Below is the GWAS methods I originally wrote for John, with new additions for greenup and flowering. I also added a section after that with relevant methods from the other 4WAY papers for your reference if you need them. We did discuss 4WAY greenup and flowering in Lowry’s paper.

The only differences between the GWAS and 4WCR plantings is the interplant spacing (1.77 m in GWAS vs 1.56 m in 4WCR) and the borders (Blackwell grown from Ernst Seed in the GWAS and Alamo grown from rhizomes obtained from a legacy stand in Temple, Texas for the 4WCR).

**GWAS METHODS AS I WROTE THEM FOR JOHN’S PAPER, He condensed these New additions in Blue**

Collections (permits, dates, etc.)

This diversity panel was assembled with switchgrass accessions from a wide variety of sources over the course of three years from 2015 to 2018. Plants were assigned an accession number for each unique geographic location of origin. When multiple individuals were collected from a single location, an additional individual number was assigned. When the accession source material was a seed, each individual seed grown from a packet was considered a unique individual. When the accession material was rhizome plugs, individuals were denoted as rhizome material from a unique plant collected at each geographic location. Seed sources were obtained GRIN and various collaborators. Rhizome material was obtained from established experimental gardens from various collaborators and from many natural environments across the species range. For all collections from natural populations, collecting was done by our staff or collaborators and requisite permits were obtained from appropriate local government entities in all cases.

Plant growth/propagation/planting/establishment

Plants obtained from seed were generated by sowing 10-15 seed in 9 cm square blow molded pots (BWI) containing a mixture of ProMix BX potting soil (Premier Tech Horticulture) and Turface MVP calcined clay (Turface Athletics). Sown seed was vernalized for 7 days at 4C, then pots were placed in a lit greenhouse with 14-hour day length and 30C/22C day/night temperature. Seedlings were thinned at the three leaf stage to one plant per pot and allowed to grow until the 5 tiller stage, then plants were transferred to a 5 gallon blow molded pot containing finely ground pine bark mulch (Lone Star Mulch, Austin, TX) and time release fertilizer (Osmocote 14-14-14, ScottsMiracleGro) and watered frequently. Plants obtained from rhizome plugs were grown by placing the field collected rhizome mass and its associated tillers in 5 gallon blow molded pot containing finely ground pine bark mulch (Lone Star Mulch, Austin, TX) and time release fertilizer (Osmocote 14-14-14, ScottsMiracleGro) and watered frequently.

Individuals obtained from both seed and rhizome plugs were propagated by clonal division over the course of three years from 2016 to 2018. For propagation, sizable portions of rhizomes and associated tillers were separated into smaller portions with a knife, with care taken not to dislodge soil from the existing associated root mass. Each resulting rhizome mass and its associated tillers (5-20 tillers) were placed in a new pot containing finely ground pine bark mulch (Lone Star Mulch, Austin, TX) and time release fertilizer (Osmocote 14-14-14, ScottsMiracleGro) and watered frequently. This process was repeated every 3-6 months depending on growth rate of the propagants. Five gallon blow-molded pots (BWI) were used for all preliminary propagations and plants were placed in 1 gallon blow-molded pots (BWI) for the final propagation before planting. The target for clonal division was ten or more individuals per unique accession. All plant growth during the propagation phase occurred in an outside propagation yard located in Austin, TX and plants were watered daily by sprinkler. Cleary 3336F systemic fungicide (Cleary Chemicals, LLC) was applied to the plants with a backpack sprayer as necessary to control fungal pathogens.

Field sites were prepared for planting with a rotary tiller to a depth of 6 inches and covered completely with 90 g weight woven ground cover (Dewitt Sunbelt). Ground cover segments were overlapped by 30 cm and secured to the soil with 15 cm 9-gauge steel staples. Plants were planted by hand in a honeycomb configuration with a 1.25 m grid spacing resulting in a 1.77 m plant to plant distance. Plants were planted to a depth where the rhizome mass was 3 cm below the soil surface. 25 cm holes were cut in the ground cover fabric for each plant at the time of planting and holes are enlarged each spring to a distance of 15 cm from the crown margin to allow for crown expansion. Plants were well watered with a hose at the time of planting and watered multiple times as necessary by hand, sprinkler or drip irrigation in the three months following planting to ensure establishment. No further irrigation was supplied to plants after the initial establishment watering period. Above ground portions of all plants were left to stand over the winter of 2018/2019 and removed in the spring of 2019 before spring tiller emergence.

Greenup/ Survival//Flowering/Biomass phenotyping

Plants were observed weekly in the spring for the presence of new tiller growth. Once any new tiller growth was observed in the field, all plants were individually assessed every two days for the presence of new emerged tillers. Spring greenup (GR50) was denoted as the day approximately 50% of the plants existing crown area contained newly emergent tillers.

Plants were considered to have experienced winter mortality during the 2018/2019 winter season when no new tiller emergence was seen from plant crowns by June 1st of 2019. The dead plant crowns were excised from the experiment and replaced with plants of the Blackwell switchgrass cultivar in July and September of 2019.

Plants were observed weekly in the summer for the presence anthesis. Once any tillers undergoing anthesis were observed in the field, all plants were individually assessed every two days for the presence of tillers undergoing anthesis. Anthesis (FL50) was denoted as the day when 50% of the plants tillers had panicles undergoing anthesis.

Biomass data was obtained from all living individuals during harvest in October and November of 2019. To collect biomass data, all tillers on each plant were tied securely with bailing twine and tagged and then each bundled plant was cut with a sickle bar mower (BCS America). Plants with an estimated mass of less than 750 g were placed in paper bags and dried whole at 60-70C until no additional moisture loss was occurring and then weighed for total dry biomass. Plants with an estimated mass of greater than 750 g were weighed in the field for wet biomass on a hanging scale with a +/- 5 g resolution. To determine biomass of these plants, approximately 500g of whole tillers were sub-sampled from each plant and dried 60-70C until no additional moisture loss was occurring and then weighed for dry biomass. The wet-biomass of the whole plant sample was then multiplied by the percent moisture in the sub-sample to approximate total dry biomass.

**RELAVANT 4WCR METHODS FROM OTHER PAPERS**

**Milano 2016; The Genetic Basis of Upland/Lowland Ecotype Divergence in Switchgrass (Panicum virgatum)**

This paper contains methods for creation of 4WCR population.

**VanWallendael 2020; Geographic variation in the genetic basis of resistance to leaf rust between locally adapted ecotypes of the biofuel crop switchgrass (Panicum virgatum)**

To identify loci controlling variation in rust progression, we used a previously developed four-way phase-known (pseudo-testcross) mapping population derived from both upland and lowland genotypes (conceptual map in Fig. 1). For full details of the development of the mapping population see Milano et al. (2016). We clonally divided the outbred populations by manually split-ting rhizomes at the Brackenridge Field Laboratory in Austin, TX, USA. In May–July 2015, the F0, F1and F2clones were pot-ted, moved by truck and transplanted into the field at 10 sites throughout the USA (Lowry et al., 2019). Thus, at each site, we planted five or more clones of each of the four F0genotypes, 15individuals of each of the two F1populations and 431 individuals from the F2generation……………. We assigned plants randomly to a honey-comb design, with 1.56 m between each plant. To reduce edge effects, we planted a border of lowland plants around the plot that were not measured experimentally. We watered plants by hand in 2015, when necessary to facilitate establishment. Weedcloth was installed to cover the ground between plants and reduce weed pressure. After 2015, we removed weeds using pre-emergent herbicides and physical pulling but did not otherwise man-age plots

**Lowry 2019; QTL × environment interactions underlie adaptive divergence in switchgrass across a large latitudinal gradient**

The details of creation of the genetic mapping population are described in Milano et al. (2016). Briefly, the genetic mapping population was produced by initial crosses between AP13×DAC6(A×B) and WBC3×VS16 (C×D). The F1 hybrids of each of those crosses were then intercrossed reciprocally to produce the four-way outbred mapping population. The four-way population, grandparents, and F1 parents were propagated clonally in 3.8-L pots at the Brackenridge Field Laboratory, Austin, TX in 2014–2015. Plants were transported to each of the 10 field sites by truck and planted at each site in May–July of 2015. Each field was covered with one layer of weedcloth (DeWitt). Holes were cut into the weed cloth for planting of the experimental plants. Plants were randomized haphazardly into a honeycomb design, where each plant had four nearest neighbors, all located at 1.56 m away from each other. To prevent edge effects, a row of plants derived from the lowland Alamo cultivar were planted at every edge position of the plot. Plants were hand-watered following transplantation as needed through the summer of 2015. Plants were not measured until the spring of 2016 to allow them to become established through one winter first. The five phenotypes for this study were assessed as follows. Green-up time was scored as the Julian date at which point a plant had sprouted new tillers from 50% of the area of the crown from the previous season. Flowering time was scored as the point when 50% of the tillers of the plant had panicles undergoing anthesis. The number of green tillers were counted within a few weeks after the 50% flowering date. Height was measured from the base of the plant to the uppermost point of the canopy. At the end of each season, plants were tied upright as a bunch and harvested with a sickle bar mower. Wet biomass was quantified in the field. A subsample of each plant was also weighed and then dried at 55 °C until completely dry and weighed again. Percent water content for each subsample was then used to calculate the dry biomass of each plant.