Evaluating methods to establish biodiverse pasturelands with native grasses and wildflowers

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ABSTRACT

Virginia's pasturelands are dominated by tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons. [Poaceae]), a competitive, non-native grass. Tall fescue has limited value for wildlife and insect pollinators, and it can pose issues for livestock if infected with an endophyte that produces a toxic alkaloid. Potential exists for developing more biodiverse pasturelands that would help reduce problems associated with tall fescue while increasing ecosystem services associated with improved wildlife and pollinator habitat. Native warm-season grasses (NWSG) and wildflowers (WF) have potential forage and conservation benefits; however, more research is needed to determine effective strategies that land managers can use to establish these species. This article summarizes 4 field experiments designed to evaluate establishment methods for NWSG and WF in tall fescue pasturelands. Experiments were established from 2016 to 2020 in central and western Virginia. Three evaluated pre-emergent imazapic herbicide applications while the fourth evaluated other methods, including glyphosate application, glyphosate combined with raking, prescribed fire, and tillage, to establish NWSG and WF. In the 3 experiments evaluating imazapic applications, the herbicide consistently suppressed wildflowers, with the exception of *Rudbeckia hirta* L. (Asteraceae), *Desmanthus illinoensis* (Michx.) MacMill. ex B.L. Rob. & Fernald (Fabaceae), *Coreopsis lanceolata* L. (Asteraceae), and *Leucanthemum vulgare* Lam. (Asteraceae). In the fourth experiment, tillage was the most effective method to establish wildflowers. Overall, we found imazapic can improve NWSG establishment, but suppresses most WF species tested in this environment. Methods to achieve more consistent WF establishment are needed if adoption of more biodiverse pasturelands becomes a future management goal.

Ghajar SM, Wagner JF, O'Rourke M, Tracy BF. 2022. Evaluating methods to establish biodiverse pasturelands with native grasses and wildflowers. Native Plants Journal 23(1):65–74.

KEY WORDS
imazapic, glyphosate, tillage, pasture, tall fescue, wildflowers, native warm-season grasses

NOMENCLATURE
USDA NRCS (2021)

doi:10.3368/npj.23.1.65

Native meadows dominated by warm-season grasses and wildflowers were common in the Southeast before European colonization because of the use of prescribed fire by Native peoples (Tompkins and others 2010). Since colonization by Europeans, however, meadowlands not converted into cropland or other uses have been supplanted with introduced forage species from Eurasia and Africa.

One especially successful introduction was tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons. [Poaceae]), a common cool-season grass that now occupies an estimated 14 million ha (35 million ac) in the Southeast (Oliver and others 2000). Tall fescue is productive, stress tolerant, and high in nutrition for most classes of livestock in the spring, fall, and if stockpiled properly, winter. However, tall fescue is often infected with a fungal endophyte (*Neotyphodium coenophialum* [Clavicipitaceae]) that produces a toxic alkaloid, ergovaline, leading to reduced gains and, potentially, veterinary issues for cattle in summer, costing producers billions of dollars a year in the US (Strickland and others 2009). Tall fescue also causes reproductive issues in horses (Cross 2015), as well as having higher carbohydrate levels than is safe for many horses during its peak growing seasons in spring and fall (Siciliano and others 2017). Consequently, some livestock managers are interested in incorporating novel forages into their grazing systems to mitigate fescue's drawbacks (Keyser and others 2019).

In addition to fescue's agronomic issues, the grass presents many difficulties for maintaining or enhancing ecosystem services. Tall fescue is highly competitive, and research suggests it has an inhibitory effect on native plant species (Renne and others 2004). The competitiveness of tall fescue reduces plant diversity and consequently lowers habitat value for grassland bird species and pollinators (Barnes and others 1995; Jokela and others 2016). In particular, native pollinator species, including bees, butterflies, moths, and flies, have been declining, in part, due to reductions in habitat diversity. The establishment of pollinator-friendly habitat on farms can be useful to their preservation (Vaughan and Skinner 2008).

Converting some fescue swards into grasslands dominated by native warm-season grasses (NWSG) and wildflowers (WF) may provide economic benefits for livestock production in summer, while enhancing the quality and diversity of habitat available for grassland obligate birds and pollinators.

ESTABLISHING NATIVE WARM-SEASON GRASSES AND WILDFLOWERS

In spite of the potential benefits of incorporating NWSG and WF into working grasslands, these species have a reputation for being difficult to establish, which remains one of the largest barriers to their incorporation into agricultural operations (Keyser and others 2019). Newly established stands are often visibly weedy, as NWSG tend to invest much of their growth into establishing prolific root systems their first year, with aboveground growth less visible for producers (Keyser and others 2012). Additionally, many graziers in the South have pastures dominated by tall fescue, which is difficult to eradicate long term without periodic management. Current recommendations by Extension programs in the South advise prolonged site preparation, with an herbicide application on the site in the fall, a cover crop over the winter, and another herbicide application in the spring just before planting (Keyser and others 2012). Specialized seed drills must be used with many NWSG species because of the elongated seed awns—a hair-like projection attached to the seedcoat—which do not flow through standard drill boxes. If no seed drill is available, land managers may broadcast seeds but must increase their seeding rate and seed costs to maximize their chances of successful stand establishment (Seymour and Seymour 2004).

Given these obstacles to NWSG establishment, reducing competition from weedy species to improve the likelihood of success for land managers is vital. Prairie restoration programs have successfully used the herbicide imazapic to facilitate establishment of native species, as many NWSG are resistant to its effects, while many invasive or exotic species—especially cool-season grasses such as tall fescue—are highly susceptible to it (Barnes 2004). Imazapic is an herbicide of the imidazolinone family, characterized by a mechanism of action that inhibits the amino acid synthesis that plants require for growth (da Costa Marinho and others 2019). Absorbed through leaves, stems, and roots, it can be used as a foliar spray or applied to soil for pre-emergent or post-emergent control. Imazapic persists in soil, but the duration of its persistence varies by soil characteristics and climate (Sheley and others 2007).

Some research on imazapic use in restoration of native grasses demonstrated that an initial application of another herbicide, glyphosate, followed by applying imazapic at the low rate of 0.067 kg ai per ha during seeding, can be effective for weed control and seedbed preparation (Barnes 2004). Other studies in the upper South found that a spring prescribed fire followed by a pre-emergent application of imazapic at seeding improved native grass establishment (Washburn and others 2002). In Virginia, we could find only one small-plot study on imazapic and NWSG establishment, and it found the herbicide to be effective for establishing native grasses on a site in the northern Shenandoah Valley (Priest and Epstein 2011). Given its highly variable effects and persistence depending on extant species composition and soil types, further research is needed to determine its effectiveness in NWSG and WF establishment. This article reports on 4 field experiments designed to evaluate the effectiveness of imazapic and glyphosate herbicides along with other non-chemical methods for establishing NWSG and WF in pasturelands.

Overall objectives of these experiments were to:

1. Evaluate effectiveness and determine optimal rates of pre-emergent imazapic herbicide application for establishing stands of NWSG and WF mixtures for possible use in pastureland, and
2. Compare glyphosate application with non-chemical methods to establish WF stands.

Specific objectives of each experiment are described in Table 1.

MATERIALS AND METHODS

Study Sites

Experiments I, II, and III were conducted at the Virginia Tech Shenandoah Valley Agricultural Research and Extension Center (AREC) in Steele's Tavern, Virginia (37.930278 N, 79.213889 W; elevation 540 m [1782 ft]). Soils at the site are Frederick and Christian silt loams, which are fine, mixed, semiactive, mesic Typic Paleudults and fine, mixed, semiactive, mesic Typic Hapludults, respectively (Web Soil Survey 2021).

Experiment IV was conducted at Virginia Tech's Kentland Farm in Blacksburg, Virginia (37.199722 N, 80.565278 W; elevation 545 m [1799 ft]). Soils on the site include the Unison (fine, mixed, active, mesic Typic Hapludults) and Braddock (fine, mixed, semiactive, mesic Typic Hapludults) series (Web Soil Survey 2021).

Experiment I

The first experiment evaluated pre-emergent imazapic applications to pasture-scale plots planted with NWSG alone and a NWSG + WF mixture. Six, 0.8-ha (2 ac) plots were established at the Virginia Tech Shenandoah Valley AREC in Raphine, Virginia, in June 2017. Three plots were planted with a NWSG-only mix of *Andropogon gerardii* Vitman, *Schizachyrium scoparium* (Michx.) Nash, and *Sorghastrum nutans* (L.) Nash, and the remaining 3 with a combined NWSG and WF mix of the above three grasses and an additional 15 wildflower species (hereafter referred to as WF mix) (Table 2). Both mixtures were planted at a rate of 10.7 kg/ha (9.5 lb/ac). Glyphosate was applied to the plots at a rate of 2.3 l/ha (1 qt/ac) prior to establishment to kill extant vegetation in preparation for seeding.

One wk after the plots were planted, imazapic at a rate of 0.15 l/ha (2 oz/ac) was applied to a 3 m (10 ft) wide swath around the perimeter of each plot. Once seeded species were established, the plots were grazed by 8 mature cows between May and September in 2018 and 2019. Cows were rotated onto the plots every 30 to 32 d during the grazing season.

We collected species density, percent ground cover, and aboveground biomass of NWSG, wildflowers, and weeds in fall 2017 and May and July 2018. Data collection for the NWSG plots was discontinued after 2018, but continued in the WF mix plots in 2019. We assessed plant species composition and ground cover visually using a modified Daubenmire method (Daubenmire 1959). Ten quadrats 0.25 m^2 were placed randomly in both the herbicide treatment and the control treatment of each of the plots. Aboveground biomass was measured by hand clipping to 1 cm and separating quadrat samples into native grasses, weeds, and wildflowers, then drying in a forced-air forage oven at 55 °C (131 °F) for 48 h.

Experiment II

We designed the second experiment to determine an optimal application rate for successful NWSG/WF establishment. Experiment II was a split-plot design comparing 4 rates of imazapic on 2 seed mixes similar to those of the large plot

TABLE 1

Summary of the 4 experiments, including objectives, seeding treatments, and establishment treatments evaluated.

	Objective	Seeding treatments	Establishment treatments
Experiment I	Evaluate effectiveness of imazapic applications in pasture-sized plots sown with a mix of NWSG (alone) or a mix of NWSG + WF.	NWSG (alone) Mix of NWSG + WF	Control: no imazapic Imazapic: 0.15 l/ha (2 oz/ac)
Experiment II	Compare establishment of NWSG and WF at 4 different rates of imazapic application.	NWSG (alone) Mix of NWSG + WF	Control: no imazapic Low: 0.15 l/ha (2 oz/ac) Medium: 0.29 l/ha (6 oz/ ac) High: 0.73 l/ha (12 oz/ac)
Experiment III	Evaluate how imazapic applications might interact with spatially segregating NWSG and WF species at planting.	NWSG + WF planted together NWSG + WF planted spatially separate	Control: no imazapic Imazapic: 0.44 l/ha (6 oz/ac)
Experiment IV	Evaluate 2 glyphosate application treatments and 2 other non-chemical strategies for establishing wildflowers.	Mix of NWSG + WF	Glyphosate Glyphosate and raking of thatch Tillage Prescribed fire

Notes: NWSG, native warm-season grasses; WF, wildflowers.

TABLE 2

Species composition of seed mixes used in the 4 experiments.

Genus species	Family	Authority	Common name	Experiment I	II	III	IV
Agastache foeniculum	Lamiaceae	(Pursh) Kuntze	Marsh blazing star	X		X	
Andropogon gerardii	Poaceae	Vitman	Big bluestem	X	X	X	
Avena sativa	Poaceae	L.	Oat (companion crop)	X	X		
Baptisia australis	Fabaceae	(L.) R. Br.	Blue false indigo		X		
Bidens aristosa	Asteraceae	(Michx.) Britton	Western tickseed			X	X
Chamaecrista fasciculata	Fabaceae	(Michx.) Greene	Partridge pea		X		X
Coreopsis lanceolata	Asteraceae	L.	Lanceleaf coreopsis	X	X	X	X
Coreopsis tinctoria	Asteraceae	Nutt.	Plains coreopsis				X
Desmanthus illinoensis	Fabaceae	(Michx.) MacMill. ex B.L. Rob. & Fernald	Illinois bundleflower	X	X		
Echinacea purpurea	Asteraceae	(L.) Moench	Purple coneflower	X	X	X	X
Gaillardia aristata	Asteraceae	Pursh	Blanketflower	X	X		
Gaillardia pulchella	Asteraceae	Foug.	Indian blanket	X	X	X	
Helianthus maximiliani	Asteraceae	Schrad.	Maximilian sunflower	X	X	X	X
Leucanthemum maximum	Asteraceae	(Ramond) DC.	Shasta daisy	X	X		
Leucanthemum vulgare	Asteraceae	Lam.	Oxeye daisy	X	X		
Liatris spicata	Asteraceae	(L.) Willd.	Anise hyssop	X			
Linum perenne	Linaceae	L.	Perennial blueflax	X	X	X	
Monarda fistulosa	Asteraceae [Lamiaceae]	L.	Wild bergamot				X
Ratibida pinnata	Asteraceae	(Vent.) Barnhart	Grey-headed coneflower	X	X	X	
Rudbeckia hirta	Asteraceae	L.	Black-eyed Susan	X	X	X	X
Schizachyrium scoparium	Poaceae	(Michx.) Nash	Little bluestem	X	X		X
Solidago nemoralis	Asteraceae	Aiton	Gray goldenrod				X
Solidago rigida	Asteraceae	L.	Stiff-leaved goldenrod			X	
Sorghastrum nutans	Poaceae	(L.) Nash	Indiangrass	X	X	X	
Tradescantia ohiensis	Commelinaceae	Raf.	Ohio spiderwort		X		
Tridens flavus	Poaceae	(L.) Hitchc.	Purpletop			X	

experiments (Table 2). The experiment also was conducted at Virginia Tech's Shenandoah AREC in Raphine, Virginia. Plots were seeded in June 2018. We seeded 6 blocks with either a NWSG-only mix of *Andropogon gerardii*, *Schizachyrium scoparium*, and *Sorghastrum nutans* (NWSG treatment) with a cover crop of *Avena sativa* L., or another combined a NWSG and WF mix of the above 3 grasses and an additional 15 wildflower species (Table 2). The NWSG + WF and NWSG mixes were planted at a rate of 13.5 kg/ha (12 lb/ac) and 45 kg/ha (40 lb/ac), respectively, with 30% of the latter being NWSG and 70% the cover crop.

Each block had 4 plots of 1.4 m × 4.6 m (4.5 × 15 ft) dimensions with rates of imazapic applied at 0.15 l/ha (2 oz/ac; hereafter referred to as Low), 0.29 l/ha (6 oz/ac; hereafter Medium), and 0.73 l/ha (12 oz/ac; hereafter High), plus a control with no imazapic. We sprayed the lot area with glyphosate and tilled before seeds were broadcast by hand immediately prior to imazapic application. The seedbed was rolled to ensure adequate seed-to-soil contact.

We sampled small plots in the imazapic rate experiment for species ground cover using the same methods as Experiment I. Aboveground biomass and percent cover were collected once in both 2018 and 2019 at the end of each growing season. One 0.1 m^2 quadrat (20 × 50 cm) was placed randomly in each plot at least 10 cm (4 in) away from the edges of the plot to prevent potential edge effects. We harvested the biomass by clipping to ground level and then hand-separating into native grasses, weedy species, and wildflowers (in the WF treatment only). Biomass samples were dried in a forced-air oven at 55 °C (131 °F) for 48 h.

Experiment III

We established Experiment III at the Virginia Tech Shenandoah Valley AREC in June 2020. The experiment consisted of a randomized complete block arrangement of 2 planting treatments and a split-plot treatment of imazapic application. Each treatment was replicated 4 times, and plot size was 3 × 12 m (10 × 40 ft). Two planting treatments consisted of NWSG and WF combined within the plot, or NWSG and WF planted in spatially separated adjacent strips (1.5 m [5 ft] each) within the plot. The NWSG species included *Andropogon gerardii, Schizachyrium scoparium,* and *Sorghastrum nutans* whereas the WF species mix included 11 wildflowers (Table 2). The NWSG species and most of the WF species have been reported to exhibit resistance to imazapic herbicide (Beran and others 1999). The NWSG and WF mixes were planted at 12 and 4 kg/ha (11 and 3.5 lb/ac), respectively, on 15 June 2020 using a Dew Drop Drill (Little Sioux Prairie Company, Spencer, Iowa). One wk after planting, we applied imazapic + surfactant to half of each plot at a rate of 0.44 l/ha (6 oz/ac) using a backpack sprayer. The remaining half of each plot received no imazapic (control). On 4 September 2020, we evaluated NWSG and WF establishment by counting all planted species that had emerged in treatment plots. We also estimated percent ground cover of weeds (species that were not sown) within one 0.25m^2 quadrat in each plot using similar methods as Experiments I and II.

Experiment IV

We established Experiment IV at Virginia Tech's Kentland Farm in Blacksburg, Virginia, in 2017 and tested a variety of establishment strategies as alternatives to imazapic. Experiments consisted of a randomized strip-plot design with 4 blocks. Each block contained 4 plots, 2 × 6 m (6.6 × 19.8 ft) in size, each assigned to whole plot treatment of 1) glyphosate application; 2) glyphosate with raking of thatch; 3) prescribed fire; and 4) tillage. Plots were established into a sward dominated by cool-season introduced grasses and forbs, which had been managed by periodic mowing for years prior to the experiment. The glyphosate treatments consisted of 3 applications at 4.67 l/ha (2 qt/ac)—one in March, April, and May of the year of establishment. For the glyphosate + raking treatment, thatch was removed from plots with both a York rake and a mower-mounted de-thatcher 1 wk before seeding. The prescribed fire and tillage treatments were conducted the same day as the de-thatching, 1 wk prior to seeding. Plots were seeded in May 2017. We tilled plots with a Kuhn EL 62 with a maximum tillage depth of 18 cm (7 in). Seeds were broadcast by hand and included a mix of 9 WF species and 2 native grass species (Table 2). Plots were mowed to a 30 cm (12 in) height in late fall or winter using a Woods 121 Rotary mower (Woods Equipment, Oregon, Illinois).

The plots in the experiment were sampled for percent cover twice per growing season in 2017, 2018, and 2019 and once for aboveground biomass each September at the end of the growing season. We collected cover and aboveground biomass data from within 1 m^2 (10.2 ft^2) quadrats (n = 3) placed randomly on each plot. Both aboveground biomass and percent cover were further designated as either wildflowers or weeds for the analyses. Samples were dried to a constant weight in a forced-air oven at 55 °C (131 °F) before weighing.

Statistical Analysis

We analyzed cover values for desirable species at the species level in addition to being combined into index variables (native grasses, weedy species, and wildflowers) to compare overall success of each establishment method. For Experiment I, aboveground biomass and species composition data were analyzed with *t*-tests within years to determine differences in cover and biomass of desirable and weedy species. Desirable species were defined as those seeded in the plots, and weeds were every other species found in the plots. For Experiment II, data were analyzed with ANOVA by year to compare biomass and cover of desirable and weedy species among treatments. Pairwise comparisons of imazapic rates were conducted with Tukey's HSD in Experiment II to determine the optimal rate for NWSG or WF establishment. Grass-only plots and WF plots were analyzed separately. In Experiment III, data on percent weed cover and number of sown NWSG and WF species per plot were analyzed using ANOVA to examine the planting treatment, imazapic application main effects, and their interaction. Similarly, in Experiment IV, data were analyzed with ANOVA by year to compare cover and biomass of WF and weedy species among establishment treatments. Tukey's HSD was also used to compare WF establishment treatments in Experiment IV. In Experiment IV, NWSG were omitted from analysis because of poor establishment. Statistical differences were considered significant at $P \leq 0.05$ and analyses were conducted with JMP, Version 15.

RESULTS

Experiment I

In plots planted with the NWSG+WF mix, imazapic application consistently reduced cover of WF relative to controls each year of the study (Figure 1). In plots receiving imazapic, only *Rudbeckia hirta* L. (Asteraceae), *Desmanthus illinoensis* (Michx.) MacMill. ex B.L. Rob. & Fernald (Fabaceae), and *Coreopsis lanceolata* L. (Asteraceae) were found in 2017, and *Linum perenne* L. (Linaceae) and *Ratibida pinnata* (Vent.) Barnhart (Asteraceae) were also found in 2018. Except for 2018, NWSG cover was not affected by imazapic application. Imazapic had a suppressive effect on weed cover in 2017 relative to controls, but this trend was reversed in 2018 and 2019 (Figure 1).

Wildflower biomass followed similar trends to cover. In 2017, WF aboveground biomass was greater in the control plots (65.3 g/m) compared with plots treated with imazapic

(0.3 g/m) ($P = 0.0029$). Aboveground biomass of weedy species was lower in imazapic plots at 23.9 g/m compared with 71.1 g/m in the control plots ($P = 0.0013$).

In plots seeded with only NWSGs, imazapic plots had approximately 2× higher NWSG cover than controls had ($P = 0.0073$) and significantly lower weed cover in 2017 ($P < 0.0001$; Figure 2). In 2018, weed cover was still 2× higher in control plots compared with imazapic plots ($P = 0.0003$).

In 2017, native grass biomass did not differ between treatments, but this changed in 2018 when native grasses had almost twice the biomass in imazapic plots (228.6 g/m) compared with control plots (138.6 g/m; $P = 0.0007$). Imazapic suppressed weed biomass in 2017 ($P = 0.017$), but no treatment differences were noted for 2018.

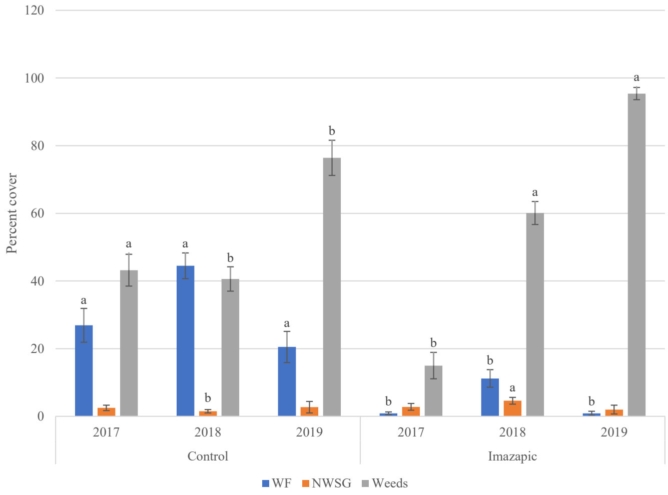

Figure 1. Mean percent cover of wildflowers (WF), native warm-season grasses (NWSG), and weedy species from Experiment I. In plots planted with the NWSG+WF mix, imazapic application consistently reduced cover of WF relative to controls each year of the study. Different letters among treatments indicate statistical differences (Tukey's HSD, $P < 0.05$). Totals do not equal 100% cover because of the omission of percent bare ground from this analysis.

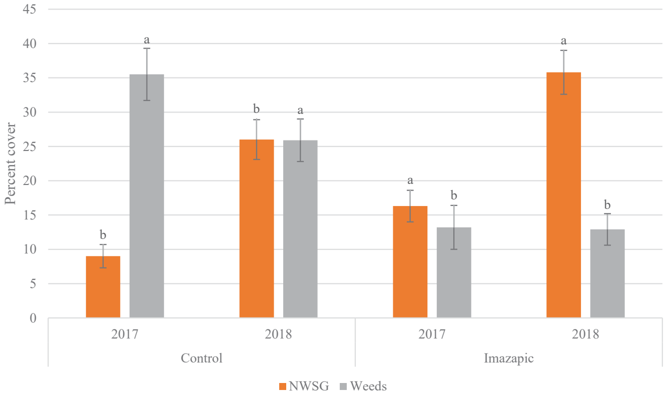

Figure 2. Mean percent cover of native grasses and weedy species in plots seeded with the native grasses only from Experiment I. Imazapic application increased NWSG cover and reduced weedy species cover relative to the control in both years of the experiment. Different letters among treatments indicate statistical differences (Tukey's HSD, $P < 0.05$). Totals do not equal 100% cover because of the omission of percent bare ground from this analysis.

Experiment II

In plots sown with NWSG and wildflowers, native grass biomass was higher in plots with the Medium treatment than in the control or Low treatment. Weedy biomass and WF cover and biomass did not differ statistically but declined with increased imazapic rates: Weedy cover was lowest in the Medium treatment at 2.4% cover, intermediate in the Low and High treatments, and highest in the control plots at 40.5% cover (Figure 3).

None of the seeded wildflower species established in any plot receiving the High treatment. Only *Rudbeckia hirta* established in the Medium imazapic treatment, while *Rudbeckia hirta, Desmanthus illinoensis*, and *Leucanthemum vulgare* Lam. (Asteraceae) were found in the Low treatment plots.

Biomass and cover (Figure 4) did not differ among treatments in plots sown only with NWSGs alone, as imazapic effectively suppressed weeds at most application rates.

Experiment III

Figure 5 summarizes first-year establishment results from the experiment. Weed cover was unaffected by planting treatment ($P = 0.33$) but was lower in split plots that received imazapic ($P = 0.01$). Although numerically higher under imazapic application, the number of established NWSG plants was not significantly affected by herbicide application ($P = 0.11$). The number of WF plants in imazapic treatments was approximately fourfold less (mean $n = 10$) than control plots (mean $n = 41$). More NWSG and WF plants ($n = 13$ and 33, respectively) established when planted together compared with being spatially separated (mean 7 and 17, respectively) ($P < 0.05$). No significant interactions between treatments were noted for the

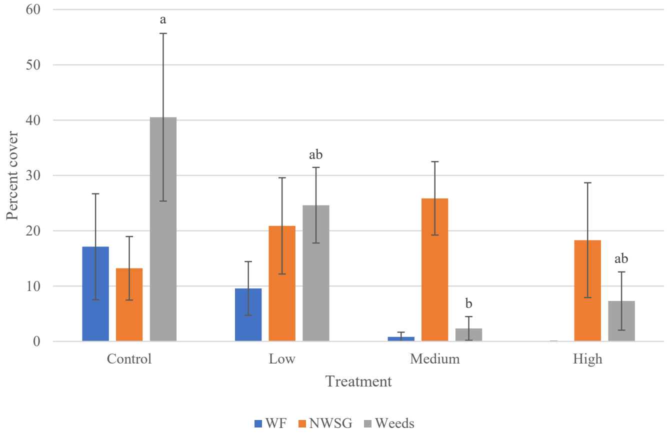

Figure 3. Percent cover of NWSG, WF, and weeds by imazapic rate from Experiment II. The Medium treatment had the lowest weedy species cover, while the no-imazapic control treatment had the highest. Different letters among treatments indicate statistical differences (Tukey's HSD, $P < 0.05$). Totals do not equal 100% cover because of the omission of percent bare ground from this analysis.

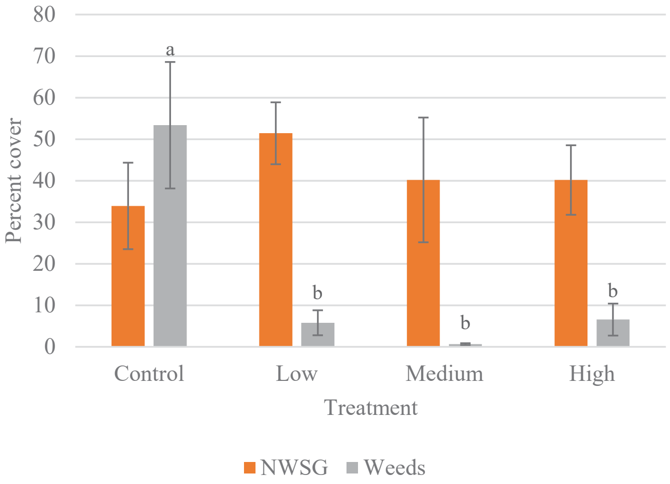

Figure 4. Percent cover of NWSG and weeds by imazapic rate in the plots seeded only with native grasses in Experiment II. The Medium treatment had lowest weedy species cover, while the no-imazapic Control treatment had the highest. Different letters among treatments indicate statistical differences (Tukey's HSD, $P < 0.05$). Totals do not equal 100% cover because of the omission of percent bare ground from this analysis.

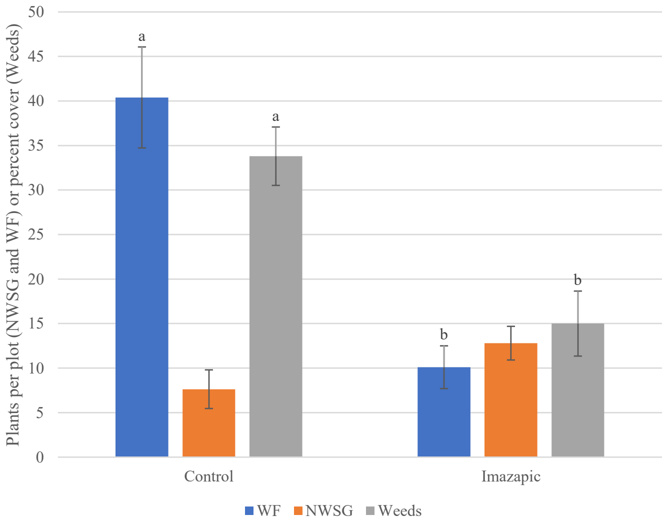

Figure 5. Percent cover of weedy species or number of NWSG and WF plants in control and imazapic plots in Experiment III. Both WF and weedy species cover were higher in the no-imazapic control treatment than in the imazapic treatment. Different letters among treatments indicate statistical differences (Tukey's HSD, $P < 0.05$). Totals do not equal 100% cover because of the omission of percent bare ground from this analysis.

variables examined. All 3 NWSG species were found in plots, and *Sorghastrum nutans* was most common. Of 10 WF species, 8 had established in the first year with *Rudbeckia hirta* and *Helianthus maximiliani* Schrad. (Asteraceae) being the most abundant.

Experiment IV

In the establishment year, weedy species cover was lowest in the tilled plots (49%) compared with other treatments, which ranged from 67.4% to 84.6% ($P < 0.0001$) (Figure 6). Weed cover was 2× higher in the year of establishment than in following years. That year, the tilled plots also had the highest WF cover at 24.2%, compared with a range of 5.5% to 13.5% in the other treatments ($P < 0.0001$). Wildflower cover was highest in tilled and glyphosate treatments and lowest under prescribed fire during 2019 ($P = 0.0006$). Weedy species cover was still highest in the glyphosate/rake treatment and lowest under tillage in the final year, at 47.3% and 29.8%, respectively ($P = 0.0002$) (Figure 6).

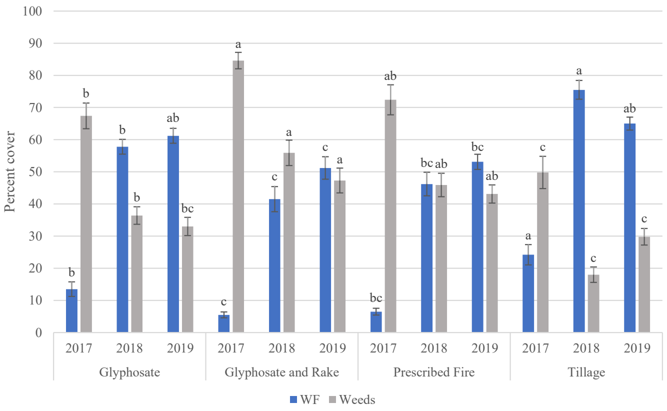

Figure 6. Percent cover of wildflowers and weedy species between establishment treatments in Experiment IV. Levels not connected by the same letter are significantly different. Totals do not equal 100% cover because of the omission of bare ground and native grasses from this analysis. Different letters among treatments indicate statistical differences (Tukey's HSD, $P < 0.05$). Totals do not equal 100% cover because of the omission of percent bare ground from this analysis.

Aboveground biomass results were similar to those of cover. In 2017, weedy species biomass was lowest in tilled plots at 40.3 g/m, and wildflower biomass was highest in the tilled plots at 86.5 g/m ($P = 0.0006$). The next year, the tillage treatment continued to have the highest WF biomass at 75.5 g/m while other treatments ranged from 41.5 g/m in the glyphosate/rake treatment to 57.7 g/m in the glyphosate-only treatment ($P = 0.0072$). In the final year of the experiment, 2019, biomass differences were less pronounced among treatments although WF biomass was greater in the tilled plots than in other treatments ($P = 0.085$). No differences in weedy biomass were noted among the treatments in 2019.

DISCUSSION

Effectiveness of Imazapic for Native Grass/ Wildflower Establishment

We propose that native warm-season grasses and wildflowers can be incorporated into tall fescue swards to create more biodiverse pastures that will benefit ecosystem services such as beef cattle production and pollination. Establishing these species can be challenging in part because of heavy weed pressure that can competitively exclude NWSG and WF species.

Imazapic herbicide has shown to be effective at helping to improve NWSG establishment, but studies on WF establishment have produced more variable results. Prior research on the effect of imazapic on wildflowers have indicated that some WF species are resistant to imazapic, whereas other studies indicate that wildflowers have little to no resistance (Beran and others 2000; Norcini and others 2003; Wiese and others 2011). Results from our experiments also suggest that wildflower responses to imazapic application can be variable. For example, low rates of imazapic application in Experiment I suppressed the establishment of supposedly imazapic-resistant wildflowers for years. Similarly, in Experiment III, WF species were consistently suppressed by imazapic. In contrast, we did find that some wildflower species successfully established when imazapic was applied at a low rate of 0.15 l/ha (2 oz/ac). These variable results could reflect differences in soils. Loux and Reese (1993) found that imidazolinones persisted longer in Hoytville clay soils than in Crosby silt loam, and that persistence of imazethapyr increased as pH decreased in the silt loam soil. Their study also determined that imazaquin had increased persistence as pH decreased in clay soil.

Norcini and others (2003) had similarly varying results in a study using native wildflowers in pots, with herbicide rate, WF species, potting medium, and even seed source within the same species affecting the stunting effects of imazapic on establishment. Stunting effects were greater in commercial potting medium than in sandy soils and greater on a wild ecotype of *Rudbeckia hirta* than on those grown from a commercial seed source. Bahm and Barnes (2011) tested the response of 22 native forb species to the pre-emergent application of 2 rates of imazapic (0.15 l/ and 0.29 l/ha) on sites in Kentucky and Indiana, finding wide variations in susceptibility between species even at low rates, and variation between sites for herbicide effectiveness. They recommended only 5 of the 22 species tested for planting on sites with imazapic application, including *Amorpha canescens* Pursh (Fabaceae), *Aster novae-angliae* (L.) G.L. Nesom (Asteraceae), *Baptisia alba* (L.) Vent. (Fabaceae), *Desmodium illinoense* A. Gray (Fabaceae), and *Solidago rigida* L. (Asteraceae). Our findings were similar in terms of WF resistance, with only 3 species establishing at the 0.15 l/ha rate of imazapic: *Rudbeckia hirta*, *Desmanthus illinoensi*, and *Leucanthemum vulgare*.

Research in Western rangelands reveals similar variability. An expansive study using 5 rates of imazapic application in salt desert shrub and Wyoming big sagebrush ecosystems found high site and species variation in imazapic impacts as well, and the authors hypothesized that variations in precipitation, soil organic matter, and disturbance history may factor into the variability (Morris and others 2009). A study conducted in Oregon investigating the use of imazapic for controlling exotic annuals and restoring native rangeland species also found variation in the impacts of post-emergent imazapic between sites and species, suppressing native and non-native annuals for 3 to 4 seasons depending on site, while having minimal effects on all but 2 perennial native species (Elseroad and Rudd 2011). In our study, higher imazapic rates did not measurably or observationally result in better establishment of native grasses relative to the lower 0.15 l/ha rate in grass-only plots. However, in the plots with the seed mix of both WF and grass species, native grasses established better at 6 oz and 10 oz rates, at which almost no wildflowers established.

One potential drawback to the use of imazapic as an establishment tool might be delayed weed invasion after the establishment year. Native grasses responded favorably to the weed suppression of imazapic at the pasture-scale with consistently greater biomass and cover in all experiments. The exception was the native grasses in WF plots in the final year of Experiment I, which were lower in cover and biomass in the imazapic plots relative to the control. Similarly, in the second and third years of the large WF plots, weedy species cover was higher in the imazapic treatment because the suppression of wildflowers by the herbicide left a void for weedy species to fill. We believe herbicide residual activity in soil wore off by spring the following year and allowed cool-season exotics such as *Trifolium repens* L. (white clover [Fabaceae]) and *Elymus repens* (L.) Gould (quackgrass [Poaceae]) to establish.

In the control plots, the thriving wildflowers competed with weedy species and grasses alike, suppressing both (Figure 7).

If a land manager is more interested in establishing a pollinator meadow than a pasture, the results in the control plots may be desirable. If a pasture is desired, however, the seed mixes we used will need to be modified to increase the proportion of grasses to wildflowers.

Establishment of Native Grass/Wildflower Stands without Imazapic

Experiment IV compared several establishment methods as alternatives to imazapic applications. Our results from the imazapic experiments indicate that seeding wildflowers into a seedbed prepared with imazapic is contraindicated at rates higher than 0.15 l/ha, and even lower rates may be deleterious. Alternatives such as preparing a seedbed with tillage may be more successful in reducing weed pressure while promoting WF abundance. We found that among the treatments evaluated in this 3-y study, tillage appeared to be most effective for successful WF establishment. Past research on the effectiveness of tillage as a WF establishment strategy is conflicting, with some studies reporting better establishment with tillage and others with no-till approaches (Aldrich 2002; Skousen and Venable 2008; Angelella and others 2019).

Grassland bird habitat restoration projects in the Southeast have long advocated the periodic use of tillage or discing as an ecological disturbance to promote forbs and graminoids found to be beneficial to grassland birds (Madison and others 2001).

Figure 7. A cow in one of the grazed no-imazapic WF plots in Experiment I in the third year of establishment. Photo by Benjamin Tracy

The same programs often advise periodic use of prescribed fire—which many native grass and WF species evolved with—in order to promote their establishment or abundance. Concordantly, our results found prescribed fire to be an effective method for site preparation if the goal is WF establishment. Past research on the use of fire and glyphosate or imazapic to convert exotic grass swards into NWSG meadows found that the most effective method of establishing NWSG was a spring prescribed fire followed by an imazapic application and seeding (Washburn and others 2002). Tillage followed by imazapic application also has been effective for conversion of exotic grass swards into native meadows (Barnes 2004). Other studies have found that tillage without imazapic was more effective than mowing or herbicide applications for roadside restoration projects in West Virginia (Skousen and Venable 2008). Conflicting results across various experiments indicate that complex environment × species interactions likely impact the effectiveness of establishment protocols.

CONCLUSIONS

Greater adoption of native grasses and wildflowers in pasture systems may help increase their provision of ecosystem services. We presented data from 4 field experiments designed to evaluate ways to establish native grasses and WF mixtures in Virginia. Our results indicate imazapic is an effective tool for reducing weedy species competition with NWSG in Virginia, but imazapic should be used with caution on sites where the goal is to establish wildflowers unless further research can identify imazapic-resistant WF genotypes. Imazapic effectively suppresses weedy competition in the establishment year, allowing native grass stands to mature enough to outcompete weedy species. For WF establishment, we found tillage was an effective alternative to herbicides. In grazed plots, imazapic-treated plots had higher weed pressure by the end of our experiment because of the suppression of wildflowers by the herbicide, which left a niche for weedy species to fill. In summary, for pure stands of NWSG, imazapic is an effective tool for weed control and stand establishment; however, other seedbed preparation measures such as tillage should be used when wildflower establishment is a goal, as imazapic reduces germination and results in stunting in many wildflower species.

ACKNOWLEDGMENTS

Velva Groover assisted with data collection and record-keeping for Experiment I and IV. Experiment IV was supported by the USDA National Institute of Food and Agriculture, Hatch project 1007077 to ME O'Rourke. The United States Department of Agriculture is an equal opportunity provider and employer. The use of trade names or commercial products in this publication is to provide information for the reader and does not imply recommendation or endorsement by the USDA. We have no conflicts of interest to report.

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