Abstract: Production of biomass feedstock crops could produce substantial environmental benefits, but these will be sharply reduced if these crops become invasive. Switchgrass (Panicum virgatum L.) is being bred for biomass production; these selective efforts may enhance invasive traits. To inform the assessment of invasive risk, undomesticated switchgrass strains were used as a baseline for comparison with strains bred for biomass production. In a three-year field experiment, we compared juvenile plant densities and survival, persistence of established plants, and aboveground biomass between selectively bred (cultivar) and undomesticated switchgrass strains. Cultivars had modestly greater third-year biomass and first-year plant densities than commercial ecotypes but lower survival and persistence; consequently, third-year plant densities did not significantly differ between cultivars and commercial ecotypes. Higher initial establishment and subsequent self-thinning in cultivars resulted in stands that were similar to those of commercial ecotypes. Therefore, our results do not suggest that the breeding of current cultivars of switchgrass enhanced juvenile and young-stand traits associated with invasiveness. Because biomass yields were not greatly different between cultivars and commercial ecotypes, use of the latter in biomass grasslands could provide functional benefits, including an enhanced habitat for native biodiversity and reduced pathogen loads, without incurring large losses in biomass production.

Keywords: biomass; native bioenergy crop; switchgrass; Panicum virgatum; cultivar; ecotype; population source; weed risk assessment; bioenergy risk assessment

1. Introduction

Interest in energy independence, rural development, sustainability, and climate-change mitigation is stimulating production of biomass feedstock crops for an emergent bioeconomy [1]. By enabling replacement of some fossil fuel-based energy, materials, and chemicals with alternatives derived from biomass feedstocks, an advanced bioeconomy could confer substantial social and environmental benefits. However, large-scale cultivation of biomass crops exposes ecosystems and society to risks associated with invasion by these crops [1,2]. Invasive species can reduce the biodiversity and resilience that contribute to ecosystem stability and functioning, thereby impairing the provision of ecosystem services [3,4].

Invasion-associated risks arise in part because the characteristics that define ideal biomass crops largely overlap with characteristics that are correlated with invasiveness in plants [5–7]. Further, the anticipated cultivation of biomass crops on large spatial and long temporal scales will increase propagule pressure on areas adjacent to cultivation fields and transportation routes, thereby amplifying invasive risk [7,8].

To date, invasive risks of candidate biomass species were largely evaluated using semi-quantitative weed risk assessments (WRAs), e.g., [9–14]. Broadly speaking, WRAs...
are conducted by combining existing information on species' traits, distribution, history of introduction, and invasive impacts to generate a score indicating relative invasive risk [9, 13]. However, WRAs were critiqued on multiple grounds, including reliance on history of invasion elsewhere [14] and evidence that WRAs are insensitive to infraspecific variation in risk factors [15–17]. These weaknesses of WRAs are particularly pertinent to candidate biomass feedstock crops, which generally have short histories of introduction outside their native ranges and may contain many unique genotypes and cultivated varieties (cultivars) due to plant improvement efforts [15, 17]. In response, some have advocated for tiered or nested approaches to the assessment of invasive risk in biomass crops [6, 16], in which preliminary findings from WRAs would be modified by empirical testing, among other measures.

Generally, WRAs performed for non-native candidate biomass crops concluded that invasive potential is high, leading to calls for the use of native rather than introduced species on the grounds that nativeness inherently reduces invasive risk. Native species, however, can become functionally invasive if hybridization occurs among previously allopatric genotypes, if new genotypes emerge, or if environmental contexts substantially change [18–21]. These dynamics may well occur during the large-scale cultivation of native biomass crops [22]. Further, selection for agronomically desirable traits in native biomass species via plant breeding will likely enhance similarities to invasive phenotypes [5, 22, 23].

In the case of switchgrass (Panicum virgatum L.), the need for empirical tests to inform the assessment of invasive risk is particularly salient. A perennial grass native to most of the conterminous U.S., switchgrass has emerged as a promising source of lignocellulosic biomass from the standpoints of both sustainability and agronomics. Relative to other candidate biomass crops, switchgrass can provide substantial ecosystem services; has few known insect pests; establishes easily; and has long-lived stands, large potential yields, and high nutrient- and water-use efficiencies [24]. Concerns regarding invasion risks arise because these valuable agronomic characteristics are shared with many invasive plant species, as are certain other switchgrass traits including C_4 photosynthesis, polyploidy, pre-senescence translocation of nutrients, high plant densities, and vegetative reproduction [5, 24, 25]. At present, dedicated biomass feedstock strains of switchgrass are in active development [26–28]. Consequently, research on the invasion risk of switchgrass is needed urgently.

Research on switchgrass invasive potential has yielded mixed results. There is little evidence of switchgrass escape from cultivation [2, 24] or of the formation of persistent seedbanks: Only 0.17% of experimentally sown ‘Cave in Rock’ switchgrass seeds were present at the end of a three-year experiment [29]. No migration of cultivated switchgrass into adjacent fields was observed over a period of ten years [30]. However, a shorter-term experiment observed switchgrass escape into adjacent plots that were maintained as both low- and high-competition receiving areas [31], and a greenhouse study reported that ‘Forestburg’ switchgrass was more competitive than invasive Miscanthus sinensis [32]. Under greenhouse and field conditions, switchgrass cultivars selected for invasive-related traits produced more biomass, seed, or both than wild populations [33–35]. Semi-quantitative WRAs found a high risk of switchgrass invasiveness but only outside its historic range [5, 12, 36–39].

In addition to the ambiguity of these various assessments, dedicated biomass feedstock strains of switchgrass will differ from extant cultivars due to selective breeding by functional-genomic and transgenic means [26–28]. Because the potential invasiveness of such strains is unknown and because large-scale cultivation of switchgrass raises the possibility of escape and establishment along transport routes, concerns about the invasive potential of switchgrass remain [7, 36, 40, 41]. These concerns are heightened by limitations of the available evidence, which is largely based on comparisons of modest numbers of strains in which genetic and maternal environmental effects are confounded.

Development of dedicated biomass strains of switchgrass is still in progress (however, see [27]), and thus it is not yet possible to compare multiple such strains to undomesticated
populations. However, insight into the effects of selective breeding on switchgrass invasive potential may be gained by comparing numerous undomesticated strains, which are considered noninvasive in their native range [6], with cultivars that were selectively bred for traits that are known to be related to invasiveness [42,43]. Such comparisons are largely lacking from the published literature: Although other studies utilized multiple cultivars and undomesticated strains when evaluating invasive-related traits in switchgrass, trait values were not analyzed as a function of cultivar versus undomesticated status [44–53].

Our goal was an empirical evaluation of effects of selective breeding on traits associated with invasiveness. Specifically, we compared numerous representatives of two broadly-defined selection regimes—forage cultivars and undomesticated strains—with respect to the invasiveness-associated traits of plant density, juvenile survival, persistence of established plants, and aboveground biomass at early stages of stand development (years 1–3). This research differs from previous studies by using commercially produced switchgrass ecotypes as the undomesticated comparators. This method avoids a problem common to studies that compare cultivars and wild populations (e.g., [54]), namely, that sowing wild-harvested seeds directly into an experiment confounds genetic effects with those of the maternal environment, potentially influencing the effect size of the cultivar-undomesticated comparison. Commercial ecotypes are grown in agronomic production fields, i.e., in maternal environments that are more similar to those of cultivars; however, they contrast to cultivars in not being subjected to intentional selection. We compared cultivars and commercial ecotypes at four monospecific field sites for three years; congruent with the paradigm of switchgrass as an environmentally sustainable biomass crop that is adapted to agriculturally marginal conditions, we used no agrichemical inputs. We hypothesized that cultivars would exhibit greater invasive potential through higher values in all observed traits.

2. Materials and Methods

Switchgrass (Poaceae) is a perennial C₄ (warm-season) grass species; in North American tall- and mixed-grass mesic prairies, it is generally sub-dominant to Andropogon gerardii, Sorghastrum nutans, and Schizachyrium scoparium [55]. Switchgrass is hermaphroditic, largely self-incompatible, sexually and vegetatively reproductive, and tolerant to a wide range of soil, moisture, and temperature conditions [24]. Two distinct biotypes have been recognized. The upland biotype occurs primarily in drier and northerly sites, can be tetraploid or octoploid, and generally produces less biomass than the exclusively-tetraploid lowland biotype, which is primarily found in floodplains and in southern portions of the range [56].

Twelve switchgrass strains were used in this experiment (Table 1); six are commercially produced ecotypes not subjected to deliberate selection (hereafter termed as “commercial ecotypes”), and six are upland biotype, forage cultivars [57]. The 12 strains were chosen to span a substantial geographic range of origin (37.5–46.9° N, 88.2–100.9° W) while being broadly adapted to growing conditions in Minnesota. Because the processes used to develop the cultivars varied (Table 1), they were variously characterized in the agronomic literature as “unimproved” (‘Cave in Rock’, ‘Dacotah’, ‘Forestburg’) or “significantly selected or bred” (‘Summer’, ‘Sunburst’) relative to wild populations [58]. However, all cultivars used in this experiment were selected for traits associated with invasive potential, including high levels of vigor, leafiness, heavy seed weight, and biomass production [42,43,57,59–61]. The bioenergy-specific cultivar ‘Liberty’ was not available for inclusion in this study, as it was released subsequent to the start of our experiment [27].
Table 1. Passport information for six cultivars (italicized) and six commercial ecotypes of switchgrass that were evaluated for plant density, juvenile survival, persistence, and aboveground biomass at four evaluation sites in Minnesota, USA (2010–2012). Some information is not available for commercial ecotypes.

| Strain               | Ploidy [62] | Release Date, Site of Origin, and Development History                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|----------------------|-------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ‘Dacotah’ (1)        | 4×          | 1989. Progenies from a wild population (Morten Co., ND) were grown in open-pollinated nurseries with other accessions for three generations. Ten individuals were chosen for uniform type and color, leafiness, level of vigor, high seed yields. Progenies from their bulked seed underwent two years of natural selection for hardiness in an increase field; then, 300 random individuals were established as a permanent breeder seed block [59] |
| ‘Dacotah’ (2)        | 4×          | 1989. Progenies from a wild population (Morten Co., ND) were grown in open-pollinated nurseries with other accessions for three generations. Ten individuals were chosen for uniform type and color, leafiness, level of vigor, high seed yields. Progenies from their bulked seed underwent two years of natural selection for hardiness in an increase field; then, 300 random individuals were established as a permanent breeder seed block [59] |
| ‘Summer’             | 4×          | 1963. Seed collected from a wild population (Otoe Co., NE). Mass selections were made for earliness, leafiness, and rust resistance. Progenies of these plants were grown in nurseries; selected individuals were polycrossed. Resulting seed was used to establish the foundation field [57, 63]                                                                                                                                  |
| ‘Cave-in-Rock’       | 8×          | 1973. Collected from one site (Hardin Co., IL). Seeds from the accession were planted at an evaluation site in Missouri. Plants with phenotypes superior to other accessions were used to establish a breeder block; bulked seed was the basis of foundation field [57]                                                                                                                                  |
| ‘Sunburst’           | 8×          | 1983. Accessions from native prairies (Union Co., SD) were grown in open-pollinated nurseries. Two cycles of phenotypic recurrent selection for level of vigor, leafiness, and seed weight resulted in ten superior plants. Eighty half-sibs from each family were reared in a common nursery (800 plants from ten families). Their bulked seed comprised the first generation of breeder seed [43] |
| ‘Forestburg’         | 8×          | 1987. Four accessions (Sanborn Co., SD) were composited and grown in increase fields. Random individuals were moved to a different site and evaluated for morphological characteristics; off-type individuals were rogued from the population. A total of 500 remaining plants comprised breeder seed block [42] |

Kossuth
LaCrosse
Isanti (1)
Isanti (2)
Iowa
Clay

Kossuth County, IA, USA
LaCrosse County, WI, USA
Isanti County, MN, USA
Multiple counties, IA, USA
Clay County, MN, USA

The commercial ecotypes were accessioned from one or more wild populations, then increased in open-pollinated fields with no deliberate selection.

Note: Due to mislabeling during production, “Dacotah” was represented by two strains, each produced by a different grower. The two Isanti strains originate from different locations in Isanti Co., MN and were produced by different growers.

Test sites were established at each of the four locations in Minnesota, USA (Morris, St. Paul, Staples, and Waseca; Figure 1, Table 2) in May–June 2010, using a completely randomized design. Site histories varied: Morris was a dairy pasture; St. Paul contained short-term research projects; and Waseca and Staples were previously in corn-soy rotation. All sites were tilled in autumn 2009, at which time the Morris site received one application of cattle manure. In spring 2010, soil samples were collected and analyzed (Table 2), and all sites were prepared following recommendations for grassland establishment on previously cultivated sites [64]. The experiments were conducted on fertile or sandy (Staples) agricultural soils, and no herbicides or additional fertilizers were applied. The 12 switchgrass strains were then each broadcast into six randomly assigned, 9 m² replicate plots per site at a rate of 2 g m⁻² (17.5 lbs ac⁻¹) of pure live seed (PLS). We opted for a sowing density based on mass, rather than number of seeds, because the former method is more consistent with general agronomic practice. Additionally, the latter method indirectly favors strains with large seeds (i.e., ‘Sunburst’), as seed mass positively correlates with seedling vigor in switchgrass [60]. Prior to sowing, seeds were stored at room temperature (21 °C) for 90 days to reduce dormancy via after-ripening [65] but were not stratified. All sites were mowed in 2010 to suppress annual weeds [64]. Cirsium arvense, a state-listed noxious weed, was manually removed as necessary.
Table 2. Characteristics of evaluation sites used in a 2010–2012 field experiment to assess the effect of two selection regimes (cultivar, commercial ecotype) on invasive characteristics of switchgrass in developing stands.

| Site    | Location | Temp.¹,² (ºC) | Precip.¹,² (cm) | Soil Series (Type) | Soil Chemistry | Soil Family and Subgroup                        |
|---------|----------|---------------|-----------------|-------------------|----------------|-----------------------------------------------|
| Morris  | 45.59º N 95.88º W | 12.1 [11.5] 24.2 [24.1] | 38 [37] | Doland (silt loam) | 5.4 7.5 10 157 | Fine-loamy mixed Udic Haploboroll |
| St. Paul| 44.99º N 93.17º W | 14.1 [13.8] 24.8 [24.4] | 51 [44] | Waukegan (silt loam) | 3.8 5.6 70 124 | Fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls |
| Staples | 46.38º N 94.81º W | 10.4 [10.7] 22.7 [23.2] | 43 [40] | Verndale & Oylen (sandy loams) | 1.6 7.3 43 88 | Coarse-loamy mixed Udic Argiborolls |
| Waseca  | 44.08º N 93.53º W | 13.3 [13.0] 25.2 [24.9] | 49 [47] | Webster (clay loam) | 4.9 6.9 38 207 | Fine-loamy, mixed, superactive, mesic Typic Endoaquolls |

¹ Temperatures and precipitation values are three-year (2010–2012) averages of growing season (May–September) lows and highs (temperature), and totals (precipitation). ² Brackets denote 30-year normal (1981–2010) averages. OM: organic matter. P: Bray phosphorus (ppm). K: ammonium acetate potassium (ppm).

During the study period, average growing season (May–September) conditions were slightly warmer and wetter than the 30-year climate normal averages (Table 2) at three sites. The exception occurred at Staples, where the minimum and maximum growing season temperatures, averaged over all three years (2010–2012), were 0.5 ºC cooler than the normal averages. Precipitation levels during the critical germination period of June and July 2010 were near or above the 30-year normal averages, except at Morris where July precipitation was 60% of normal. The only significantly dry periods occurred at Waseca in August 2011 and at all sites in September of 2011 and 2012, with precipitation at 20–30% of normal.

We compared switchgrass cultivars and commercial ecotypes with regard to invasive potential using four metrics: plant density, juvenile survival, persistence of established
plants, and aboveground (AG) biomass. To assess the effect of selection regime on plant density, we counted the individuals present in one randomly placed 0.25 m$^2$ quadrat within each 9 m$^2$ plot during the first (2010), second (2011), and third (2012) years of the experiment. To reduce edge effects, quadrats were placed no closer to plot perimeters than 0.15 m. Quadrat location was re-randomized every year to avoid overlap with previous positions. To differentiate individuals, we located the crown of each plant (genet) and only counted as separate individuals those with clearly distinct, spatially differentiated crowns. During the course of the experiment, all 12 strains exhibited upright, bunching architecture, and we observed no evidence of rhizomatous spread that could have blurred distinctions among individuals. To determine whether selection regime affected invasive-related traits differently across early life stages, we analyzed juvenile survival (density$_{year\ 2}$−density$_{year\ 1}$) and persistence of established plants (density$_{year\ 3}$−density$_{year\ 2}$) separately, using profile analysis [66]. Third-year AG biomass provided an integrated measure of switchgrass performance as well as information directly relevant to bioenergy production. Biomass was harvested from a 1 m$^2$ quadrat [67] within each of three replicate plots per strain per site after all strains senesced in 2012. All standing vegetation within the quadrat was clipped at 2 cm above the soil surface, sorted in the field to separate switchgrass from other species, and dried for 72 h at 60 °C prior to weighing. As above, all quadrats were placed randomly but at least 0.15 m from plot perimeters. Because weed presence differed among sites, we assessed switchgrass AG biomass both in absolute terms (g m$^{-2}$) and as the switchgrass proportion of whole-plot AG biomass, including weeds. Due to early snowfall, neither third-year plant densities nor biomass were measured at Staples in 2012.

To evaluate differences in switchgrass performance between selection regimes (cultivars, commercial ecotypes), we analyzed invasiveness-related traits that were measured once (juvenile survival, persistence, absolute, and proportional biomass) using generalized linear models (glm) in R [68,69], which fits models using maximum likelihood estimation. Plant density, which was assessed repeatedly, was analyzed using linear mixed effects models (lmer) in R [70]. This method appropriately accounts for within-site autocorrelation as well as unbalanced data [71]. The models for survival, persistence, and biomass included selection regime, evaluation site, and their interaction as fixed main effects. The model for plant density additionally included year and all two- and three-way interactions as fixed effects. The random term in all mixed-effects models was “plot”, which took a unique value for the 288 plots sampled collectively across the four sites. To estimate F-tests and p-values, and to conduct post-hoc tests of the mixed-effects models, we used lmerTest in R [72].

The 12 experimental strains, which may be of interest with regard to continued germplasm development, originated from different geographic locations. Accordingly, in secondary analyses, we examined the effects on the measured traits of strain and of latitude and longitude of origin, using lmer (plant density) or glm (juvenile survival, persistence, absolute and proportional biomass). The latitude/longitude models included selection regime, evaluation site, and their interaction as fixed main effects. The model for plant density additionally included year and all two- and three-way interactions as fixed effects. The random term in all mixed-effects models was “plot”, which took a unique value for the 288 plots sampled collectively across the four sites. To estimate F-tests and p-values, and to conduct post-hoc tests of the mixed-effects models, we used lmerTest in R [72].

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3. Results

When data were pooled across sites and, as applicable, across years, mean values of plant density and biomass were greater in cultivars than in ecotypes. Juvenile survival and persistence, however, were greater in ecotypes (Table 3).
Table 3. Means of invasive-related traits measured in developing switchgrass stands at multiple locations in Minnesota, USA, 2010–2012. Data were pooled across sites and, for plant density, across years. Parentheses denote standard errors; italics denote n.

| Selection Regime | Plant Density (Plants m$^{-2}$) | Survival (Plants m$^{-2}$) | Persistence (Plants m$^{-2}$) | Absolute Biomass (g m$^{-2}$) | Switchgrass Proportion of Biomass |
|------------------|-------------------------------|-----------------------------|-------------------------------|-------------------------------|----------------------------------|
| Cultivar         | 71.3 (3.21); 396             | −59.2 (6.32); 144           | −8.48 (2.92); 108             | 343 (43.8); 54                | 0.594 (0.0486); 54              |
| Ecotype          | 39.2 (1.92); 396             | −11.8 (4.64); 144           | −0.111 (2.40); 108            | 264 (36.7); 54                | 0.499 (0.0518); 54              |

Note: Refer to text regarding calculation of survival, persistence, and proportion of biomass.

3.1. Effects of Selection Regime

Switchgrass selection regime affected some but not all of the invasive-related traits that were measured. Selectively-bred cultivars exceeded commercial ecotypes in first-year plant density (Table 4, Figure 2, Figure S1 in Supplementary Materials) but had lower juvenile survival (density$_{\text{year 2}}$–density$_{\text{year 1}}$) and lower established-plant persistence (density$_{\text{year 3}}$–density$_{\text{year 2}}$). In aggregate, we did not detect a significant difference in plant densities between the two groups by the third year, despite marked declines in cultivar plant density between years 1 and 2 (Figure 2). In contrast, cultivars exceeded commercial ecotypes with regard to both absolute and proportional AG biomass (Tables 3 and 4, Figure 3), two measures that were strongly correlated ($r = 0.82$).

Table 4. Results of tests of fixed effects on measures of invasive-related traits in developing switchgrass stands under different selection regimes, sites, and years. Subscripts denote d.f. $p$: ns, not significant; * < 0.1; ** < 0.05; *** < 0.01; **** < 0.001; ***** < 0.0001.

| Predictors $^*$ | Plant Density | Juvenile Survival | Persistence | Aboveground Biomass | Switchgrass Proportion |
|-----------------|---------------|-------------------|-------------|--------------------|-----------------------|
|                 |               |                   |             | Absolute           | Proportion             |
| SR              | 71.9, 283     | 36.5, ****        | 5.01, *     | 4.13, *            | 4.63, *               |
| S               | 19.9, 287     | 3.29, ns          | 3.24, ns    | 61.62, ***         | 1352, ****            |
| Y               | 62.1, 491     | —                 | —           | —                  | —                     |
| SR $\times$ S   | 0.719, 287    | 2.97, ns          | 5.42, *     | 1.09, ns           | 0.759, ns             |
| S $\times$ Y    | 31.4, 491     | —                 | —           | —                  | —                     |
| SR $\times$ S $\times$ Y | 4.134, 491 | —                 | —           | —                  | —                     |
| Str $\times$ Str | 2.357, 491   | —                 | —           | —                  | —                     |
| S               | 11.1, 284     | 68.6, ****        | 8.73, ns    | 88.61, ****        | 69.3, ****            |
| Y               | 19.4, 251     | 3.58, ns          | 3.19, ns    | 105, ****          | 202, ****             |
| Str $\times$ S  | 82.7, 83      | —                 | —           | —                  | —                     |
| Str $\times$ Y  | 1.523, 251    | 38.5, ns          | 28.5, ns    | 22.4, ns           | 18.9, ns              |
| Lat             | 5.512, 484    | —                 | —           | —                  | —                     |
| Long            | 18.7, 270     | 3.21, *           | 0.071, ns   | 5.20, *            | 10.11, **             |
| SR              | 12.2, 270     | 6.24, *           | 0.33, ns    | 7.571, **          | 12.7, ***             |
| S               | 11.0, 270     | —                 | —           | —                  | —                     |
| Y               | 0.357, 281    | 0.777, ns         | 1.06, ns    | 10.32, **          | 4.08, ns              |
| Lat $\times$ Long | 4.08, *    | —                 | —           | —                  | —                     |
| Lat $\times$ SR | 5.532, 509    | —                 | —           | —                  | —                     |
| Long $\times$ SR | 7.641, 270    | —                 | —           | —                  | —                     |
| S $\times$ Lat  | 6.63, o       | 8.52, *           | 0.43, ns    | 1.49, ns           | —                     |
| Y $\times$ Lat  | 5.262, 509    | —                 | —           | —                  | —                     |
| Y $\times$ Long | 5.062, 509    | —                 | —           | —                  | —                     |
| S $\times$ Y    | 4.982, 509    | —                 | —           | —                  | —                     |
| Lat $\times$ Long $\times$ SR | 10.32, 272**** | —                 | —           | —                  | —                     |
| Lat $\times$ Long $\times$ Y | 4.942, 509 | —                 | —           | —                  | —                     |
| Lat $\times$ S $\times$ Y | 3.178, 404 | —                 | —           | —                  | —                     |
| Long $\times$ S $\times$ Y | 3.228, 420 | —                 | —           | —                  | —                     |

Note: SR: selection regime (cultivar or commercial ecotypes); S: evaluation site; Y: year (1st, 2nd, or 3rd year of growth, 2010–12). Str: switchgrass strain. Year and associated interactions were not included in the models for survival (2011 plant density–2010 plant density), persistence (2012 density–2011 density), or biomass (3rd year only). Tests on density: F-statistics, lmer. Tests on survival, persistence and biomass: $\chi^2$, glm.
3.2. Effects of Strain within Selection Regime

Analyses of the individual strains revealed that they differed in invasiveness-related traits and that experimental site and geographic origin had certain detectable effects (Table 4). Plant densities were consistently lowest at Morris and highest at St. Paul and Staples (Supplementary Figure S1); biomass was highest at Waseca (Figure 3), and neither juvenile survival nor established plant persistence differed significantly among sites (Table 4). Strains within both groups (cultivars, commercial ecotypes) exhibited considerable variation in all traits measured (Supplementary Figures S2 and S3). The strain x site interaction was significant for plant density but not for either juvenile survival or persistence, indicating that although sites varied with regard to the number of plants...
present, the magnitude of interannual changes in plant densities were similar across sites (Table 4, Supplementary Figure S1).

3.3. Effects of Geographic Origin

The relationship to geographic origin varied among the invasiveness-related traits. The weak positive association between plant density and latitude and longitude of origin was slightly stronger for ecotypes than cultivars (Table 4), but this effect was obscured by lower ecotype densities overall. For both ecotypes and cultivars, the relationship between density and geographic origin was negligible by year three (Table 4). This declining relationship is illustrated in Figure 4A, which, for most cultivar points of origin, shows a decrease in bubble diameter between the left-hand panel (2010) and the right-hand panel (2012). Specifically, juvenile survival increased with latitude of origin but decreased with longitude of origin, jointly resulting in an average decrease in survival of 4.5 individuals m$^{-2}$ for every 100 km in a northwesterly distance from the origin site. Both absolute and proportional switchgrass biomass had weak negative associations with geographic origin, decreasing on average by 31 g m$^{-2}$ (0.14 tons ac$^{-1}$) and 3.8% per 100 km northwest, respectively (Table 4, Figure 4B). We detected no difference between cultivar and commercial ecotypes in their responses to geographic origin, possibly due to an uneven distribution of sites of origin across the geographic gradient (Figure 4), resulting in confounding of selection regime with geographic origin.

Figure 4. Bubble plots indicating mean performance of six cultivar (dark gray circles) and six commercial ecotypes (light gray circles) of switchgrass. Bubble position indicates longitude and latitude of origin. Bubble sizes indicate (A) plant densities (m$^{-2}$) during the first three growing seasons, averaged across four (2010, 2011) or three (2012) evaluation sites (site effect $p > 0.05$, per-site, per-strain $n = 6$) and (B) third-year aboveground biomass (g m$^{-2}$) at three evaluation sites (site effect $p = 0.006$, per-site, per-strain $n = 3$). Black squares denote location of evaluation sites. Nine sites of origin per panel are shown because three pairs of strains originate from the same approximate area (‘Forestburg’ and ‘Summer’, two strains of ‘Dacotah’, and two commercial ecotypes from Isanti County, MN, USA). Black circles denote 1 SE.
4. Discussion

Despite calls for proactive risk assessments prior to the large-scale production of dedicated biomass feedstock crops including switchgrass, and for tiered multi-method assessments, empirical evaluation of the invasive potential in these crops is limited [7,40,74]. To evaluate aspects of invasive potential in switchgrass, we assessed plant density, juvenile survival, persistence, and aboveground biomass in numerous selectively-bred cultivars and undomesticated commercial ecotypes. In these developing stands, we found that cultivars had a higher yield; however, the difference was modest when expressed at spatial scales relevant to biomass production systems (0.82 Mg ha$^{-1}$ or 0.36 tons ac$^{-1}$). Cultivar plant densities originally far exceeded the 40% threshold (48 plants m$^{-2}$) that indicate successful stand establishment [75] and were initially greater than those of the undomesticated ecotypes. Lower juvenile survival and persistence in cultivars, however, resulted in third-year densities that were similar between the two selection regimes. This pattern suggests that initial advantages conferred to cultivars by high rates of germination and seedling establishment eroded over time.

Jointly, these results suggest that the upland-biotype forage cultivars used in this experiment are not likely to be more invasive than their undomesticated counterparts during the initial establishment through early maturation phases of switchgrass stand development. This conclusion aligns with reports of similar values in both upland cultivars and wild switchgrass populations for at least some invasiveness-related traits during the first one or two growing seasons [33,34]. Additionally, we ran a concurrent experiment at the same evaluation sites with the same switchgrass strains and found no evidence of differential effects of cultivars versus commercial ecotypes on species diversity or yield in developing prairie communities [76]. It bears noting, however, that switchgrass stands generally require three years or longer to mature fully and that characteristics such as rooting depth and tiller number increase with age [24,77]; therefore, invasiveness-related traits in mature switchgrass stands may differ from those in younger ones. To our knowledge, no comparisons of these traits have been published for stands of cultivar and undomesticated switchgrass aged four years or older.

While we did not find notable between-group differences in the measured invasiveness-related traits, we also did not find that cultivars were associated with a substantial yield advantage. In fact, incorporation of undomesticated strains into biomass plantings might increase yield, given our finding that some commercial ecotypes produced more biomass than some cultivars at any given site, even without the agrichemical inputs that are necessary for long-term productivity of the cultivars used in this study [78]. Insofar as sustainability is an objective of the emerging bioeconomy, our results suggest that undomesticated strains of switchgrass may offer some benefits compared to cultivars.

The sharp declines in plant density and the strongly negative correlation between first-year plant density and persistence ($r = -0.87$) were particularly evident in the cultivars and are consistent with density-dependent mortality or self-thinning [79]. Winter mortality might also have contributed to density declines, given that some of our translocation distances exceeded the 500 km maximum (~5° latitude) recommended when moving switchgrass populations north of their areas of origin [48,65]. However, if this was the case, we would likely have observed greater density declines in southern-origin switchgrass than in their northern counterparts. Instead, we observed smaller density declines among southerly-originating strains than among northerly-originating strains, as have other studies [80,81]. Our findings contrast with those reported by Schmer and others (2010) [67], who observed steady increases in cultivar plant densities from years 1 through 5. The dissimilarity in results may be due to different weed management (herbicide use by Schmer and others vs. no herbicide in our experiment) and seeding rates, which were approximately three times higher in our study. Future assessments of invasive potential in switchgrass could include experimental treatments intended to enhance or increase stand performance, including supplemental seeding, application of fertilizer, and use of herbicide for weed control.
The range of biomass values reported here generally aligns with those reported in other field studies conducted in Minnesota and South Dakota, USA [80–82]. Published values of switchgrass yield vary widely, reflecting differences in performance among evaluation sites, years, and strains [82], and because of interactions between test site location, geographic origin, phenology, and precipitation regime [24,47,54,63,81,83–86]. Soil characteristics such as type and acidity are not considered to be strong drivers of such differences [77,85]; however, interactions between soil texture and moisture can strongly affect switchgrass performance at various life stages [87]. Soil textures at our sites ranged only from sandy loam to clay loam, but combinations of soil texture and moisture may have contributed to inter-site variation. Another likely source of variation was different intensities of weed competition, which reflected differences among sites in land use history.

To ensure that production of biomass feedstock crops is sustainable, associated risks must be managed, and several courses of action could contribute to such efforts with regard to switchgrass. First, switchgrass stands for biomass production could make use, wholly or partially, of commercial ecotypes rather than selectively-bred cultivars. While we did not find notable between-group differences in the measured invasiveness-related traits, we also did not find that cultivars were associated with a substantial yield advantage. In fact, we found that some commercial ecotypes produced more biomass than some cultivars at any given site, indicating that the use of undomesticated switchgrass might actually increase yield in biomass plantings. As a precautionary approach, use of undomesticated strains, presumed to be more genetically variable than cultivars, could facilitate adaptation to climate change [88], reduce gene-flow risks [2], and decrease the likelihood of pest or pathogen outbreaks by increasing stand heterogeneity [89]. If mixtures are sown, within-stand hybridization between cultivar and undomesticated switchgrass could be minimized by utilizing strains with temporally differentiated phenologies. Second, the potential for weediness could be assessed at various stages of the germplasm development process, with materials that pose severe risk being excluded from further domestication [23]. Third, the cultivation of sterile switchgrass would greatly reduce the risk of escape via propagule pressure [5], but this presents practical difficulties.

Our interest was to respond to calls for tiered approaches to invasive risk assessment in biomass crops (e.g., [16]) by comparing switchgrass derived through two broadly-defined selection regimes, each represented by numerous strains. Some literature (e.g., [58,62]) characterized switchgrass cultivars as essentially equivalent to or representative of their wild-source populations because comparatively few cycles of selection have been imposed on switchgrass during its relatively short domestication history. According to this view, our consideration of cultivars with varied selection histories collectively may have obscured pertinent variation. We did not attempt to differentiate among cultivars because selection intensity, which critically affects response to selection [90], is rarely quantified in cultivar registration documents.

5. Conclusions

Our study exemplifies the tiered or nested multi-method approach to the assessment of invasive risk in biomass crops in which preliminary findings from WRAs are modified by empirical testing, among other measures. Our findings, taken together with previous studies, are consistent with an assessment of modest or limited invasion risk from improved switchgrass forage cultivars, and moreover suggest that the invasive risk of these cultivars may be no greater than that of their undomesticated, commercially produced counterparts.

The development of biomass feedstock strains of switchgrass includes conventional plant breeding, biotechnology, or both. Efforts to assess the ecological risks associated with these dedicated biofeedstocks are in their infancy and must grapple with novel complexities and sources of uncertainty related to invasiveness, gene flow and introgression, and ecosystem function. Proactively developing a robust, multi-tiered risk assessment framework and managing risks associated with novel strains of native species is crucial to realizing the many environmental and social benefits of the emerging bioeconomy.
Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su13095045/s1, Figure S1: Plant density per year per site by selection regime; Figure S2: First- and third-year plant density per site by switchgrass strain; Figure S3: Third-year aboveground biomass per site by switchgrass strain.

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