Evaluation of Variation in Switchgrass (*Panicum virgatum* L.) Cultivars for Rust (*Puccinia emaculata*) Resistance[1]

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Abstract

Eleven switchgrass cultivars (eight ornamental and three agronomic) were inoculated with 40 switchgrass rust isolates collected from the southeastern U.S. to study host resistance, rust virulence and host/pathogen interactions by measuring urediniospore germination percentage, latent period, and the number of uredia and urediniospores produced per cm^2 of leaf surface. In general, ornamental switchgrass cultivars had reduced number of uredia and urediniospores produced per cm^2 than did agronomic cultivars. Rust isolates were variable for virulence in culture (on grass blades in petri dishes); however they could not be segregated into groups based on collection locations or years. The results of this study will provide information concerning durable horizontal resistance in switchgrass for the ornamental industry.

Index words: Switchgrass, leaf rust, resistance.

Species used in the study: switchgrass (*Panicum virgatum* L.), rust (*Puccinia emaculata*).

Significance to the Horticulture Industry

Switchgrass is used as an ornamental grass and as a biofuel feedstock. Leaf rust, caused by *Puccinia emaculata Schwein*., may reduce the crop yield by up to 55% (Skyes et al. 2016). Researchers have reported on rust resistance in agronomic cultivars/populations or ornamental cultivars (Gustafson et al. 2003, Jacobs et al. 2004, Uppalapati et al. 2013). This research focused on evaluating agronomic and ornamental cultivars for their susceptibility to rust isolates collected in seven states from either agronomic and/or ornamental plantings. The team found that some ornamental cultivars were more resistant than agronomic cultivars and cultivars resistant to some rust isolates were susceptible to other isolates of rust. This study provides information on how more durable resistant cultivars can be developed and explain why resistance may fail at some locations.

Introduction

Switchgrass (*Panicum virgatum* L.) is a warm-season perennial grass that is native to North America. It has been traditionally used for hay and bank stabilization (Lemus et al. 2002) and used more recently as a biomass crop for ethanol and butanol (Bouton 2008, Cherney et al. 1991). As an ornamental, switchgrass cultivars have been selected for leaf blade coloration, height and form.

Due to different propagation methodology, switchgrass cultivars were divided into agronomic and ornamental cultivars. Agronomic cultivars, such as 'Kanlow' and 'Cave-In-Rock' ('CIR'), were developed using traditional breeding methods for cross-pollinated crops, which has led to genetically different individuals within the cultivar. Based from their ploidy level and habitat preference, agronomic switchgrass cultivars can be grouped into two ecotypes: lowland and upland ecotypes (Porter 1966, Hultquist et al. 1996). Lowland cultivars (i.e. 'Kanlow') are mostly tetraploid (2n= 4X= 36) and generally planted in wetter environments and well adapted to the southern USA; upland cultivars (i.e. 'CIR') are tetraploid or octoploid (2n= 8X= 72), and usually found in drier locations in the central and northern USA (Barnett and Carver 1967, Brunken and Estes 1975, Hultquist et al. 1996, Nielsen 1944, Zhang et al. 2011). The origins of ornamental switchgrass cultivars, such as 'Cloud Nine' and 'Thundercloud', were selected by identifying an individual switchgrass plant with a desirable trait, such as leaf blade color, height, or form. The individual was then propagated vegetatively (clonally).

Many pathogens have been reported as disease-causing agents of switchgrass, including bacteria and nematodes (Cassida et al. 2005, Mekete et al. 2010), viruses (Sill and Pickett 1957, Stewart et al. 2015) and fungal pathogens (rust, smuts, leaf spots, crown and root rots) (Gravert and Munkvold 2002). Among these pathogens, only head smut (*Tilletia maclaganii*) (Thomsen et al. 2008), anthracnose (*Colletotrichum navitas*) (Crouch et al. 2009), and rust (*Puccinia emaculata*) (Skyes et al. 2016) have been observed to reduce switchgrass biomass. Although three other rust pathogens, *P. graminis*, *P. huberi*, and *Uromyces graminicola* have been reported on switchgrass (Arthur 1934, Gravert and Munkvold 2002, Ramachar and Cummins 1963, 1965, Cummins 1971), *P. emaculata* is the most widely distributed across the U.S. (Frazier et al. 2013, Hagan and Atridge 2013, Hirsch et al. 2010, Lenné 1990, Gilley et al. 2013, Gravert and Munkvold 2002, Zale et al. 2008). Rust resistance variation among switchgrass cultivars has been reported (Gustafson et al. 2003, Jacobs et al. 2004, Li et al. 2009, Uppalapati et al. 2013). These studies focused on either agronomic cultivars/populations (Gustafson et al. 2003, Uppalapati et al. 2013) or

[1]Received for publication May 8, 2019; in revised form August 26, 2019. The authors gratefully acknowledge research funding from TN AgResearch, Knoxville, TN 37996.

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ornamental cultivars (Jacobs et al. 2004) and rust severity rating observations were reported on a single date. These single, end-of-season ratings (from 1 to 9) were used to indicate whether cultivars were resistant or susceptible (McNeal et al. 1971). However, as a polycyclic epidemic, these ratings cannot provide information about the latent period, which is a crucial index of host resistance for the pathogen with a secondary disease cycle. In addition, these studies did not take account the potential of different genotypes (races) of rust occurring in geographic areas in different years. Differences in rust susceptibility were considered to be a result of differing genetics in the host without attention given to the possibility of variable genetics within rust populations. Therefore, the objective of this study was to differentiate the variability of rust resistance / tolerance in 12 switchgrass cultivars including ornamental, lowland and upland cultivars using 40 rust isolates collected throughout the southeastern U.S. in a lab setting. This study would also provide insight into the variation among rust isolates for the ability to rapidly reproduce after developing a food relationship (successful infection) with the host.

Material and Methods

Switchgrass cultivars. Eleven cultivars of *Panicum virgatum* were used, including 'Kanlow' (lowland switchgrass cultivar), 'Summer' and 'Cave-In-Rock' ('CIR') (upland switchgrass cultivars), and eight ornamental cultivars ('Badland', 'Cheyenne Sky', 'Cloud Nine', 'Dallas Blue', 'Dewey Blue', 'Prairie Sky', 'Thundercloud' and the wild type *P. virgatum*). The seeds of the three agronomic switchgrass cultivars were obtained from the U.S. National Plant Germplasm System (NPGS: https://www.ars-grin.gov/npgs/) and 10 seedlings for each cultivar were randomly selected from germinated seeds and grown in each 4 L (1 gal) pots. The ornamental switchgrass cultivars, 10 pots for each cultivar, were ordered from Hoffman Nursery (Rougemont, NC). Plants were kept in the greenhouse at 25 C (77 F) with a 16-h light/8-h dark cycle. Randomly chosen leaf segments, 4 cm (1.6 in) long from each cultivar, were used to evaluate spore germination, latent period, and urediniospore production.

Fungal isolates collection and confirmation. Forty isolates of switchgrass rust, collected across the southeastern U.S., were used in the study (Table 1). All rust isolates were confirmed to be *P. emaculata* using morphological characters and molecular assays. The molecular confirmation was conducted by extraction of rust genomic DNA with DNeasy Plant Mini Kit (Qiagen, Valencia, CA) according to manufacturer's instructions, followed by amplifying ITS sequences with forward primer ITS1-rustF10d (5′ TGAACCTGCAGAAGGATCATTA 3′) (Barnes and Szabo 2007, Uppalapati et al. 2013) and reverse primer RUST1 (5′ GCTTACTGCCTTCCT CAATC 3′) (Kropp et al. 1995, Uppalapati et al. 2013). PCR assays were conducted in a 25 µl reaction volume with diluted DNA 2 µl (5 ng/ µl), GoTaq master mix (Promega, Madison, WI) 10 µl, each of the primers 1.5 µl, dimethyl sulfoxide (DMSO) 1.5 µl, and autoclaved double distilled water 8.5 µl. The assays were performed in a thermo cycler (Eppendorf AG, Hamburg, Germany) with the following conditions: 95 C for 5 min followed by 35 cycles of 95 C for 30 s, 50 C for 30 s, 72 C for 120 s, and a final extension at 72 C for 10 min. Five microliters of PCR products were electrophoresed with 1% agarose gel and visualized with a 2000 Gel Documentation System (BIO-RAD, Hercules, CA). The PCR products were directly sent to a molecular cloning laboratory (MCLAB, San Francisco, CA) for cleaning and sequencing. The sequencing results were then compared and confirmed to be *P. emaculata* ITS sequences with the Basic Local Alignment Search Tool (BLAST) in the GenBank database from the National Center for Biotechnology Information (NCBI: https://www.ncbi.nlm.nih.gov/) website.

Inoculum and inoculation. To enhance genetic purity of these isolates, a single pustule from each collected rust sample was isolated and urediniospores from that pustule

Table 1. Location and cultivar of switchgrass (*Panicum virgatum* L.) that each isolate of *Puccinia emaculata* (total 40) was collected from. Cultivars (bold) are clonal-nursery derived plants. The switchgrass cultivars in bold indicates the ornamental cultivars.

Rust Isolate	Switchgrass Cultivar	Location	Collected Year
AR-01	**Dallas Blue**	Fayetteville, AR	2008
LA-0902	**Dallas Blue**	Mobile, AL	2009
MS-09099	Alamo	MSU research plot, MS	2009
MS-0911	Cycle 2	MSU research plot, MS	2009
NC-01	**Heavy Metal**	Hoffman Nursery, NC	2008
NC-03	**Northwind**	Hoffman Nursery, NC	2008
NC-05	**Cloud Nine**	Hoffman Nursery, NC	2008
TN03	**Dewey Blue**	Knoxville, TN	2008
TN 10-4	Alamo	Madisonville, TN	2010
TN 10-5	Alamo	Madisonville, TN	2010
TN 10-6	Alamo	Madisonville, TN	2010
TN-0918	**Northwind**	Knoxville, TN	2009
TN-0919	**Dallas Blue**	Knoxville, TN	2009
TN-0920	**Thundercloud**	Oak Ridge, TN	2009
TN-0921	Alamo	Knoxville, TN	2009
TN-0922	**Rotstrahlbusch**	Crossville, TN	2009
TN-0923	**Dallas Blues**	Jackson, TN	2009
TN-0924	**Rotstrahlbusch**	Jackson, TN	2009
TN-0925	**Northwind**	Knoxville, TN	2009
TN-0926	Alamo	Knoxville, TN	2009
GA12-01	**Dallas Blues**	Atlanta, GA	2012
GA12-02	**Dallas Blues**	Atlanta, GA	2012
GA12-03	**Dallas Blues**	Atlanta, GA	2012
GA12-04	**Dallas Blues**	Atlanta, GA	2012
SC12-01	Alamo	Florence, SC	2012
SC12-02	Alamo	Florence, SC	2012
SC12-03	Alamo	Florence, SC	2012
SC12-04	Alamo	Florence, SC	2012
AL-BL	**Badland**	Brewton, AL	2012
AL-C9	**Cloud Nine**	Brewton, AL	2012
AL-CS	Unknown	Brewton, AL	2012
AL-DaB	**Dallas Blue**	Brewton, AL	2012
AL-DeB	**Dewey Blue**	Brewton, AL	2012
AL-HM	**Heavy Metal**	Brewton, AL	2012
AL-NW	**Northwind**	Brewton, AL	2012
AL-PF	Unknown	Brewton, AL	2012
AL-PS	**Prairie Sky**	Brewton, AL	2012
AL-PV	***Panicum virgatum***	Brewton, AL	2012
AL-SH	**Shenandoah**	Brewton, AL	2012
AL-TC	**Thundercloud**	Brewton, AL	2012

were used to inoculate leaf segments of the susceptible cultivar 'Thundercloud' in Petri dishes containing moistened filter paper. The inoculated leaf blades were incubated in the laboratory at 20 ± 2 C (68 F) with 10 h light per 24 h (four 40W residential fluorescent bulbs from 45 cm above leaf segments). Every two weeks, fresh leaf segments would be inoculated with urediniospores from the previous culture and then incubated for a two-week interval. Surplus urediniospores that were not used to inoculate new cultures were harvested with a small paint brush and stored in the freezer (-20 C) for later usage.

Leaf segments of different switchgrass cultivars were inoculated with separate suspensions of 40 rust isolates in concentration of 1×10^5 spores·ml^{-1} of water-Tween 20 (0.001% v/v) with fingertip sprayers (Lehman and Shaner 1997). Final deposition of spores on the leaf segments were approximately three spores per mm^2 by measuring the spore density on 1.5% water agar on glass slides setting alongside with leaf segments during inoculation. For each host cultivar and rust isolates combination, three inoculated leaf segments were placed on two layers of moistened paper towels with adaxial surface up in 9-cm-diameter petri dishes. The petri dishes were incubated in laboratory at 20 ± 2 C with 10 h light per 24 h as described above. Percentages of spore germination, latent period, number of uredia·cm^{-2} of leaf tissue, and number of urediniospores per uredium were measured.

Germination of urediniospore. A urediniospore was considered germinated when a germ-tube length was at least half the width of a urediniospore. At 12 h after inoculation (HAI), the percentage of germinated urediniospores was assessed on 10 random samples of 10 conidia for each sample on leaf segment.

Latent period. Latent period is defined as the time between when infection process begins (appressorium formation) and the uredia are first observed on inoculated leaf pieces. Uredia formation was examined on leaf pieces under a dissecting microscope daily after inoculation.

Uredium and urediniospore production. Twenty-one days after inoculation, for each host cultivar and rust isolate combination, three 1 cm^2 areas on leaf segments were randomly chosen and the numbers of uredia were counted using a stereo microscope. For each of the 1 cm^2 areas, 5 uredia were randomly picked and transferred into a 50µl water and 0.001% Tween 20 mixture. The urediniospore numbers were measured using a hemacytometer with a compound microscope and the average number of urediniospore produced by a uredium was calculated. For each of the 1 cm^2 areas, the average number of urediniospores in a uredium experiment was repeated four times. And the average number of urediniospores produced per 1 cm^2 leaf segment was calculated by multiplying the number of uredia per square centimeter by the average urediniospores number per uredium.

Statistical analysis. The whole experiment described above was repeated two times. The data were analyzed as a randomized complete block design with samples. The repeated experiments were considered a block effect. The experimental unit was a leaf segment, and the measurements coming from three 1-cm^2 areas on the leaf were considered as samples. The blocks were considered as random effects, and treatments were considered as fixed effects. After transformation of the data (arcsine for percentage data and common logarithm for count data), the effects of block, switchgrass cultivars, rust isolates, and their interactions on conidia germination, latent period, conidia production were determined by analysis of variance (ANOVA), using the Mixed model procedure of SAS, version 9.3 (SAS Institute Inc. 2011). Mean separation for each variable were conducted with the Tukey's HSD test at $\alpha = 0.05$ using the PDMIX800 macro (Saxton 1998). The effects of switchgrass cultivar by rust isolate interaction were presented by heatmap using package 'gplots' in R.

Table 2. The percentage germination rate of switchgrass rust (*Puccinia emaculata*) on 11 switchgrass cultivars (*Panicum virgatum* L.) 24 h after inoculation. Different letters in letter group indicate the effects are different according to Tukey's HSD test at $\alpha = 0.05$.

Cultivar	Germination Rate	Standard Error	Letter Group
Badland	0.9999	0.05517	A
Dallas Blue	0.9997	0.05517	A
Thundercloud	0.9991	0.05517	AB
Cheyenne Sky	0.9985	0.05517	ABC
Summer	0.9983	0.05517	ABC
P. virgatum	0.9975	0.05517	ABC
Cave-In-Rock	0.9936	0.05954	ABCD
Kanlow	0.9901	0.05517	BCD
Dewey Blue	0.9890	0.05517	CD
Prairie Sky	0.9879	0.05517	CD
Cloud Nine	0.9800	0.05517	D

Results and Discussion

Forty rust isolates were identified as *Puccinia emaculata* with blasting ITS sequences from the NCBI website and looking at morphological characters. No isolate was identified as *Uromyces graminicola*, which is a common rust on switchgrass in northeastern states.

Significant differences in germination percentage of switchgrass rust were observed among switchgrass cultivars ($p < 0.0001$), which ranged from 99.99% (Badland) to 98% (Cloud Nine) (Table 2). Significant differences were also found among 40 switchgrass rust isolates for their germination percentage ($p < 0.0001$) (data not shown). Isolate NC01 had the highest germination rate with 99.95% and MS0911 had the lowest with 96.73% (data not shown). Although differences in urediniospore germination percentage may be negligible in affecting the rate of an epidemic, germination percentage can be used metric to separate switchgrass cultivars for resistance or for the virulence of rust isolates. Furthermore, the interactions between switchgrass cultivars and rust isolates were also significant ($p < 0.0001$) (data not shown).

Significant differences in latent period were observed among switchgrass cultivars ($p < 0.0001$). The ornamental cultivar Cloud Nine had the shortest latent period (9.8 days) and another ornamental cultivar Cheyenne Sky has

Table 3. The latent period of switchgrass rust (*Puccinia emaculata*) in days on 11 switchgrass cultivars (*Panicum virgatum* L.). Different letters in letter group indicate the effects are different according to Tukey's HSD test at $\alpha = 0.05$.

Cultivar	Latent Period	Standard Error	Letter Group
Cheyenne Sky	14.4	0.003171	A
P. virgatum	12.6	0.003171	B
Kanlow	12.0	0.003171	C
Badland	11.9	0.003171	C
Dewey Blue	11.9	0.003171	C
Thundercloud	11.4	0.003171	D
Prairie Sky	10.3	0.003171	E
Dallas Blue	9.8	0.003171	F
Summer	9.8	0.003171	F
Cave-In-Rock	9.4	0.003171	G
Cloud Nine	9.2	0.003171	H

the longest latent period (14.4 days) (Table 3). From the perspective of latent period, there was no difference between agronomic and ornamental cultivars. However, among agronomic cultivars, the lowland cultivar Kanlow had a significantly longer latent period when compared to latent periods of the upland cultivars Summer and CIR. In addition, significant differences in latent period were also found among rust isolates ($p < 0.0001$), which range from 12.2 days (MS0911) to 10.1 days (TN0803) (Table 4). Furthermore, the interactions between switchgrass cultivars and rust isolates were also significant ($p < 0.0001$) (Fig. 1). For instance, changing from rust isolate AL-DaB to TN0924, on the cultivar Prairie Sky resulted in a decrease in latent period whereas the latent period increased when changing between the two rust isolates on the cultivar Summer. The significant interaction effects suggested race-specific resistances in these switchgrass cultivar-rust isolate combinations with longer latent period, such as the Cheyenne Sky and MS0911 combination, Dallas Blue and AL-NW combination, etc. (Fig. 1).

Average numbers of pustules per cm^2 produced on host leaf surfaces were significantly different among switchgrass cultivars ($p < 0.0001$), where numbers of pustules per cm^2 ranged from 29.5 (Summer) to 3.6 (Prairie Sky) (Table 5). Overall, ornamental cultivars had smaller number of pustules per cm^2 when compared to the number of pustules per cm^2 on agronomic cultivars. In addition, significant differences in number of pustules per cm^2 varied for rust isolates ($p < 0.0001$) and ranged from 22.8 (TN0920) to 13.7 (AR01) (Table 6). Interactions between switchgrass cultivars and rust isolates were also significant ($p < 0.0001$) (Fig. 2). For instance, changing from rust isolate AL-BL to TN10-4, on CIR, the number of pustules per cm^2 decreased while the numbers increased on Dallas Blue. The significant interaction effects also suggested there might be some race-specific resistances in these switchgrass cultivar-rust isolate combinations with smaller number of pustules per cm^2, such as Cheyenne Sky and MS0911 combination, Dallas Blue and AL-NW combination, etc. (Fig. 2). These findings were in coincidence with results in the previous latent period experiment (Fig. 1).

Significant differences in number of urediniospores per cm^2 were observed on leaves among switchgrass cultivars ($p < 0.0001$), which ranged from 44,228.3 (Cloud 9) to 653.1 (Prairie Sky) (Table 7). Overall, ornamental cultivars produced less urediniospores per cm^2 compared with agronomy cultivars. In addition, significant differences in number of urediniospores per cm^2 which each rust isolates

Table 4. The latent period of 40 switchgrass rust isolates (*Puccinia emaculata*) in days on switchgrass (*Panicum virgatum* L.). Different letters in letter group indicate the effects are different according to Tukey's HSD test at $\alpha = 0.05$.

Isolate	Latent Period	Standard Error	Letter Group
MS0911	12.2	0.004281	A
AL-PV	11.7	0.004281	AB
NC05	11.6	0.004281	BC
AL-PF	11.5	0.004281	BCD
MS0909	11.4	0.004281	BCDE
LA0902	11.4	0.004281	BCDE
AL-PS	11.4	0.004281	BCDEF
AL-NW	11.3	0.004281	BCDEFG
NC01	11.3	0.004281	BCDEFGH
GA1202	11.3	0.004281	BCDEFGHI
SC1203	11.2	0.004281	BCDEFGHIJ
NC03	11.2	0.004281	BCDEFGHIJ
TN0924	11.2	0.004281	CDEFGHIJ
GA1204	11.1	0.004281	CDEFGHIJ
TN0918	11.1	0.004281	DEFGHIJK
GA1201	11	0.004281	EFGHIJKL
AL-BL	11	0.004281	EFGHIJKL
AR01	11	0.004281	EFGHIJKL
AL-HM	10.9	0.004281	FGHIJKLM
SC1202	10.9	0.004281	GHIJKLM
TN0926	10.9	0.004281	GHIJKLM
AL-CS	10.9	0.004281	GHIJKLM
TN10-6	10.9	0.004281	GHIJKLM
TN0923	10.9	0.004281	GHIJKLM
AL-TC	10.9	0.004281	HIJKLM
AL-DaB	10.8	0.004281	HIJKLM
TN0922	10.8	0.004281	HIJKLM
TN0919	10.8	0.004281	IJKLM
AL-DeB	10.8	0.004281	JKLMN
AL-C9	10.8	0.004281	JKLMN
TN0925	10.7	0.004281	JKLMN
TN0921	10.7	0.004281	JKLMN
GA1203	10.6	0.004281	KLMNO
TN10-4	10.5	0.004281	LMNO
TN0920	10.5	0.004281	LMNO
TN10-5	10.5	0.004281	MNOP
AL-SH	10.5	0.004281	MNOP
SC1201	10.3	0.004281	NOP
SC1204	10.3	0.004281	OP
TN0803	10.1	0.004281	P

Table 5. Number of pustules per cm^2 of switchgrass rust (*Puccinia emaculata*) on 11 switchgrass cultivars (*Panicum virgatum* L.). Different letters in letter group indicate the effects are different according to Tukey's HSD test at $\alpha = 0.05$.

Cultivar	Pustules·cm^{-2}	Standard Error	Letter Group
Summer	29.4875	0.3574	A
Cave-In-Rock	24.4458	0.3574	B
Dewey Blue	22.5500	0.3574	C
Badland	21.8667	0.3574	C
Dallas Blue	21.6917	0.3574	C
Thundercloud	19.7958	0.3574	D
Kanlow	17.5792	0.3574	E
Cheyenne Sky	12.4500	0.3574	F
P. virgatum	12.2042	0.3574	F
Cloud Nine	9.2792	0.3574	G
Prairie Sky	3.5875	0.3574	H

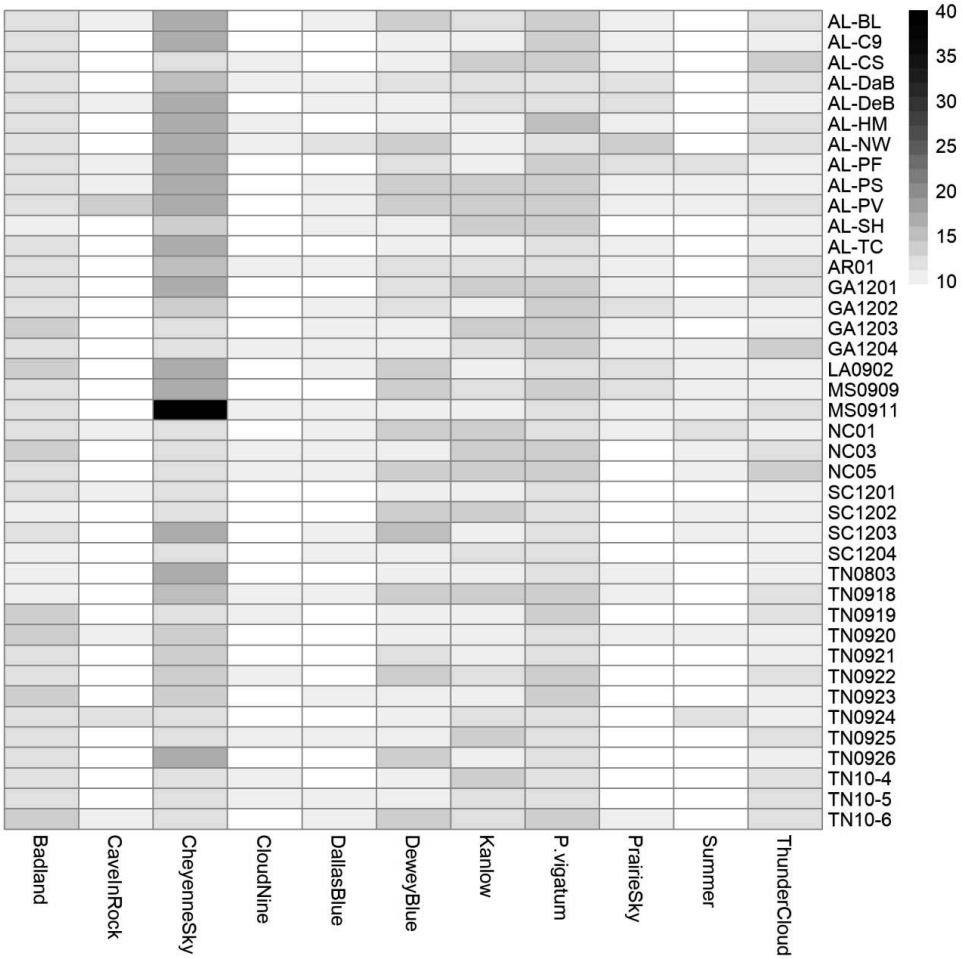

Fig. 1. Black and white heatmap for the effects of switchgrass cultivar (x axis) and rust isolate (y axis) interaction on latent period for rust on switchgrass leaves. Darker colors indicate which switchgrass cultivar and rust isolate combinations have longer latent periods than those combinations with light colors.

produced were also found ($p < 0.0001$), ranging from 20,835.3 (NC03) to 3250.9 (AL-PS) (Table 8). Furthermore, the interactions between switchgrass cultivars and rust isolates were also significant ($p < 0.0001$) (Fig. 3). For instance, changing from rust isolate TN0920 to TN10-4, on CIR, the number of urediniospores per cm^2 increased while they decreased on Cheyenne Sky. The significant interaction effects suggested there might be some race-specific resistances in switchgrass cultivar-rust isolate combinations with a smaller number of urediniospores per cm^2, such as Cheyenne Sky and TN10-4 combination, Cloud Nine and MS0909 combination, etc. (Fig. 3), although these combinations might have a shorter latent period (Fig. 1) and a larger number of pustules per cm^2 (Fig. 2).

Although four rust species, *P. emaculata*, *P. graminis*, *P. huberi*, and *U. graminicola*, were reported to infect switchgrass (Arthur 1934, Gravert and Munkvold 2002, Ramachar and Cummins 1963, 1965, Cummins 1971), *P. emaculata* is the most widely distributed across the USA. The urediniospores of *P. graminis* and *P. huberi* are oval while the urediniospores *P. emaculata* and *U. graminicola* of are globose in shape and are most identical and therefore, cannot be used to differentiate these two species (Kenaley et al. 2016). Teliospore morphology can be used to separate *P. emaculata* and *U. graminis* since teliospores of *P. emaculata* have one cell whereas teliospores of *U. graminis* have two cells. All 40 isolates, collected from the southeastern US, were morphologically and molecularly confirmed as *P. emaculata* and none as *U. graminicola*. This finding corresponds with the statement that *U. graminicola* on switchgrass is usually more common in northern states and rarely found in southern states (Kenaley et al. 2016). In northern states, such as New York, Iowa, Nebraska and South Dakota, mixtures of these two species of rust fungi have been detected on switchgrass.

Successful spore germination and hyphal penetration are the first two steps in the pathogen infection process, which sounds simple and easy but has tremendous signal interactions between the pathogen and the hosts. The infection process continues when fungal spores detect and recognize the biochemical, topographical and thigmotropic signals of the host surfaces (Hoch et al. 1987, Mellersh and Heath 2003). In our rust and switchgrass interaction complex, although variations in rust germination percentage were observed among different switchgrass cultivars, the smallest germination percentage was still high (98% on Cloud Nine). No significant differences were identified among rust isolates for their germination. Although these

Table 6. Number of pustules per cm^2 of 40 switchgrass rust isolates (*Puccinia emaculata*) on switchgrass cultivars (*Panicum virgatum* L.). A different letter in letter group indicates the isolates are different according to Tukey's HSD test at $\alpha = 0.05$.

Isolate	Pustules·cm^{-2}	Standard Error	Letter Group
TN0920	22.7639	0.6525	A
GA1204	22.4861	0.6525	AB
AL-SH	22.0833	0.6525	ABC
NC01	22.0833	0.6525	ABC
AL-BL	21.6667	0.6525	ABCD
NC03	21.5417	0.6525	ABCDE
TN0921	21.4722	0.6525	ABCDEF
TN0919	21.3889	0.6525	ABCDEF
TN0922	21.3611	0.6525	ABCDEF
AL-C9	21.0417	0.6525	ABCDEFG
AL-CS	20.6528	0.6525	ABCDEFGH
GA1202	20.3889	0.6525	ABCDEFGH
TN0924	19.5000	0.6525	ABCDEFGHI
MS0911	19.3889	0.6525	ABCDEFGHIJ
NC05	19.2083	0.6525	ABCDEFGHIJ
TN0918	19.2083	0.6525	ABCDEFGHIJ
SC1201	19.0694	0.6525	BCDEFGHIJK
AL-TC	18.7361	0.6525	CDEFGHIJKL
GA1203	18.6806	0.6525	CDEFGHIJKL
TN10-5	18.5278	0.6525	CDEFGHIJKL
AL-DeB	18.4444	0.6525	DEFGHIJKL
TN10-4	18.2778	0.6525	DEFGHIJKL
SC1202	18.2500	0.6525	DEFGHIJKL
MS0909	18.1111	0.6525	DEFGHIJKL
GA1201	18.0417	0.6525	EFGHIJKL
SC1204	17.9306	0.6525	FGHIJKL
TN0925	17.8889	0.6525	FGHIJKL
AL-PF	17.6111	0.6525	GHIJKLM
TN0923	17.4583	0.6525	GHIJKLM
AL-NW	17.3194	0.6525	HIJKLM
TN10-6	17.2917	0.6525	HIJKLM
SC1203	16.7500	0.6525	IJKLMN
TN0803	16.7361	0.6525	IJKLMN
AL-HM	16.5278	0.6525	IJKLMN
AL-PV	16.3194	0.6525	IJKLMN
TN0926	15.8333	0.6525	JKLMN
AL-DaB	15.4861	0.6525	KLMN
LA0902	15.1528	0.6525	LMN
AL-PS	14.2500	0.6525	MN
AR01	13.6806	0.6525	N

Table 7. Number of urediniospores per cm^2 of switchgrass rust (*Puccinia emaculata*) produced on 11 switchgrass cultivars (*Panicum virgatum* L.). Different letters in letter group indicate the effects are different according to Tukey's HSD test at $\alpha = 0.05$.

Cultivar	Spores·cm^{-2}	Standard Error	Letter Group
Cloud Nine	44228.3	0.02691	A
Summer	24400.6	0.02724	BC
Kanlow	24165.7	0.02691	BC
Dallas Blue	20960.4	0.02691	C
Dewey Blue	9107.5	0.02691	D
Thundercloud	9099.1	0.02691	D
P. virgatum	7469.6	0.02691	D
Badland	6864.4	0.02691	D
Cheyenne Sky	2937	0.02691	E
Cave-In-Rock	2193.8	0.02691	F
Prairie Sky	653.1	0.02691	G

Table 8. Number of urediniospores per cm^2 of 40 switchgrass rust (*Puccinia emaculata*) isolates produced on switchgrass (*Panicum virgatum* L.). Different letters in letter group indicate the effects are different according to Tukey's HSD test at $\alpha = 0.05$.

Isolate	Spore·cm^{-2}	Standard Error	Letter Group
NC03	20835.3	0.04912	A
SC1204	18754.3	0.04912	AB
SC1202	18569.5	0.04912	AB
NC01	16734.0	0.04912	ABC
TN10-5	15925.8	0.04912	ABCD
TN0921	15268.6	0.04912	ABCD
AL-CS	14862.8	0.04912	ABCD
TN0922	14397.9	0.04912	ABCD
SC1201	14171.0	0.04912	ABCDE
AL-SH	13176.5	0.04912	ABCDEF
NC05	12661.9	0.04912	ABCDEF
AL-C9	12488.2	0.04912	ABCDEF
AL-TC	12053.1	0.04912	ABCDEFG
GA1202	12006.0	0.04912	ABCDEFG
GA1201	11587.8	0.04912	ABCDEFG
AL-DeB	11585.1	0.04912	ABCDEFG
TN0924	11574.4	0.04912	ABCDEFG
AL-BL	10122.8	0.04912	BCDEFGH
GA1204	9749.9	0.04912	CDEFGHI
AL-HM	9680.5	0.04912	CDEFGHIJ
MS0911	9458.0	0.04912	CDEFGHIJK
AR01	9447.1	0.04912	CDEFGHIJK
GA1203	9287.5	0.04912	CDEFGHIJKL
TN0926	8957.8	0.04912	CDEFGHIJKL
TN0919	8766.0	0.04912	DEFGHIJKL
TN10-6	8517.3	0.04912	DEFGHIJKL
AL-NW	7550.9	0.05113	EFGHIJKLM
TN0925	7481.7	0.04912	FGHIJKLM
TN0923	6532.8	0.04912	GHIJKLMN
LA0902	5917.0	0.04912	HIJKLMNO
AL-PF	5361.7	0.04912	IJKLMNO
TN0920	5277.2	0.04912	IJKLMNO
TN0803	5178.5	0.04912	JKLMNO
TN0918	5176.1	0.04912	JKLMNO
AL-DaB	5071.1	0.04912	KLMNO
SC1203	5047.8	0.04912	LMNO
TN10-4	4348.1	0.04912	MNO
MS0909	3934.6	0.04912	NO
AL-PV	3754.0	0.04912	NO
AL-PS	3250.9	0.04912	O

isolates were collected from different states, agronomical cultivars or ornamental cultivars, and some isolates had been in culture (leaf blades in petri dishes) for four years or longer than other isolates, we observed no difference in spore germination on leaf surfaces of agronomic and ornamental cultivars.

In wheat, 75 leaf rust (*P. triticina*) resistance (*Lr*) genes have been described (Singla et al. 2017). Although most *Lr* genes were related with race-specific resistance which has short durability due to the rapid evolution of new rust strains, some International Wheat and Maize Improvement Center (CIMMYT) released cultivars combining several slow-rusting genes and demonstrated near immune resistance response to wheat leaf rust (Singh and Rajaram 1991, Singh et al. 1998, 2005). A similar breeding strategy was also applied to control stripe rust and stem rust in wheat (Ellis et al. 2014, Singh et al. 2000). Selections for slow rusting can use the area under the disease progress curve (AUDPC) which measures disease severity or incidence

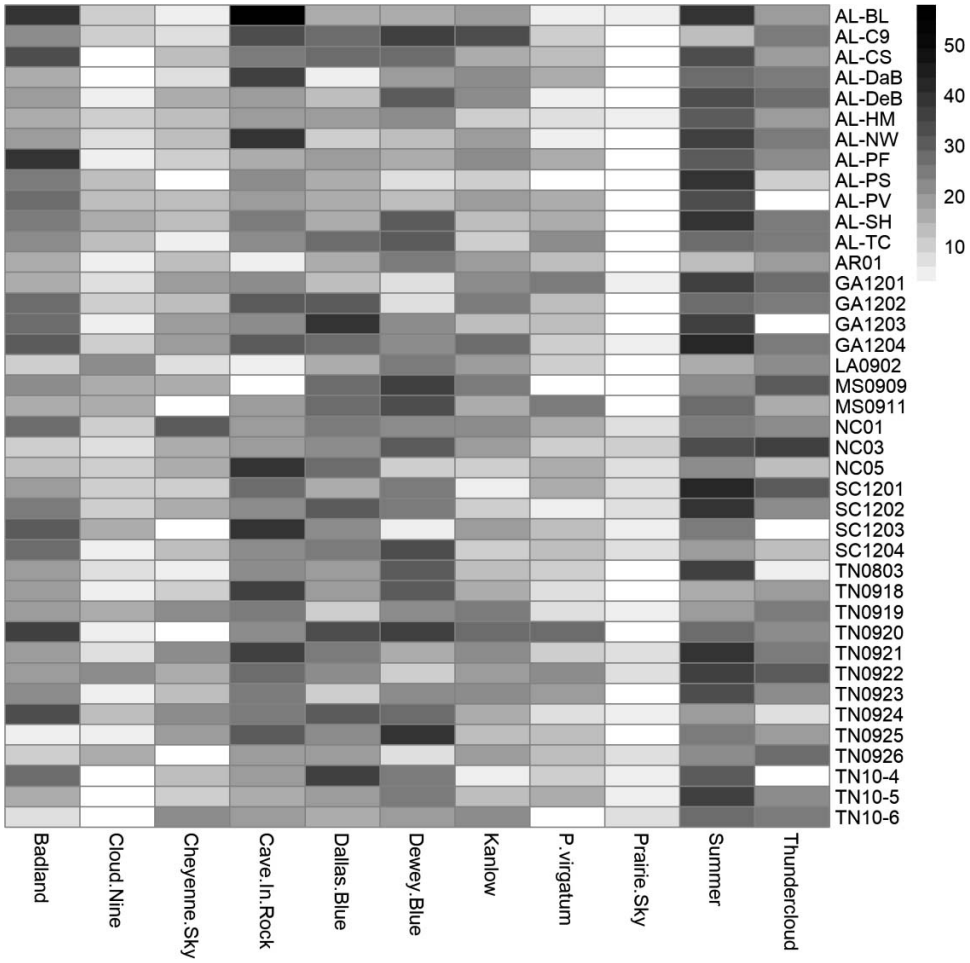

Fig. 2. Black and white heatmap for the effects of switchgrass cultivar (x axis) and rust isolate (y axis) interaction on number of rust pustules produced per cm^2 on switchgrass leaves measured on 21 days after inoculation. Darker colors indicate these switchgrass cultivar and rust isolate combinations have more of pustules per cm^2 than those combinations with light colors.

multiple times over the grown season in the field (Taye et al. 2014). With a significant negative correlation of latent period and AUDPC, using latent period can also assist in selecting slow-rusting resistant cultivars in the greenhouse (Johnson and Wilcoxson 1978, 1979, Singh et al. 2015).

Switchgrass rust is a polycyclic plant pathogen and the wind borne urediniospores can have multiple and repeated infections on the same host plant under favorable environmental conditions. Host cultivars with longer latent periods, an important aspect of slow rusting, would significantly reduce the number of cycles of infection, which would result in slower rates of disease development. To date, no study has identified resistance in switchgrass based on the latent period for rust development, although an increased latent period would contribute to durable resistance. Cultivars with a longer latent period, such as Cheyenne Sky and wild type *P. virgatum*, could be used as a genetic resource for slow rusting and provide slow rusting genes for rust resistance breeding in switchgrass. For agronomic cultivars, the lowland cultivar Kanlow showed a significantly increased latent period than was observed for upland cultivars Summer and CIR (Table 3). These observations match the findings that upland cultivars were more susceptible to rust than lowland cultivars (Cornelius and Johnston 1941, Gustafson et al. 2003, Uppalapati et al. 2013).

Variations were observed among 40 rust isolates in latent period, number of uredia and urediniospores produced per cm^2. Some isolates are more virulent than others with significantly shorter latent period (TN0803, SC1204), more numbers of uredia per cm^2 (TN0920, GA1204), or more number of urediniospores produced per cm^2 (NC03, SC1204). Although no clear pattern was observed to group these isolates by different collecting years or locations, genetic variations among 40 rust isolates and cultivar-specific interactions between switchgrass and rust were observed. With some simple sequence repeats (SSRs) (Wadl et al. 2011), the population genetics of switchgrass rust isolates could be studied to understand the genetic diversity, gene flow, origin and epidemiology of the rust.

The present study is the first report that germination percentage, latent period, number of uredia and urediniospores produce per cm^2 leaf surface of rust isolates is variable on different agronomic and ornamental switchgrass cultivars. This continuous resistance throughout the whole infection process on switchgrass cultivars other than the single overall end-season disease rating value could

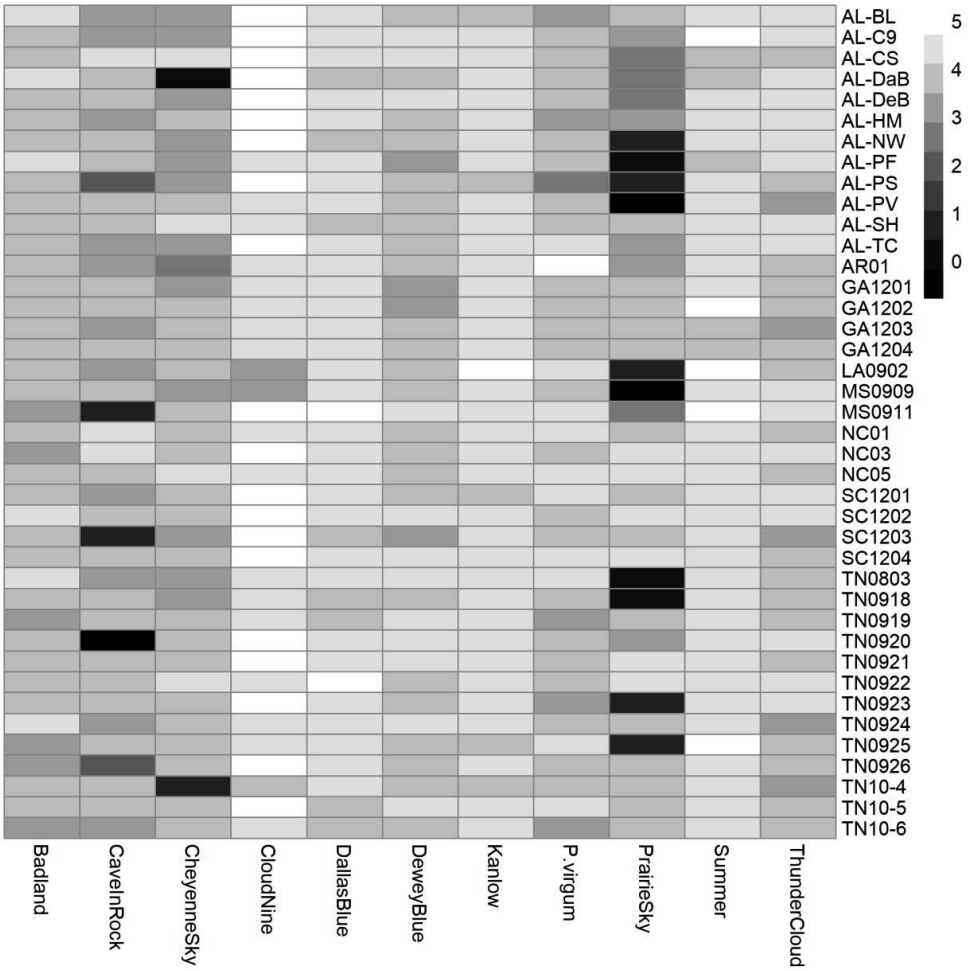

Fig. 3. Black and white heatmap for the effects of switchgrass cultivar (x axis) and rust isolate (y axis) interaction on number of urediniospores produced per cm^2 (log10 transformed) on switchgrass leaves measured on 21 days after inoculation. Darker colors indicate these switchgrass cultivar and rust isolate combinations have less urediniospores per cm^2 than those combinations with light colors.

provide more details and new resources for developing rust-resistant switchgrass cultivars to achieve sustainable control of switchgrass rust. On the other hand, before the new rust resistant switchgrass cultivars are developed or released, the high genetic diversity in rust population should be kept in mind, otherwise rust resistant cultivars would be short lived due to variation for virulence in rust populations.

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