CROP BREEDING, GENETICS & CYTOLOGY

Genetic Variation for *Puccinia emaculata* Infection in Switchgrass

D. M. Gustafson, A. Boe,* and Y. Jin

ABSTRACT

Seed yields, forage quality, and biomass of switchgrass (*Panicum virgatum* L.) can be negatively impacted by diseases. Genetic vulnerability is a concern when one switchgrass cultivar is grown in monoculture for long periods of time. Thus, genetic variation is key to improvement in pest resistance through selection. Our objectives were to develop a rust rating system for *Puccinia emaculata* Schwein and to determine genetic variation for *P. emaculata* infection among and within four improved switchgrass populations from the northern and central Great Plains. Populations were evaluated in replicated family-row nurseries at two locations (Aurora and Kimball) in eastern South Dakota. Disease ratings were taken after heading in 2000 and 2001 utilizing a 0 (highly resistant) to 9 (highly susceptible) scale. Population mean rust ratings averaged across families and years ranged from 3.7 to 8.0, and 3.2 to 5.0, at Aurora and Kimball, respectively. Significant variation among populations and among and within families within populations was observed for disease ratings. Data suggested the presence of both additive and nonadditive genetic variation and indicated selecting the best individuals from the best families for resistance to the rust disease may be an effective approach in utilizing genetic variation for improvement in switchgrass for the northern and central Great Plains.

Switchgrass, a perennial warm-season grass native to a large portion of North America, is used for forage production, erosion control, and as a renewable biomass energy source. Seed yields, forage quality, and biomass of switchgrass can be negatively impacted by diseases (Gravert et al., 2000). Many fungal diseases have been reported to affect switchgrass. According to Farr et al. (1989), 42 species of fungi on switchgrass have been identified in the United States, 13 of which have been reported in Iowa as of 1999 (Gravert and Munkvold, 2002). Three of the fungal diseases are caused by rust fungi: *Uromyces graminicola* Burrill, *Puccinia graminis* Pers.: Pers., and *P. emaculata* Schwein.

Several studies have examined the effects of rust fungi on switchgrass. Cornelius and Johnston (1941) evaluated 34 accessions of switchgrass from the Great Plains and found collections from North Dakota and Nebraska were highly susceptible to *U. graminicola.* On the other hand, collections from lowland in Oklahoma and southern Texas were highly resistant. Barnett and Carver (1967) later classified the taller, coarser-stemmed plants from the south, which are generally more rust resistant, as lowland types and the short, finer-stemmed plants from the north, which are generally more rust susceptible, as upland types.

Eberhart and Newell (1959) recorded degrees of rust infection for *P. virgatum* utilizing a 1-to-9 scale. On the basis of collections obtained throughout Nebraska, it was determined that heritability estimates for rust infection ranged from 0.96 in green type plants to 0.00 in blue-green type plants. Heritability estimates from another study conducted by Newell and Eberhart (1961) showed significant additive genetic variation, indicating progress could be made by selection within superior strains of the grass.

More recently, the rusts *P. emaculata*, *P. graminis*, and *U. graminicola* have been reported with switchgrass in Iowa (Gravert and Munkvold, 2002). However, severe outbreaks have not been common in that area to date. Rust infection for *P. emaculata* was not present at a high incidence during 1999 in Iowa, but appeared to be more prevalent during 2000 (Gravert and Munkvold, 2002). *U. graminicola* and *P. panici* Diet. (*P. emaculata*) have also been listed as occurring on switchgrass in South Dakota (Mankin, 1969).

During 2000 and 2001, switchgrass at several locations in South Dakota was infected with a high incidence of *P. emaculata* (= *P. panici*), identified by the morphological characters of the species. The teliospores of *P. emaculata* are two-celled and pedicel is brown. In comparison, teliospores of *U. graminicola* are one-celled and the pedicel is nearly colorless (Arthur, 1934, p. 127–129).

Considering that typically one switchgrass cultivar is grown in an area, genetic vulnerability is a concern for breeders. Also, stands of switchgrass normally remain in production up to 20 or more years; thus, failure to plant a high-yielding and disease-resistant cultivar may result in substantial losses to producers (Hopkins et al., 1995). Therefore, genetic variation is important for providing improvement in rust resistance through selection. Our objectives were to develop a rust rating system for *P. emaculata* and to determine genetic variability for *P. emaculata* disease response among and within four switchgrass populations with origins from either the northern or central Great Plains.

Plant Science Dep., South Dakota State Univ., Brookings, SD 57007-2141. This research was sponsored by the U.S. Dep. of Energy's Biomass Program through contract DE-AI05-900R21954 with Oak Ridge National Laboratory (ORNL). The ORNL is managed by UT-Battelle, LLC, for the U.S. Dep. of Energy under contract DE-AC05-00OR22725. South Dakota Agric. Exp. Stn. Journal Series No. 3310. Received 14 May 2002. *Corresponding author (arvid_boe@sdstate.edu).

Published in Crop Sci. 43:755–759 (2003).

MATERIALS AND METHODS

Plant Materials and Nursery Design

Half-sib families from switchgrass populations native to the northern and central Great Plains were transplanted at two locations in South Dakota during 2000. Fourteen families developed by Dr. K.P. Vogel, USDA-ARS, Lincoln, NE., 19 families developed by Dr. C. Taliaferro, Oklahoma State University, and 30 families from each of 'Summer' (Alderson and Sharp, 1994) and 'Sunburst' (Boe and Ross, 1998) were used in this research. We expected 'Cave-In-Rock' to be the most rust resistant cultivar that had potential for persistence and long-term biomass production in the northern Great Plains. Therefore, it was used as a check at both locations.

The experiment consisted of eight plants per family row and five plants per family row at Aurora and Kimball, SD, respectively, in randomized complete block designs with two replications. Inter- and intrarow spacings were 1 m and 30 cm, respectively. Data were collected in 2000 and 2001 at each location. Soil at Aurora is a Brandt (Fine-silty, mixed, superactive, frigid Calcic Hapludolls) and at Kimball an Eakin (fine-silty, mixed, mesic, Typic Argiustolls).

Infection Type Disease Rating System

A disease rating system for *P. emaculata* infection was developed based on the wheat stripe rust (*P. striiformis* f. sp. *Tritici)* system (McNeal et al., 1971, p. 42). Extensive work determining ratings for cereal rusts has provided model infection type systems. Standard scoring systems for stem and leaf rusts of cereals utilize a 0 to 4 infection type rating system or by observations of a percentage of possible tissue rusted. The wheat stripe infection type system is a model system for *P. emaculata* because it utilizes a 0 to 9 disease rating system, which is advantageous in genetic studies. In addition, *P. striiformis* pustules are linear and *P. emaculata* pustules also form lines between the veins. The primary symptom difference between the two rusts is that much less chlorosis and/or necrosis is observed to occur on switchgrass when infected with *P. emaculata*.

Healthy and diseased switchgrass leaves were collected, pressed, and analyzed to determine an appropriate 0 to 9 scale (Fig. 1). A description of the rating system is given in Table 1.

P. emaculata was prevalent in 2000 and 2001 at Aurora; however, no visible symptoms or signs were observed in 2000 at Kimball. Populations were in various stages of seed development (Moore et al., 1991) depending on their maturity at the time of disease assessment. Scoring of *P. emaculata* (0–9) was on a whole-plant basis (Roelfs et al., 1992, p. 46). Data were recorded during October of the two consecutive years and were subjected to analysis of variance utilizing a mixed model. Families were assumed to be fixed as they were previously selected for divergent morphological traits, biomass, and/or other agronomic traits. Replications and years were assumed to be random effects. Where needed, approximate *F* values with approximate degrees of freedom were calculated according to Satterthwaite (1946).

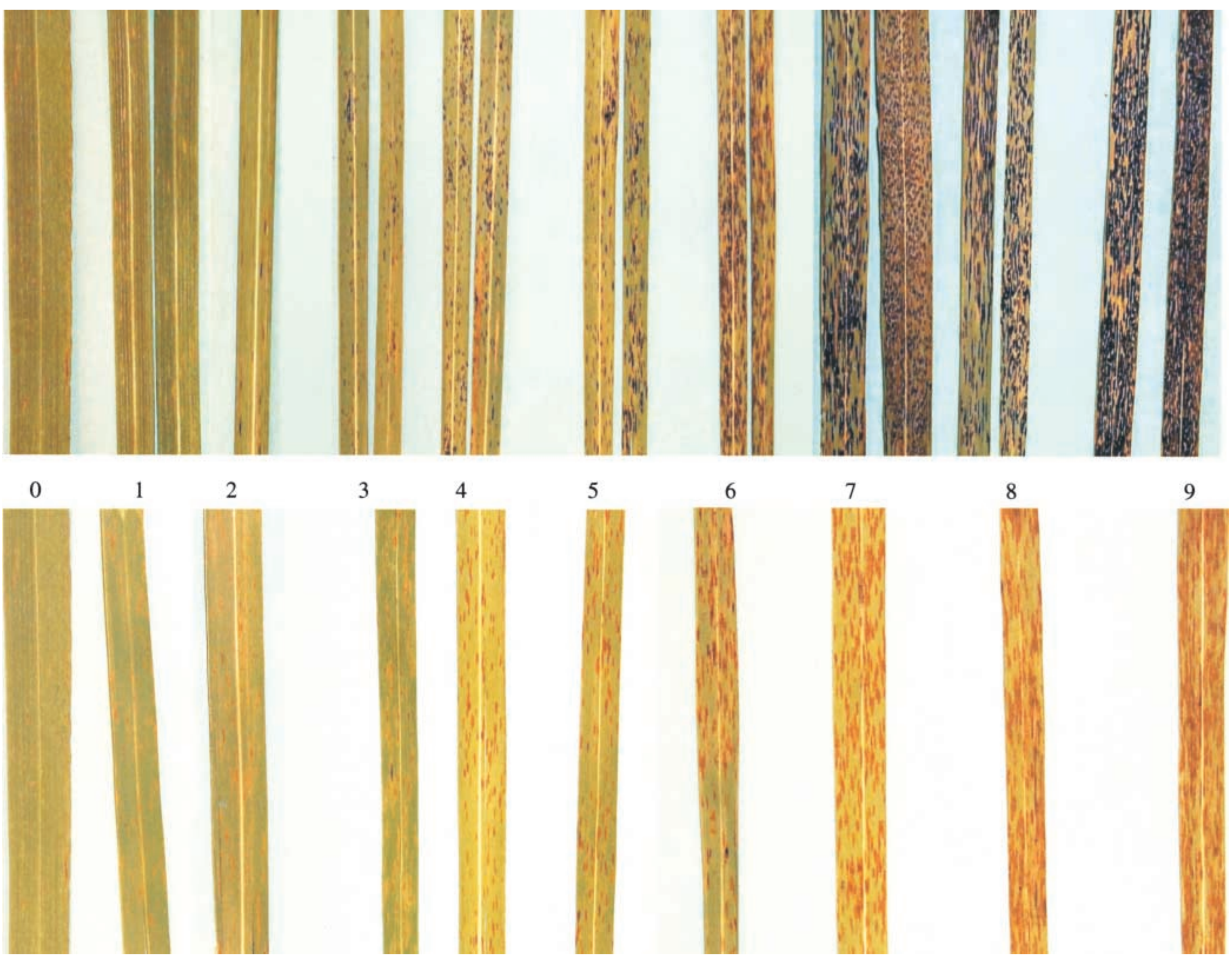

Fig. 1. Leaf blades representing the range in infection types for *Puccinia emaculata* telia (top) and uredinia (bottom) infesting switchgrass in eastern South Dakota.

Table 1. Host response and rust rating descriptions used in the switchgrass *Puccinia emaculata* system.†

Host response (Class)	Rust rating	Disease symptoms
Highly resistant	0	No visible symptoms.
Resistant	1	Light necrotic and/or chlorotic areas, no sporulation.
	2	Trace sporulation and/or light necrotic and/or chlorotic areas.
Moderately resistant	3	Trace-light sporulation, light necrotic and/or chlorotic areas.
	4	Light sporulation (spores may be present on sheath), light necrotic and/or chlorotic areas may/may not be present.
Moderately susceptible	5	Moderate sporulation (spores generally also present on sheath), necrotic/chlorotic areas may/may not be present.
	6	Moderate-heavy sporulation (spores generally present on sheath), necrotic/chlorotic areas may/may not be present.
Susceptible	7	Heavy sporulation (spores generally present on sheath, may be present on stems), necrotic/chlorotic areas generally not present.
	8	Heavy-abundant sporulation (spores generally present on sheath, may be present on stems), necrotic/chlorotic areas generally not present.
Highly susceptible	9	Abundant sporulation without necrosis/chlorosis, spores generally present on sheath, may be present on stems.

† On the basis of the wheat stripe rust system (McNeal et al., 1971, p. 42).

RESULTS AND DISCUSSION

Analysis of variance for rust ratings of individual plants within families of each population evaluated for 2 yr at Aurora is summarized in Table 2. Variation among families was significant ($P < 0.05$) for Summer and highly significant ($P < 0.01$) for Sunburst, indicating the presence of additive genetic variation for rust reaction within both cultivars. No significant differences were found among families within each of the Nebraska and Oklahoma populations. In addition, significant ($P < 0.01$) variation among individuals within all four populations suggested differences in rust reaction could also be influenced by nonadditive gene effects. The analysis of variance for rust ratings of individual plants within plots for the check cultivar Cave-In-Rock further emphasized this point. No significant variation was found among individuals within Cave-In-Rock plots, nor was the replication × year interaction significant (data not shown).

The family component of variance should be equal to one-fourth of the additive variance (Falconer, 1981). Therefore, the rest of the genetic variation within the four populations, additive and nonadditive, is due to differences among individuals within families. Significance of the family by replication and family × year × replication mean squares indicated that families failed to rank similarly across replications and family × replication plots failed to rank similarly across years. However, the changes in rank across years were predominantly within a host response/class (Table 1).

Population mean rust ratings (Table 3), averaged across families and years, ranged from 3.7 (moderately resistant) for Nebraska elite to 8.0 (susceptible) for Sunburst. In comparison, the mean averaged across years for Cave-In-Rock was 3.4. In 1999, Gravert and Munkvold (2002) found a prevalence of *P. emaculata* in 47% of the Cave-In-Rock biomass production fields sampled in south-central Iowa. However, a systematic survey determining specific rust ratings was not conducted.

The superior level of resistance in the Nebraska material and the apparent lack of additive genetic variation in that material for rust reaction is likely due to the fact that the Nebraska population was previously selected for low rust symptoms. (K.P. Vogel, Dec. 2001, personal communication). In addition, the origins of the Nebraska and Oklahoma elites are natural populations of more southern origin than populations from which Sunburst and Summer were developed, thus they would be expected to have a higher level of resistance to rust (Cornelius and Johnston, 1941; Newell and Eberhart, 1961). The significant among and within family components of variance for rust ratings in Sunburst and Summer at Aurora suggested that progress could be made by selecting best plants within best families for both of those populations. Despite the fact that Sunburst had high susceptibility, rust ratings of individual plants varied within families, ranging from 4 to 9 (data not shown). Therefore, some improvement in rust resistance in this population can be expected.

Although the four switchgrass populations were evaluated at Kimball during 2000 and 2001, *P. emaculata* was not observed there in 2000. However, moderate infection was observed in 2001. Mean rust ratings averaged across families ranged from 3.2 (moderately resistant) for Oklahoma elite to 5.0 (moderately susceptible) for Sunburst (Table 3). The mean rust rating for the

Table 2. Significance of pertinent mean squares from analysis of variance for rust ratings at Aurora (2000 and 2001) and Kimball (2001), SD.

	Mean Squares†							
	Aurora						Kimball	
Population/Cultivar	F	Y	F × R	F × Y	F × R × Y	I(F)	F	F × R
Nebraska	3.84	75.24	4.29	3.59	1.83**	1.08**	4.71**	4.30**
Oklahoma	16.54	2.39	11.09**	5.53*	2.03**	0.97*	4.98**	2.62**
'Summer'	10.42*	131.76	4.14*	2.26	1.69**	1.17**	6.43**	4.27**
'Sunburst'	4.72**	75.40	1.85	1.27	1.64**	0.73**	6.30**	5.52**

* Significant at the $P = 0.05$ level.
** Significant at the $P = 0.01$ level.
† F, Family; R, Replication; Y, Year; I(F), Individuals within Families.

Table 3. Mean rust ratings for four switchgrass populations and the Cave-In-Rock check at Aurora (averaged across 2000 and 2001) and Kimball (2001), SD.

	Rust rating†	
Population/Cultivar	**Aurora**	**Kimball**
Nebraska	**3.7 ± 0.20**	**3.8 ± 0.32**
Oklahoma	**5.2 ± 0.21**	**3.2 ± 0.20**
'Summer'	**7.3 ± 0.15**	**4.4 ± 0.17**
'Sunburst'	**8.0 ± 0.15**	**5.0 ± 0.19**
'Cave-In-Rock' (check)	**3.4 ± 0.08**	**4.3 ± 0.37**

† Mean ± SE.

check cultivar Cave-In-Rock was 4.3. Highly significant mean squares for among families and the family × replication interaction were observed (Table 2), indicating significant differences among average performances of families but also the failure of families to rank identically across replications.

Compared to Aurora, the observed mean rust ratings at Kimball were lower, except for the Nebraska population. The presence of another disease may have impacted the severity of *P. emaculata* during 2001 at Kimball. A leaf spot disease was observed on predominantly Sunburst, Summer, and the Oklahoma population. The symptoms included centrally bleached lesions surrounded by purple margins. The necrotic center was dotted with tiny black pycnidia. These symptoms and signs suggest *Selenophoma* sp., which has been reported on switchgrass in eastern South Dakota (Mankin, 1969), or *Phoma* sp., which is a common disease on cultivated switchgrass in Iowa (Gravert and Munkvold, 2002).

The presence of a competing disease could account for lower rust infection at Kimball compared with Aurora. Another possibility could be that environmental conditions were less favorable for rust infection. Kimball was warmer and drier than Aurora during the growing season of 2001. These environmental differences also affected biomass production. All four populations yielded ≈30% lower at Kimball compared with Aurora in 2001 (data not shown).

Despite obfuscation of rust symptoms, phenotypic selection within populations for rust resistance may be an effective approach in this environment since among and within families mean squares were highly significant. Of course, selection would be more effective if based on more than 1 yr of rust expression at that location.

Recurrent phenotypic selection has also been used as a breeding method for increasing rust resistance for other perennial grasses. Although this technique has been predominantly used for cool-season turfgrass species (Meyer, 1982), including perennial ryegrass (*Lolium perenne* L.) (Rose-Fricker et al., 1986; Welty and Barker, 1992), Kentucky bluegrass (*Poa pratensis* L.) (Watkins et al., 1981), timothy (*Phleum pratense* L.) (Braverman, 1966), and tall fescue (*Festuca arundinacea* Schreb.) (Welty and Barker, 1992), it may also have application for warm-season grasses like switchgrass.

Cool-season turfgrass cultivars are mostly evaluated and selected for resistance in field and turf plots under natural disease conditions, which has resulted in new, unique cultivars (Meyer, 1982). However, field evaluations alone may not be reliable in years when environmental conditions do not favor disease development as evident at Kimball in 2000. More progress in selecting for rust resistance in switchgrass could be made if dependable inoculation methods are developed for controlled environments.

CONCLUSION

Considerable variation for rust infections was observed among and within four populations of switchgrass at two diverse locations in South Dakota. We adapted a scale developed for wheat to describe and quantify the variation in symptoms we observed under natural rust infections in family-row nurseries in South Dakota. That scale should be useful to other pathologists and geneticists/plant breeders interested in quantifying rust diseases on perennial warm-season grasses.

Selection among populations for resistance to *Puccinia emaculata* would certainly be the most effective approach in utilizing genetic variation for improvement in disease resistance of switchgrass for the northern and central Great Plains. However, other factors such as winterhardiness may require selection within cultivars/populations that are well adapted to the climatic conditions of the northern Great Plains. In that case, selection of the most resistant individuals within the best families should also result in progress.

REFERENCES

Alderson, J., and W.C. Sharp. 1994. Grass varieties in the United States. Agric. Handb. 170. USDA-SCS, U.S. Gov. Print. Office, Washington, DC.

Arthur, J.C. 1934. Manual of the rusts in United States and Canada. The Science Press Printing Co., Lancaster, PA.

Barnett, F.L., and R.F. Carver. 1967. Meiosis and pollen stainability in switchgrass, *Panicum virgatum* L. Crop Sci. 7:301–304.

Braverman, S.W. 1966. Sources of resistance to stem and leaf rust among *Phleum* spp. Plant Dis. Rep. 50:849–851.

Boe, A., and J.G. Ross. 1998. Registration of 'Sunburst' switchgrass. Crop Sci. 38:540.

Cornelius, D.R., and C.O. Johnston. 1941. Differences in plant type and reaction to rust among several collections of *Panicum virgatum* L. J. Am. Soc. Agron. 33:115–124.

Eberhart, S.A., and L.C. Newell. 1959. Variation in domestic collections of switchgrass, *Panicum virgatum.* Agron. J. 51:613–616.

Falconer, D.S. 1981. Introduction to quantitative genetics. 2nd ed. Longman Publ., London.

Farr, D.F., G.F. Bills, G.P. Chamuris, and A.Y. Rossman. 1989. Fungi on plants and plant products in the United States. APS Press, St. Paul, MN.

Gravert, C.E., and G.P. Munkvold. 2002. Fungi and diseases associated with cultivated switchgrass in Iowa. J. Iowa Acad. Sci. 109:30–34.

Gravert, C.E., L.H. Tiffany, and G.P. Munkvold. 2000. Outbreak of smut caused by *Tilletia maclaganii* on cultivated switchgrass in Iowa. Plant Dis. 84:596.

Hopkins, A.A., K.P. Vogel, K.J. Moore, K.D. Johnson, and I.T. Carlson. 1995. Genotype effects and genotype by environment interactions for traits of elite switchgrass populations. Crop Sci. 35:125–132.

Mankin, C. 1969. Diseases of grasses and cereals in South Dakota. Tech. Bull. 35. South Dakota Agric. Exp. Stn., Brookings, SD.

McNeal, F.H., C.F. Konzak, E.P. Smith, W.S. Tate, and T.S. Russell. 1971. A uniform system for recording and processing cereal research data. USDA, ARS 34–121. U.S. Gov. Print. Office, Washington, DC.

Meyer, W.A. 1982. Breeding disease-resistant cool-season turfgrass cultivars for the United States. Plant Dis. 66:341–344.

Moore, K.J., L.E. Moser, K.P. Vogel, S.S. Waller, B.E. Johnson, and J.F. Pedersen. 1991. Describing and quantifying growth stages of perennial forage grasses. Agron. J. 83:1073–1077.

Newell, L.C., and S.A. Eberhart. 1961. Clone and progeny evaluation in the improvement of switchgrass, *Panicum virgatum* L. Crop Sci. 1:117–121.

Roelfs, A.P., R.P. Singh, and E.E. Saari. 1992. Rust diseases of wheat: Concepts and methods of disease management. CIMMYT, Mexico, D.F.

Rose-Fricker, C.A., W.A. Meyer, and W.E. Kronstad. 1986. Inheritance of resistance to stem rust (*Puccinia graminis* subsp. *graminicola*) in six perennial ryegrass (*Lolium perenne*) crosses. Plant Dis. 70:678–681.

Satterthwaite, F.E. 1946. An approximate distribution of estimates of variance components. Biometrics 2:110–114.

Watkins, J.E., R.C. Shearman, J.A. Houfek, and T.P. Riordan. 1981. Response of Kentucky bluegrass cultivars and blends to a natural stem rust population. Plant Dis. 65:345–347.

Welty, R.E., and R.E. Barker. 1992. Evaluation of resistance to stem rust in perennial ryegrass grown in controlled and field conditions. Plant Dis. 76:637–641.