by Wright et al. 2021, in which they found difference in rice cultivar response to applications of florpyrauxifen-benzyl.

In conclusion, our research findings demonstrate the ability of florpyrauxifen-benzyl-containing herbicides to add value to forage systems through: (1) controlling certain broadleaf weed species with the flexibility to apply across timings and (2) preserving established white clover. Future research should further investigate the weed spectrum of florpyrauxifen-benzyl-containing herbicides, as well as evaluating the effect of various environmental factors, application timings, and clover growth stages on white clover injury.

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Tables

Table 2.1 Field trial info for broadleaf weed control trials in Virginia from 2018-2020.

Location	Coordinates	Application Date	Species Common Name(s)	Species Scientific name(s)
Single Application Study				
Meadowview, VA	36.7692, -81.8691	April 22, 2019	Canada thistle; broadleaf plantain	*Cirsium arvense* (L.) Scop.
Glade Spring, VA	36.7745, -81.8691	April 22, 2019	Canada thistle	*Cirsium arvense* (L.) Scop.
Blacksburg, VA	37.2376, -80.4700	July 19, 2019	horsenettle	*Solanum carolinense* (L.)
Raphine, VA	37.9335, -79.2109	June 4, 2020	horsenettle; white clover	*Solanum carolinense* (L.); *Trifolium repens* (L.)
Raphine, VA	37.9199, -79.2217	June 4, 2020	white clover	*Trifolium repens* (L.)
Blacksburg, VA	37.2367, -80.4675	July 28, 2020	horsenettle; white clover	*Solanum carolinense* (L.); *Trifolium repens* (L.)
Fall Versus Spring Study				
Blacksburg, VA	37.2727, -80.3637	November 16, 2018 & April 24, 2019	bulbous buttercup; white clover	*Ranunculus bulbosus* (L.); *Trifolium repens* (L.)
Amelia Court House, VA	37.2912, -77.8683	November 19, 2018 & April 17, 2019	bulbous buttercup	*Ranunculus bulbosus* (L.)
Blacksburg VA	37.2364, -80.4676	November 26, 2019 & April 6, 2020	bulbous buttercup; white clover	*Ranunculus bulbosus* (L.); *Trifolium repens* (L.)
Established White Clover Tolerance Study				
Blacksburg, VA	37.2365, -80.3638	September 3, 2020	white clover	*Trifolium repens* (L.)
Raphine, VA	37.9335, -79.2109	September 10, 2020		
Greenhouse White Clover Variety Response				
Blacksburg, VA	37.2319, -80.4347	October 17, 2020, March 1, 2021 & March 16, 2021	white clover	*Trifolium repens* (L.)

Table 2.2 Herbicide materials and sources for broadleaf weed control trials in pastures and hayfields in Virginia in 2018-2020.

Treatment	Rate(s)	Trade Name(s)	Manufacturer	Location
	g ai/ae ha^{-1}			
florpyrauxifen-benzyl + 2,4-D[a]	9	ProClova®	Corteva Agriscience	Wilmington, DE
aminopyralid + 2,4-D[b]	933 + 115	GrazonNext® HL	Corteva Agriscience	Wilmington, DE
2,4-D + dicamba[b]	1,065 + 560	Shredder® Amine 4 + Clarity®	Winfield Solutions + BASF Corporation	St. Paul, MN; Research Triangle Park, NC
2,4-D	1,065	Shredder® Amine 4	Winfield Solutions	St. Paul, MN
triclopyr + 2,4-D	560 + 1,121	Crossbow®	Corteva Agriscience	Wilmington, DE
metsulfuron[b]	7	Cimarron® MAX	Bayer CropScience	St. Louis, MO
triclopyr + fluroxypyr[b]	631 + 210	PastureGard® HL	Corteva Agriscience	Wilmington, DE

[a] Included methylated seed oil (1% v v^{-1}).

[b] Included nonionic surfactant (0.25% v v^{-1}).

Table 2.3 Broadleaf weed control from single herbicide applications in pastures and hayfields across Virginia in 2019-2020.

	White clover injury			Canada thistle control				Broadleaf plantain control			Horsenettle control		
Treatment	30 DAA [a]	60 DAA [b]	90 DAA	30 DAA	60 DAA	90 DAA	120 DAA	30 DAA	60 DAA	90 DAA	30 DAA	60 DAA	90 DAA
	---%												---
florpyrauxifen-benzyl + 2,4-D	30 D	16 B	3 C	89 A	83 AB	75 AB	69 AB	96 A	99 A	98 A	48 D	71 B	56 C
aminopyralid + 2,4-D	100 A	99 A	99 A	94 A	89 A	89 A	79 A	89 AB	92 AB	93 A	88 A	95 A	89 A
2,4-D + dicamba	86 BC	100 A	98 A	80 AB	78 ABC	73 B	63 B	90 AB	91 AB	90 AB	69 B	95 A	81 AB
2,4-D	19 D	18 B	9 C	68 B	60 D	55 CD	44 C	85 BC	85 BC	80 C	56 CD	59 C	48 C
triclopyr + 2,4-D	90 ABC	91 A	85 B	67 B	63 D	58 CD	75 AB	89 AB	85 BC	83 BC	71 BC	93 A	75 AB
metsulfuron	84 C	97 A	100 A	69 B	64 CD	50 D	38 C	94 AB	90 AB	83 BC	41 D	38 D	26 D
triclopyr + fluroxypyr	99 AB	99 A	100 A	78 AB	72 BCD	69 BC	68 AB	75 C	75 C	68 D	85 AB	91 A	74 B

[a]Means followed by the same letter are not significantly different according to Fisher's LSD ($p \leq 0.05$), within column.

Table 2.4 Additional broadleaf weed control data from field trials conducted in pastures in Virginia in 2018-2020.

Herbicide(s)	Plumeless thistle 30 DAA[a]		Plumeless thistle 60 DAA[b]		Plumeless thistle 90 DAA		Plumeless thistle 120 DAA		Wild carrot 60 DAA		Wild carrot 90 DAA		Common ragweed 30 DAA		Common ragweed 60 DAA		Common ragweed 90 DAA	
	%																	
florpyrauxifen-benzyl + 2,4-D	99	A	99	A	100	A	98	A	100	NS	100	A	100	A	100	A	100	A
aminopyralid + 2,4-D	98	A	100	A	100	A	100	A	96	NS	90	A	100	A	100	A	100	A
2,4-D + dicamba	86	AB	85	B	90	A	100	A	100	NS	73	AB	100	A	100	A	100	A
2,4-D	75	B	75	C	80	A	78	AB	88	NS	74	AB	100	A	100	A	100	A
triclopyr + 2,4-D	85	AB	85	B	90	A	95	A	95	NS	73	AB	100	A	100	A	100	A
metsulfuron	65	B	48	C	21	B	23	B	88	NS	49	B	43	B	21	B	8	B
triclopyr + fluroxypyr	65	B	60	C	55	B	50	B	100	NS	93	A	100	A	100	A	100	A

[a]Means followed by the same letter are not significantly different according to Fisher's LSD ($P \leq 0.05$), within column.
[b]Abbreviation: DAA, days after application

Table 2.5 Bulbous buttercup control and white clover injury following herbicide application made in the fall and spring in Virginia in 2018-2020.

		bulbous buttercup control							white clover control					
Treatment	Timing	30 DAF [a]	60 DAF[b]	90 DAF	120 DAF	30 DAS[c]	60 DAS	90 DAS	30 DAF	60 DAF	120 DAF	30 DAS	60 DAS	90 DAS
		%												
florpyrauxifen-benzyl + 2,4-D	fall	38 BC	70 A	81 AB	68 A	93 AB	87 AB	82 CD	15 D	12 C	2 B	0 E	0 E	0 E
	spring					99 A	99 A	96 AB				0 E	0 E	0 E
aminopyralid + 2,4-D	fall	54 A	74 A	89 A	81 A	91 AB	90 AB	90 ABC	65 A	93 A	100 A	95 A	95 A	93 A
	spring					100 A	100 A	99 A				100 A	100 A	100 A
2,4-D + dicamba	fall	50 AB	71 A	64 BC	75 A	81 BC	72 C	71 DE	63 A	93 A	100 A	34 D	25 D	20 DE
	spring					95 A	93 A	89 ABC				100 A	100 A	95 A
2,4-D	fall	24 D	56 B	56 C	39 B	60 D	48 D	38 F	33 C	68 B	4 B	11 E	0 E	0 E
	spring					73 C	77 BC	70 E				88 AB	55 CD	29 D
triclopyr + 2,4-D	fall	31 CD	65 AB	66 BC	71 A	70 CD	70 C	60 E	55 AB	93 A	98 A	67 C	50 CD	36 D
	spring					97 A	98 A	98 A				100 A	100 A	100 A
metsulfuron	fall	26 D	57 B	82 AB	70 A	93 AB	91 A	84 BC	48 B	90 A	98 A	79 BC	80 B	85 B
	spring					99 A	99 A	97 A				100 A	100 A	100 A
triclopyr + fluroxypyr	fall	41 BC	55 B	69 ABC	65 A	59 D	48 D	44 F	35 C	90 A	100 A	69 C	75 BC	80 BC
	spring					95 A	96 A	94 ABC				100 A	100 A	100 A

[a]Means followed by the same letter are not significantly different according to Fisher's LSD ($p \leq 0.05$), within column.
[b]Abbreviations: DAF, days after fall treatment; DAS, days after spring treatment.

Table 2.6 Established white clover injury and above ground biomass to postemergence herbicides in Virginia in 2020.

Treatment	rate(s)	Visible Injury										Above ground biomass					
		1WAT[a]		2WAT[b]		3WAT		4WAT		6WAT		2WAT		4WAT		6WAT	
	g ai/ae ha^{-1}	----------%										----------kg ha^{-1}----------					
nontreated		0		0		0		0		0		647	A	1736	A	2102	A
florpyrauxifen-benzyl + 2,4-D	9 + 560	46	B	38	C	37	BC	25	BC	11	CD	548	AB	823	BC	883	C
florpyrauxifen-benzyl + 2,4-D	18 + 1,120	63	A	56	B	46	B	36	B	25	B	511	AB	893	ABC	891	C
florpyrauxifen-benzyl	9	25	D	24	D	15	E	8	D	4	E	312	BC	880	ABC	883	BC
florpyrauxifen-benzyl	18	36	C	39	C	33	CD	23	C	14	C	390	ABC	748	BC	1095	BC
2,4-D	560	21	D	19	D	23	DE	14	CD	6	DE	622	AB	1442	AB	1251	BC
2,4-D	1,120	36	C	41	C	33	CD	23	C	15	C	440	ABC	1119	AB	708	C
dicamba + 2,4-D	560 + 1,120	65	A	84	A	89	A	94	A	96	A	135	C	172	C	0	D
mowing	————	————		————		————		————		————		519	AB	1598	AB	1583	AB

[a]Means followed by the same letter are not significantly different according to Fisher's LSD (P≤0.05), within column.
[b]Abbreviation: WAT, weeks after treatment.

Table 2.7 White clover above ground biomass following postemergence herbicides in greenhouse experiments.

Treatment	Rate(s)	Above ground biomass [a]	
	g ai/ae ha^{-1}	kg ha^{-1}	
nontreated	-----	3892	A
florpyrauxifen-benzyl + 2,4-D	9 + 560	1315	C
florpyrauxifen-benzyl + 2,4-D	18 + 1,120	599	D
2,4-D	560	2543	B
2,4-D	1,120	1940	BC
dicamba + 2,4-D	560 + 1,120	539	D

[a]Means followed by the same letter are not significantly different according to Fisher's LSD ($P \leq 0.05$).

Forage grass and legume response to florpyrauxifen-benzyl during establishment

Abstract. Competition from weeds is one of the greatest factors affecting forage establishment. Managing weeds during establishment can be difficult as forage species can be very sensitive to herbicides during this time, limiting their use. Field and greenhouse trials were conducted from 2018-2020 in Blacksburg, Virginia to determine the effect of new herbicides combinations, florpyrauxifen-benzyl + 2,4-D and florpyrauxifen-benzyl + aminopyralid on the establishment of tall fescue, orchardgrass, and white clover. Herbicides were applied: two weeks prior to grass seeding in the fall, at seeding, and postemergence for the forage grass establishment field trials. In addition, two separate field studies were conducted to determine the effect of the herbicides on white clover establishment when planted using a seed drill and frost-seeded. Herbicides were applied the fall prior to late-winter/spring planting, and florpyrauxifen-benzyl-containing treatments were applied at planting and postemergence. Lastly, greenhouse trials were conducted in order to evaluate white clover sensitivity to florpyrauxifen-benzyl as affected by white clover growth stage at the time of application. Growth stages evaluated included vegetative (3-4 trifoliate) and flowering. Establishment of both forage grass species was unaffected by application timing, and the only herbicide to negatively affect establishment was metsulfuron, which injured stands of tall fescue. Results were mixed for drilled white clover, with florpyrauxifen-benzyl – containing treatments resulting in variable levels of white clover injury across site-years. However, no fall-applied herbicide injured white clover. All aminopyralid-containing treatments resulted in injury to frost-seeded white clover. Greenhouse results showed that flowering white clover was more sensitive to herbicides than vegetative white clover and that injury was dependent on florpyrauxifen-benzyl rate. Overall, our data suggests that florpyrauxifen-benzyl containing herbicides can be safely used around the time of establishment of tall fescue and orchardgrass. However, more caution must be used when establishing white clover, and further research needs to be conducted to determine proper timing and rate.

Nomenclature: Tall fescue, *Lolium arundinaceum* Schreb.; orchardgrass, *Dactylis glomerata* L.; white clover, *Trifolium repens* L.

Keywords: forage establishment, herbicides, weed control

Introduction. Cool-season perennial grass species such as tall fescue (*Lolium arundinaceum* Schreb.) and orchardgrass (*Dactylis glomerata* L.) are the foundation for forage grass production across their wide range of adaptation in the United States. These grasses are nutritious, palatable to livestock, and tolerant to frequent grazing (Beck et al. 2006). In addition to cool-season, perennial forage grasses, forage legumes such as white clover (*Trifolium repens* L.) are often integrated into forage systems to provide a plethora of benefits. Benefits of forage legumes include nitrogen fixation, which can become available to neighboring plants (Brophy et al. 1987), therefore increasing overall forage production compared to grass monocultures (Carter and Scholl 1964). The increased forage production from the inclusion of legumes can ultimately lead to enhanced livestock performance, as legumes tend to have more total digestible nutrients compared to grasses (Ellis and Lippke 1976), leading to increased weight gain in livestock (Blaser et al. 1956; Golding et al. 2008; Schaefer et al. 2014), and milk protein yields (Harris et al. 1998).

The establishment phase of any crop is extremely important in ensuring a successful crop stand that will be productive throughout the life of the crop. In particular, proper weed management during establishment is important to reduce competition. Weeds emerging before or with forage grasses can outgrow the forage seedlings and result in diminished or even failed stands (Fermanian et al. 1980; Martin et al. 1982). In contrast, when weeds are successfully controlled during crop establishment, productive stands can be obtained (Cox and McCarty 1958). Many forages require extensive time and effort in the establishment phase, and the negative outcomes of crop failure can be more severe compared to annual crops (Lee 1965; Stichler and Bade 2003).

Herbicides are commonly used to control weeds prior to forage establishment (Brothers et al. 1994; Hall et al. 2020). Typically, non-residual herbicides used are postemergence and applied prior to forage seeding (Lee 1965). Following grass emergence, broadleaf herbicides may be used, but seedling grasses need to reach the tillering stage to avoid the risk of herbicide injury (Huffman and Jacoby 1984; Peters et al. 1989; Dear et al. 2006). One common practice to minimize the effect of weeds on cool-season grass establishment is to seed forages in late summer/fall in order to allow seedlings to become established prior to competition with spring/summer emerging weeds (Green et al. 2006). However, with this strategy winter weeds are able to compete with new stands, which can lead to stand reductions and possibly even stand failures. If a producer wishes to establish forage legumes, such as white clover, along with forage grasses, weed control prior to establishment becomes much more critical, as there are currently no selective herbicides that can be used early-postemergence in mixed stands of forage grasses and legumes. Because of this, producers will often seed forage grasses in the fall, and then seed legumes the following spring in order to have the ability to apply a broadleaf herbicide prior to legume seeding. Forage legumes, such as white clover, are commonly planted in the spring by using a no-till seed drill or "frost seeding". Frost-seeding involves broadcasting legume seed onto the soil surface in late winter, and the freeze-thaw cycle allows the legume seed to be worked into the upper layer of the soil and germinate (Schlueter and Tracy 2012). Frost-seeding has been shown to be an effective method of establishing legumes into existing forage grasses, which can reduce cost compared to traditional seeding methods (Gettle et al. 1996). But again, once the legume is seeded there are currently no postemergence control options for broadleaf weed species that emerge throughout the spring and summer.

Florpyrauxifen-benzyl is a synthetic auxin herbicide in the arylpicolinate family that is used for selective grass and broadleaf weed control. Currently, florpyrauxifen-benzyl is labeled in rice (Loyant®, Corteva Agriscience, Indianapolis, IN) and in combination with the herbicide aminopyralid in forage crops (DuraCor®, Corteva

Agriscience) in the United States. Aminopyralid is extremely active on broadleaf weed species, therefore DuraCor is not safe to white clover. Florpyrauxifen-benzyl combined with 2,4-D is reported to preserve established stands of white clover while controlling broadleaf weed species (Sleugh et al. 2020). Little research exists on the effect of florpyrauxifen-benzyl combined with 2,4-D used to aid in establishing forage grasses and legumes. If safe during establishment, florpyrauxifen-benzyl + 2,4-D would be a very useful tool during this critical stage of the forage stand. The objective of this research is to determine the safety of florpyrauxifen-benzyl + 2,4-D on tall fescue, orchardgrass, and white clover when applied prior to, at, and post-seeding. Additionally, the effect of white clover seeding method (drilled versus frost-seeded) was evaluated as well as the effect of florpyrauxifen-benzyl + 2,4-D on different growth stages of white clover.

Materials and methods

Forage grass establishment.

Study Sites. In order to determine the effect of herbicide and application timing on the establishment of tall fescue and orchardgrass, field trials were established in 2018 and 2019 in Blacksburg, Virginia (37.23, -80.46). Sites with low natural weed populations were selected in order to minimize weed competition. Soil type was a Fine, mixed, semiactive, mesic Typic Hapludults. 'Kentucky 31' tall fescue (Southern States Seed Division®, Richmond, VA) and orchardgrass (Alliance Seed, Winnipeg, Canada) were planted using a no-till seed drill at a rate of 16.8 and 11.2 kg ha^{-1}, respectively. Tall fescue and orchardgrass were planted in separate 3 m-wide swaths perpendicular to the length of the plot. Plot size was 3 m by 6 m. Forages were planted in the fall. Planting dates are shown in Table 3.1. Prior to planting, field sites were maintained weed free, as well as receiving a pre-plant burndown application of paraquat (Gramoxone® SL 2.0, Syngenta Crop Protection, LLC, Greensboro, NC) at 1 kg ha^{-1} to control any newly emerged weeds.

Experimental Design. The experiment was a randomized complete block design with four replications. Herbicide treatments are listed in Table 3.2 in addition to a nontreated control. Treatments were arranged in a factorial design, with factor A being herbicide, and factor B being application timing including: 2 weeks prior to planting (preplant), at planting, and in the following spring (POST). Herbicide applications were made using a 1.8 m-wide handheld backpack sprayer with 4 TeeJet (Spraying Systems Co.; Wheaton, IL) 11002XR nozzles calibrated to deliver 140 L ha^{-1} at 207 kPa.

Data Collection and Analysis. Following herbicide application, visible forage injury data were taken by species 30 and 60 days after the POST application timing on a scale of 0% to 100% with 0 being no observable injury and 100 being complete plant necrosis relative to the nontreated check as described by Frans et al. (1986). Above-ground forage biomass was taken at the end of the establishment season the following summer. Biomass was dried at 50 C for 72 hours and then weighed.

Data were subject to ANOVA and subsequent means separation using Fisher's Protected LSD test ($P \leq 0.05$) to compare across treatments using JMP Pro 15 (SAS Institute, Inc; Cary, NC). Fixed effects consisted of herbicide treatment and application timing. Year and replication were considered random effects to allow inferences to be made over a range of environments and conditions (Blouin et al. 2011).

White clover establishment.

Study Sites. Field trials were established in 2018 and 2019 adjacent to forage grass establishment sites to determine the effect of florpyrauxifen-benzyl containing herbicide products on white clover establishment by drilling and frost seeding. Two separate trials were established each year for each seeding method. Each trial used the same methods with the only exception being establishment method. 'Ladino' white clover (Southern States®, Richmond, VA) was planted using a no-till seed drill at a rate of 2.2 kg ha^{-1} for the drilled study and broadcast onto the soil surface at a rate of 3.4 kg ha^{-1} for the frost-seeded study. Plot size was 3m by 6m. Frost-seeded white clover was broadcast in late winter, and drilled white clover was planted in mid-spring (Table 3.1). Prior to planting, field sites were maintained weed free, as well as receiving a pre-plant burndown application of paraquat (Gramoxone® SL 2.0) at 1 kg ha^{-1} to control any newly emerged weeds.

Experimental Design. Both experiments were a randomized complete block design with four replications. Herbicide treatments are listed in Table 3.2 in addition to a nontreated control. Herbicide applications were made as previously described.

Data Collection and Analysis. Following herbicide applications, above-ground white clover biomass was collected at the end of the establishment growing season in late summer. Biomass was dried at 50 C for 72 hours and then weighed.

Data were subject to ANOVA and subsequent means separation using Fisher's Protected LSD test ($P \leq 0.05$) to compare across treatments using JMP Pro 15. Fixed effects consisted of herbicide treatment (herbicide by timing) and year. Year was considered a fixed effect and results are separated by year due to differences in white clover response between years.

White clover growth stage sensitivity.

Study Site. Greenhouse trials were established in 2020 and 2021 in Blacksburg, Virginia (37.23, -80.43) in order to determine white clover sensitivity to florpyrauxifen-benzyl + 2,4-D by growth stage. The two growth stages were vegetative (3-4 trifoliate leaves), and flowering.

Based on preliminary data (not shown), a sensitive white clover variety 'Patriot' (Pennington Seed Inc®, Madison, GA) was selected for greenhouse trials. White clover was seeded into 15 cm diameter pots at a seeding rate of 5.6 kg ha^{-1}. The experiment was a randomized complete block design with five replications. Treatments were arranged in a factorial design with factor A being white clover growth stage and factor B being herbicide treatment. Herbicide product and sources for all trials are listed in Table 3.3. Herbicide applications were made as previously described. The trial was repeated thrice in time.

Data Collection and Analysis. Following herbicide applications, plant height and above ground white clover biomass was collected six weeks following herbicide treatments. Data were subject to ANOVA and subsequent means separation using Fisher's Protected LSD test ($P \leq 0.05$) to compare across treatments using JMP Pro 15. Fixed effects consisted of white clover growth stage and herbicide treatment. Trial run and replication, nested within trial run, were considered random effects.

Results

Forage grass establishment.

Visible forage injury.

The only treatment to result in any visible injury to either tall fescue or orchardgrass was metsulfuron. In December, tall fescue injury was 80% and 91% from metsulfuron applied 2 weeks prior to seeding and at planting, respectively. Orchardgrass injury in December was 50% and 61%, from applications made 2 weeks prior to seeding and at planting, respectively. Injury was characterized by overall stunting and chlorosis, consistent with previous reports (Israel et al. 2016). In January, tall fescue injury had fallen to 58% and 69% from applications made prior to and at planting, respectively, while orchardgrass injury had fallen to 26% and 44%. The next visible injury ratings were taken in April, approximately 30 days following postemergence herbicide applications, and metsulfuron resulted in 16% and 4% injury in tall fescue and orchardgrass, respectively. By 90 days after the POST treatment, injury from POST applied metsulfuron in both tall fescue and orchardgrass was negligible (<6 %). Seedling recovery, as well as increased tillering from surviving plants led to overall stand recovery.

Above ground forage grass biomass.

Following all herbicide applications, above ground biomass data were taken mid-establishment season in May and at the end of the establishment season in late September for both tall fescue and orchardgrass. There were no differences in biomass for tall fescue or orchardgrass at the mid-establishment season timing. Across treatments and application timings, above ground biomass was 2692 kg ha^{-1} for tall fescue and 2670 kg ha^{-1} for orchardgrass. Additionally, there were no differences in orchardgrass biomass at the end of season timing, which had 2490 kg ha^{-1} biomass across treatments and application timings. Interactions between application timing and herbicide were significant for tall fescue at the end of season timing. Florpyrauxifen-benzyl + 2,4-D applied preplant resulted in significantly lower biomass (2174 kg ha^{-1}) compared to POST applied (4029 kg ha^{-1}). Even though there were differences between herbicide treatments, there were no significant differences between herbicide treatments and the nontreated control, which resulted in 2701 kg ha^{-1}.

White clover establishment.

Drilled establishment.

For the drilled white clover establishment studies, results were significant by year, therefore results were separated by year. In 2018, the only herbicide treatment to significantly reduce white clover biomass compared to the nontreated was aminopyralid

+ florpyrauxifen-benzyl applied postemergence which completely eliminated white clover. More injury was observed in 2019. All florpyrauxifen-benzyl-containing herbicides applied at seeding or POST completely eliminated white clover. No fall-applied herbicide caused a decrease in white clover biomass.

Frost-seeded establishment. Several herbicides resulted in decreased end-of-season biomass from frost seeded white clover establishment (Table 3.4). All aminopyralid + florpyrauxifen-benzyl treatments, regardless of application timing, reduced white clover biomass compared to the nontreated control, resulting in 342 kg ha^{-1}, 535 kg ha^{-1}, and 0 kg ha^{-1}, when applied in the fall (preplant), at planting, and POST, respectively. All other fall-applied herbicides did not reduce white clover biomass compared to the nontreated control (1927 kg ha^{-1}), although fall-applied aminopyralid + 2,4-D resulted in less than half of the nontreated control (980 kg ha^{-1}) and aminopyralid + florpyrauxifen-benzyl completely eliminated white clover.

White clover growth stage sensitivity. Height data is shown as a percentage of the nontreated control for each respective growth stage (Table 3.5). No 2,4-D treatments caused a height reduction, for either growth stage, with heights ≥ 98% relative to the nontreated. Dicamba + 2,4-D applied to both vegetative and flowering white clover completely reduced height (0%) as well as florpyrauxifen-benzyl + 2,4-D at 18 g ai ha^{-1} + 1120 g ae ha^{-1} applied to vegetative white clover. Other herbicides that resulted in the greatest decreases in height included florpyrauxifen-benzyl + 2,4-D at 9 g ai ha^{-1} + 560 g ae ha^{-1} applied to vegetative white clover (1.5%), florpyrauxifen-benzyl at 18 g ai ha^{-1} applied to vegetative white clover (3.3%).

Biomass data is also represented as a percentage of the nontreated control for each growth stage in order to make comparisons across treatments and growth stages. Treatments which resulted in the greatest decreases in biomass included: dicamba + 2,4-D applied to vegetative (0%) and flowering (0%) white clover, 2,4-D alone at 1120 g ai ha^{-1} applied to flowering white clover (18%), and florpyrauxifen-benzyl + 2,4-D at 18 g ai ha^{-1} + 1,120 g ae ha^{-1} applied to flowering white clover (18%). Treatments which resulted in the least biomass reductions include: 2,4-D at 1120 g ae ha^{-1} applied to vegetative white clover (104%), florpyrauxifen-benzyl at 18 g ai ha^{-1} applied to vegetative white clover (83%), 2,4-D at 560 g ae ha^{-1} applied to vegetative (76%) and flowering (73%) white clover, and florpyrauxifen-benzyl at 18 g ai ha^{-1} applied to flowering white clover (53%).

Discussion

With the exception of metsulfuron, all of the herbicides used were safe to establishing tall fescue and orchardgrass. Our findings are similar to those by Peters et al. 1989, in which metsulfuron resulted in 76% or greater injury to seedling tall fescue. Overall, orchardgrass tended to be more tolerant of herbicides than tall fescue, and postemergence herbicide applications tended to be less injurious than those made around to and prior to planting. Frost-seeded white clover establishment was negatively affected by application of aminopyralid + florpyrauxifen-benzyl applications, regardless of timing. However, florpyrauxifen-benzyl + 2,4-D only caused significant biomass reductions when applied postemergence, indicating that the addition of aminopyralid is likely the cause for increased injury. Results from drilled white clover establishment studies differ by year, however, fall-applied herbicide treatments were safe, regardless of herbicide in both years.

Data from the greenhouse study suggests that white clover may be more susceptible to injury when herbicides are applied at flowering compared to vegetative growth, which is similar to other research that found crops can be more susceptible to exposure to herbicide via off target movement at these growth stages (Scholtes et al. 2019). Both field and greenhouse results align with previous reports that showed little to no injury to white clover from 2,4-D applications in the field (Enloe et al. 2014).

These data suggest that florpyrauxifen-benzyl containing herbicide products can successfully be used around the time of forage grass establishment and can be safely used in the fall prior to white clover establishment via both frost-seeded and drilled methods. However, extreme caution must be exercised when determining the proper use rate, as higher rates can lead to excessive white clover injury. Because of their activity on broadleaf weed species, florpyrauxifen-benzyl-containing herbicide products can offer great value by controlling weeds around the time of establishment, and therefore lead to more productive forage stands.

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Tables

Table 3.1 Field studies site data and planting info for forage establishment trials in 2018-19 in Blacksburg, VA.

Study	Species	Seeding Rate(s)	Planting Date(s)	
		kg ha^{-1}		
Forage grass establishment	tall fescue orchardgrass	16.8 11.2	October 9, 2018	October 29, 2019
Drilled clover establishment	white clover	2.2	March 29, 2019	May 14, 2020
Frost-seeded clover establishment	white clover	3.4	February 7, 2019	February 17, 2020

Table 3.2 Herbicide treatments for forage establishment field studies in Blacksburg, Virginia in 2018-2019.

Study	Herbicide	Rate	Timing(s)
		g ai/ae ha^{-1}	
Forage grass establishment			
	florpyrauxifen-benzyl + 2,4-D	8.9 + 560	2 weeks prior to planting At planting Spring POST
	aminopyralid + 2,4-D	115 + 933	2 weeks prior to planting At planting Spring POST
	metsulfuron	7	2 weeks prior to planting At planting Spring POST
	triclopyr + fluroxypyr	560 + 1,121	2 weeks prior to planting At planting Spring POST
	florpyrauxifen-benzyl + aminopyralid	9.4 + 665	2 weeks prior to planting At planting Spring POST
Clover Establishment			
	2,4-D + dicamba	1,065+ 560	Fall
	2,4-D	1,064	Fall
	aminopyralid + 2,4-D	114 + 933	Fall
	florpyrauxifen-benzyl + aminopyralid	9.4 + 665	Fall At planting Spring POST
	florpyrauxifen-benzyl + 2,4-D	8.9 + 560	Fall At planting Spring POST

Table 3.3 Herbicide active ingredients and sources used in field and greenhouse trials to white clover in Blacksburg, VA in 2020-2021.

Active Ingredients(s)	Trade Name(s)	Manufacturer	Location
florpyrauxifen-benzyl + 2,4-D	ProClova®	Corteva Agriscience	Wilmington, DE
florpyrauxifen-benzyl	Loyant®	Corteva Agriscience	Wilmington, DE
2,4-D	Shredder® Amine 4	Winfield Solutions	St. Paul, MN
dicamba + 2,4-D	Shredder® Amine 4 + Clarity®	Winfield Solutions + BASF Corporation	St. Paul, MN; Research Triangle Park, NC
triclopyr + fluroxypyr	PastureGard® HL	Corteva Agriscience	Wilmington, DE
metsulfuron	Cimarron® MAX	Bayer CropScience	St. Louis, MO

Table 3.4 White clover above ground biomass resulting from herbicide treatments applied in the fall prior to planting, at planting, and postemergence from field experiments in Blacksburg, VA in 2018-2019.

		End of Season Above Ground Biomass [a]					
		Drilled					
Treatments	Application Timing	2018		2019		Frost-Seeded	
		----------------------------kg ha^{-1}----------------------------					
nontreated	-----------------------	1063	BC	1198	BC	1927	A
2,4-D-dicamba	Fall	767	C	1286	ABC	1988	A
2,4-D	Fall	2166	A	713	CD	1596	A
aminopyralid + 2,4-D	Fall	861	C	1031	BC	980	AB
florpyrauxifen-benzyl + aminopyralid	Fall	1978	B	2013	A	342	B
florpyrauxifen-benzyl + aminopyralid	At Planting	1144	BC	0	D	535	B
florpyrauxifen-benzyl + aminopyralid	POST[b]	0	D	0	D	0	B
florpyrauxifen-benzyl + 2,4-D	Fall	1777	AB	1652	AB	1844	A
florpyrauxifen-benzyl + 2,4-D	At Planting	1924	A	0	D	1816	A
florpyrauxifen-benzyl + 2,4-D	POST	1440	ABC	0	D	865	AB

[a]Means followed by the same letter are not significantly different according to Fisher's LSD ($p \leq 0.05$), within column.
[b]POST treatments were applied in the spring of the planting year.

Table 3.5 White clover height and above ground biomass 6 weeks following treatment in the greenhouse. Treatments were applied postemergence to vegetative and flowering growth stages.

Treatment	Rate(s)	Growth Stage[a]	Height[b]		Biomass	
	g ai/ae ha^{-1}		--------------------% of nontreated--------------------			
florpyrauxifen-benzyl + 2,4-D	9 + 560	V3	2	E	33	BC
florpyrauxifen-benzyl + 2,4-D	9 + 560	F	22	CD	32	BC
florpyrauxifen-benzyl + 2,4-D	18 + 1,120	V3	0	E	25	CD
florpyrauxifen-benzyl + 2,4-D	18 + 1,120	F	12	DE	18	D
florpyrauxifen-benzyl	9	V3	11	DE	47	BC
florpyrauxifen-benzyl	9	F	74	B	37	BC
florpyrauxifen-benzyl	18	V3	3	DE	83	AB
florpyrauxifen-benzyl	18	F	32	C	58	ABC
2,4-D	560	V3	103	A	76	ABC
2,4-D	560	F	115	A	73	ABC
2,4-D	1121	V3	99	A	104	A
2,4-D	1121	F	116	A	18	D
dicamba + 2,4-D	560 + 1121	V3	0	E	0	D
dicamba + 2,4-D	560 + 1121	F	0	E	0	D

[a]Abbreviations: V3, vegetative 3-trifoliate; F, flowering.

[b]Means followed by the same letter are not significantly different according to Fisher's LSD ($p \leq 0.05$), within column.