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**RUST AND VIRAL MOSAIC DISEASES IN BIOFUEL
SWITCHGRASS**

By

Anthony A. Muhle

A THESIS

Presented to the Faculty of
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Professor Gary Y. Yuen

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RUST AND VIRAL MOSAIC DISEASES IN BIOFUEL SWITCHGRASS

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University of Nebraska, 2019

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Switchgrass (*Panicum virgatum* L.) is a perennial warm-season monocot that is indigenous to locations in North America east of the Rocky Mountains, and is considered a model grass for biofuel feedstock production. As switchgrass production increases, diseases pose a potential threat to biomass production and ethanol extraction. The two predominant switchgrass diseases in Nebraska are rust caused by *Puccinia* spp. and a viral mosaic disease caused by *Panicum mosaic virus* (PMV) and its associated *Satellite panicum mosaic virus* (SPMV). In this thesis, one study determined how SPMV affects PMV infection and systemic spread in two populations of switchgrass at different temperatures under controlled conditions. The results from this study showed that no synergism from co-infection of PMV and SPMV occurred in switchgrass cvs. Kanlow and Summer. The study also indicated that both cultivars can equally be infected by PMV alone and the combination of PMV+SPMV, but Kanlow suppressed systemic spread of the viruses. Temperature had no effect on systemic spread of the viruses, although there was some evidence that higher temperature may have an effect on the initial infection of switchgrass plants by the PMV+SPMV combination. Another study evaluated hybrid switchgrass populations that originated from crossing of Kanlow (lowland ecotype) and

Summer (upland ecotype) for their responses to rust and viral mosaic diseases under Nebraska field conditions. The results indicated that there was large variation among switchgrass hybrid populations as to their response to rust and viral mosaic severity ratings, with populations exhibiting high resistance comparable to Kanlow and other populations exhibiting susceptibility similar to Summer. Also, there was a significant positive correlation between parent and progeny populations as to their response to rust and viral mosaic diseases. However, there was no linkage found between resistance/susceptibility to rust and resistance/susceptibility to viral mosaic. Nevertheless, hybrid populations with resistance to both rust and viral mosaic diseases were identified, these populations being excellent candidates for use in further development of biofuel switchgrasses

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DEDICATION

To my late brother Andrew M. Muhle

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CHAPTER 1:

LITERATURE REVIEW

The studies within this thesis were conducted as part of the United States Department of Energy (DOE) funded project “Genetics and Genomics of Pathogen Resistance in Switchgrass.” This thesis focuses on two diseases of switchgrass, rust caused by *Puccinia* spp, and viral mosaic caused by *Panicum mosaic virus* and its associated *Satellite panicum mosaic virus*. Therefore, this literature review will summarize aspects of research on switchgrass and these two diseases.

1.1) Switchgrass

1.1)1. Introduction

Switchgrass (*Panicum virgatum* L.) is a perennial warm-season monocot that belongs to the family *Poaceae* and is indigenous to North America east of the Rocky Mountains (Hitchcock et al., 1950). It is considered a model perennial grass for cropping as a biofuel feedstock because it is suitable for marginally productive cropland, land that is comparable to the Conservation Reserve Program (Mitchell et al., 2012). It is tolerant to abiotic stresses such as drought and salinity, and has minimal input requirements for economically acceptable stand quality and yield (Mitchell et al., 2014). Compared to other agronomical important crops, switchgrass also contributes benefits to the environment such as reducing soil erosion, improving water quality from low pesticide

and fertilizer usage, increasing soil organic carbon, and reducing greenhouse gas emissions (Gu et al., 2018). Switchgrass has gone through a slow transition over the past century and continues to show benefits for the future.

1.1)2. Agronomic uses past & present

Switchgrass was initially identified as a desirable native grass for revegetating grasslands, following the drought of the 1930s. During the late 1930's, switchgrass was being researched as a forage for livestock (Mitchell et al., 2012). Within the last 35-years, the research focus on switchgrass expanded to include biomass production for bioenergy purposes, primarily for the development of liquid fuels such as ethanol and butanol. The DOE at the Oak Ridge National Laboratory (ORNL) started a program in 1984 to screen 34 herbaceous grass species in several different states for potential biomass energy production (Wright, 2007). The results of the ORNL study revealed that switchgrass was among the top three species for biomass production, and in 1991 the DOE selected switchgrass as a model species because of the high yield potential, wide adaptability as a native species, and its conservation qualities (Wright, 2007; Vogel et al., 2011). Current switchgrass breeding efforts in Nebraska are focused on increasing biomass yield, reducing lignin content, promoting winter hardiness, and integrating pathogen resistance.

1.1)3. Morphology & physiology

Switchgrass can grow to heights between 0.5 to 3 m, depending on interactions between genotype, environment, and ecotype (Vogel et al., 2011). Switchgrass plants are categorized into lowland and upland ecotypes (Vogel et al., 2011). Lowland ecotypes

evolved to flourish in areas of lower elevation such as flood plains, or river bottoms, primarily in the southern United States (Ayyappan et al., 2017). Lowland ecotypes produce stems that are tall and coarse, leaves that are long and wide, have a high biomass potential, and are typically tolerant to most pests and diseases (Sanderson et al., 1996; Ayyappan et al., 2017). Upland ecotypes have evolved to higher elevations that usually have colder climates and drier conditions, and originated primarily in the northern United States and southern Canada (Ayyappan et al., 2017). Upland ecotypes produce narrow short stems and leaves, have low biomass production, and tend to be susceptible to pests and diseases (Sanderson et al., 1996; Ayyappan et al., 2017). Genetically, lowland ecotypes are all tetraploids ($2n=4x=36$), while upland ecotypes are either tetraploids or octoploids ($2n=8x=72$) (Vogel et al., 2011). It is possible to cross the two ecotypes if the parents are of the same ploidy level. For example, 'Liberty' is a cultivar developed by population hybridization of two tetraploid switchgrass cultivars, 'Summer' as the female parent (upland ecotype) and 'Kanlow' as the male parent (lowland ecotype) (Vogel et al., 2014). Liberty has increased biomass production, similar to Kanlow, and inherited winter hardiness, a trait similar to Summer (Vogel et al., 2014).

Most switchgrass genotypes grow in clumps or tufts, known as caespitose that produce short rhizomes, which can form a sod over time (Vogel et al., 2011). During the early stages of growth following germination, seedlings produce adventitious roots that develop into massive fibrous roots that can reach depths of 3 m (Vogel et al., 2011). The inflorescence is a panicle of 15 to 55 cm in length, which contains spikelets located at the end of long branches (Vogel et al., 2011). Switchgrass is able to spread vegetatively by

tillers and rhizomes, while seeds are produced via out crossing to further increase genetic diversity (Aurangzaib, 2015).

The ability for switchgrass to successfully thrive in marginally productive cropland is largely due to its physiology. Switchgrass utilizes the C₄ photosynthetic pathway, which is an efficient process for fixing carbon (C) for photosynthesis, particularly at higher temperatures (Vicentini et al., 2008; Vogel et al., 2011). The recommended planting date for switchgrass is 2-3 weeks before or after the recommended seeding date for maize (*Zea mays* L.), since seed germination and seedling growth are reduced when soil temperatures drop below 20°C (Mitchell et al., 2013; Vogel et al., 2011). Switchgrass seedlings emerge through the soil surface by elongation of the mesocotyl or subcoleoptile internodes, which once the soil surface is reached the mesocotyl is induced by light to halt elongation and encourage adventitious root growth (Vogel et al., 2011). It is important to plant before a period of anticipated rain to keep the soil moist to encourage seedling germination and adventitious root establishment. Since switchgrass is a perennial plant, growth during the establishment year is slower than annual grasses due to the plant's resources being dedicated to root establishment, but during spring of the following year, auxiliary buds on the crown, stem, and rhizomes provide a quick start for new growth (Vogel et al., 2011). Once the extensive root system of switchgrass is fully developed, drought tolerance improves, and within the Great Plains region the water-use efficiency can range from 3.5 to 5.0 mg biomass g⁻¹ water (Vogel et al., 2011). Near the end of the growing season, flower production is prompted due to the photoperiod sensitivity of the plant, which is based on the latitude where the plant has evolved (Vogel et al., 2011).

When cultivating a perennial plant such as switchgrass, which has the potential to be productive for 10 years or more with proper management, it is possible to see harvestable yields in the planting year (Mitchell et al., 2012). During the planting year, the perennial grass is placing most of its energy into developing the root system and crown. At the end of the growing season in the seeding year, it is reasonable to attain 50% of the yield potential when harvesting after a killing frost, with upland cultivars producing 4-5 Mg ha⁻¹ (Mitchell et al., 2012). In the second year, just 18-months after planting, switchgrass can produce 75 to 100% of the yield potential of the cultivar, with upland cultivars producing 8-13 Mg ha⁻¹ of dry matter (Mitchell et al., 2012). Generally, the highest biomass yields are obtainable from a single harvest if switchgrass is harvested at anthesis, or flowering. If harvest is delayed after anthesis there is the potential to lose 10 to 20% biomass yield up until a killing frost (Sanderson et al., 2006; Vogel et al., 2011).

1.1)4. Diseases

While research in large scale switchgrass cultivation continues, there are growing concerns that diseases could reach epidemic proportions within switchgrass, crop grown in a monoculture. A number of diseases have been reported on switchgrass. Diseases caused by fungal pathogens include but are not limited to: rust (*Puccinia* spp.), anthracnose (*Collectotrichum* spp.), smuts (*Tilletia* spp.), sharp eye spot (*Rhizoctonia cerealis*), Helminthosporium spot blotch (*Bipolaris sorokiniana*), leaf spot (*Elsinoe panici*), Phoma leaf spot (*Phoma* spp.) and Fusarium root rot (*Fusarium* spp.) (Crouch et al., 2009; Etheridge et al., 2001; Farr et al., 1989; Gravert et al., 2000; Gustafson et al.,

2003; Ma, Y., 2015; Mitchell et al., 2014; Tiffany et al., 1961; Zeiders, 1984). Viral diseases include mosaic caused by *Panicum mosaic virus* (PMV), with its satellite, *Satellite panicum mosaic virus* (SPMV), and *Switchgrass mosaic virus* (SwMV) (Agindotan et al., 2012; Stewart, 2015). The two predominant switchgrass diseases in Nebraska are rust and viral mosaic disease caused by PMV and SPMV, which are the focus of this thesis (Ma, 2015; Stewart, 2015).

1.2) Rust fungi

1.2)1. Introduction

Rust fungi are obligate pathogens that obtain their nutrients from a living host plant (Duplessis et al., 2011). Infection by rust typically appears as numerous rusty or orange colored spots that rupture the epidermis of the infected host (Agrios, 1997). Most rust fungi have evolved to be host specific with infections typically restricted to leaves and stems. Once the fungus is ready to sporulate, pustules will rupture through the epidermis of the host and usually appear as rusty, orange, or yellow spots (Agrios, 1997). Rust fungi are a vast group with about 8,400 species within the subphylum *Pucciniomycotina* (Aime et al., 2014). With such a large group there are many species of rust that can cause devastating epidemics which have the potential to lead to massive crop losses. These examples include but are not limited to wheat and barley rust (*Puccinia graminis*), coffee rust (*Hemileia vastatrix*), southern corn rust (*Puccinia polysora*), cotton rust (*Puccinia stakmani*), and cedar-apple rust (*Gymnosporangium juniperi-virginianae*) (Duplessis et al., 2011; Agrios, 1997).

A number of fungal species were reported to cause rust on switchgrass, all classified in the *Pucciniaceae* family (Demers et al., 2017). In Nebraska, *Puccinia emaculata* Schwein. and *Uromyces graminicola* Burrill are the two rust species found to infect switchgrass strains developed for bioenergy production (Ma, 2015). Demers et al. (2017) reported a taxonomical revision of the rust species on switchgrass based on comparison of gene sequences and spore morphology of rust fungi in herbarium specimens. They proposed a new classification of *Puccinia graminicola* for the fungus formerly called *Uromyces graminicola* and *Puccinia novo-panici* as the new species name for the switchgrass pathogen formerly called *Puccinia emaculata* (Demers et al., 2017); hence, all switchgrass rust fungi are now considered *Puccinia* spp. Because all of the literature on rust fungi in switchgrass reviewed here was published prior to this taxonomic revision, the species names *Puccinia emaculata* and *Uromyces graminicola* will be used in this literature review.

1.2)2. Distribution

The distribution of switchgrass rust disease extends over a wide range in North America mainly due to the host plant's ability to inhabit a large majority of the continent. The disease has been reported from Texas to South Dakota and to the east coast to New York (Kenaley et al., 2016). Switchgrass rust pathogen species are believed to geographically cover the eastern two thirds of North America, since switchgrass rust was also reported from Mexico all the way north to southeastern Canada (Demers et al., 2017). With such a wide distribution, it is believed that switchgrass rust, if unchecked, could occur in large epidemic proportion as switchgrass monoculture production

continues to grow. This is the reason why there is expressed importance to incorporate rust resistance into switchgrass breeding programs.

1.2)3. Life Cycle of Switchgrass Rust

P. emaculata and *U. graminicola*, like many rust fungi, need multiple hosts to complete their life cycles and are known as macrocyclic-heteroecious species (figure 1.1). ‘Macrocyclic’ indicates that the rust fungus has multiple spore stages, typically up to five, and ‘heteroecious’ denotes the species has to have two different unrelated host plant species to complete its life cycle (Kolmer et al., 2009). Switchgrass is the telial host on which there are three separate spore stages: uredinia, telia, and basidia. The aecial host, or alternate host, for *P. emaculata* (= *P. novo-panici*), on which the pycnial and aecial stages are produced, is in the *Euphorbiaceae* family, while the aecial host of *U. graminicola* is unknown (Demers et al., 2017; Kolmer et al., 2009).

In Nebraska, the rust infection season typically starts around mid- to late-summer (July to August), depending on climatic factors. Aeciospores from the aecial host are produced in abundance and can be dispersed great distances to reach the telial host switchgrass (Kolmer et al., 2009). Dikaryotic aeciospores land on leaves of the telial host, germinate, and infect through the stomata. Mycelium spreads through the plant cells to establish feeding sites where the fungal pathogen collects nutrients to produce the next spore stage, the uredinia stage (Kolmer et al., 2009). Urediniospores are also dikaryotic and have the ability to re-infect the same host multiple times throughout the infection season, which can lead to a quick increase in inoculum and potentially lead to an epidemic (Kolmer et al., 2009). Once the growing season comes to an end and the host

plant starts to senesce, the uredinial stage will convert into the telial stage, where dark brown or black diploid teliospores are produced, and these spores act as the overwintering spores, which are tolerant to cold and desiccation (Kolmer et al., 2009). Since most teliospores are typically immobile, depending on the species, they remain attached to the telial host and undergo meiosis to produce haploid basidiospores as temperatures begin to warm in early spring. During the basidial stage, basidiospores are unable to infect the telial host, switchgrass, and thus must be forcibly ejected and dispersed to infect the aecial host, plants in the *Euphorbiaceae* family (Kolmer et al., 2009). Basidiospores can only disperse short distances and are typically released during the night when there is high moisture, since they are fragile spores (Kolmer et al., 2009). Upon infection of the aecial host by a basidiospore, the fungus will start to produce haploid pycnia, which can consist of two or more mating types. When pycniospores are dispersed by rain or insects, they will cause fertilization of pycnia of the opposite mating type (Kolmer et al., 2009). After fertilization, the aecial stage will produce an aecium that will develop on the underside of the same leaf and give rise to the aeciospores (Kolmer et al., 2009). From here, the cycle can start all over again with the aeciospores making the journey back to the telial host, switchgrass. The localized distribution of *U. graminicola* suggests that inoculum that initiates epidemics in switchgrass by this species originates from an aecial host that is localized in distribution, while the continental distribution of *P. emaculata* could possibly reflect northward dissemination of urediniospores from southern regimes (Kenaley et al., 2016).

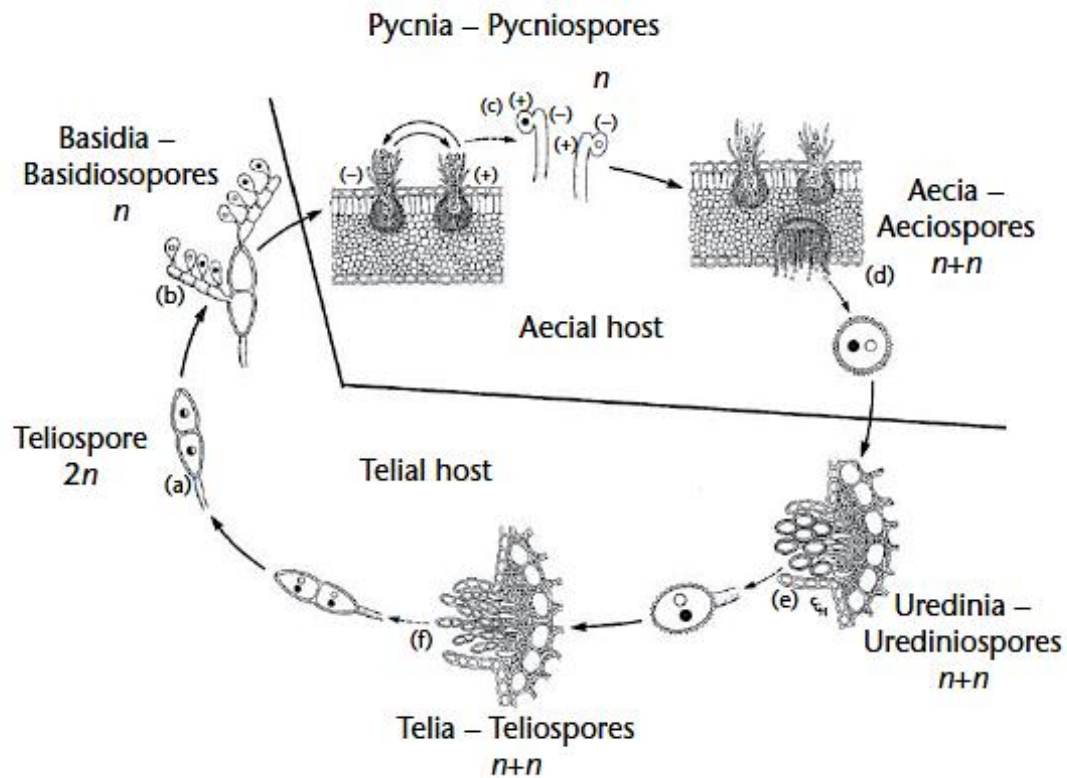


Figure 1.1. Generalized life cycle of a macrocyclic-heteroecious rust. From Kolmer et al. (2009).

1.2)4. Host resistance mechanisms & management

Rust species, especially *P. emaculata*, have the potential to be a threat to switchgrass biofuel industries (Serba et al., 2015). Sykes et al. (2016) reported that under high disease severity levels rust has the potential to reduce downstream conversion of cellulosic ethanol extraction by up to 55%. It is not economically feasible to apply chemical fungicides to manage diseases like rust in switchgrass grown for biomass production, because of the requirement of low input costs. It is also not practical to incorporate cultural practices, such as crop rotation, since switchgrass typically is kept productive for up to 10 years once planted. Other cultural practices such as rouging out

the aecial alternate host might decrease disease severity by removing the source of aeciospores, and might reduce the sexual cycle which can degrade genetic variability (Agrios, 1997). But given the amount of time and energy needed to eradicate an alternate host, this may not be economically feasible or useful.

The best way to manage pathogens such as rust in a low-input system is with host resistance. There are two main types of resistance by which plants defend themselves from potential pest and pathogens. The first type of resistance is horizontal resistance, or polygenic resistance, which involves many different genes each encoding a portion of the resistance to control a certain pathogen (Agrios, 1997). The other type of resistance is known as vertical resistance, or monogenic resistance, in which one or just a few genes are responsible for providing resistance to a certain pathogen (Agrios, 1997). Currently it is unknown what type of resistance switchgrass has to rust diseases. A resistance mechanism that switchgrass potentially uses to deal with rust infection is programmed cell death (Serba et al., 2015), a mechanism by which a host plant, upon recognizing a pathogen, will automatically kill the cells being invaded and surrounding cells to prevent further expansion of the infection.

Past breeding efforts have been utilized to improve rust resistance within switchgrass populations. Eberhart and Newell (1959) examined populations of switchgrass found in Nebraska for several characteristics, including rust resistance, and found large phenotypic variations among populations and plants within populations. Gustafson et al. (2003) evaluated rust resistance among five switchgrass populations, which included a Nebraska elite population, an Oklahoma elite population, 'Sunburst', and 'Cave-In-Rock'. The Nebraska population was observed to be moderately resistant to

rust as compared to summer and Sunburst which were susceptible (Gustafson et al., 2003). Additionally, they observed significant variation among half-sib populations with in each of the populations (Gustafson et al., 2003). This suggests that further improvement in resistance can be gained by the use of more resistant populations for further breeding and selection. Uppalapati et al. (2013) reported that two lowland populations, Alamo and Kanlow, expressed resistance to rust. Currently it is unknown what gene(s) are responsible for inducing rust resistance in these lowland cultivars. But when crossing rust-resistant cultivars such as Kanlow with rust-susceptible Summer, breeders are able to obtain progeny populations that show improved resistance to rust. Liberty exhibited intermediate rust severity, when compared with Kanlow (rust-resistant) and Summer (rust-susceptible) parent lines, in a multi-year study at five locations across northcentral United States (Muhle et al., 2017). Thus far, Liberty is the only example of a Kanlow x Summer (KxS) population that has been evaluated for rust resistance; it is unknown whether other KxS populations could have improved resistance over Liberty, or even exhibit resistance similar to Kanlow.

1.3) *Panicum mosaic virus – Satellite panicum mosaic virus Complex*

1.3)1. Introduction

Panicum mosaic virus (PMV) is a plant pathogenic virus that was first reported infecting switchgrass in Kansas in 1953 (Sill & Pickett, 1957). In Nebraska, PMV poses a possible threat to switchgrass biomass production for the use of cellulosic ethanol-based bioenergy (Stewart et al., 2015). PMV belongs in the genus *Panicovirus* in the family *Tombusviridae*, and has a positive-sense single-stranded (ss) RNA genome approximately

4,300 nucleotides (nts), which is encapsidated in a 28-30 nm icosahedral virion (Turina et al. 1998). Switchgrass grown in the field can be found infected by PMV alone or by PMV in association with *Satellite panicum mosaic virus* (SPMV) (Stewart et al., 2015). PMV is considered the helper virus for SPMV replication and movement (Scholthof, 1999).

SPMV is similar to PMV in that it has a positive-sense single-stranded (ss) RNA genome, but it differs in that the RNA genome is approximately 824 nts and is encapsidated in a 16 nm icosahedral virion (Omarov et al., 2005). In addition, there is no significant similarity between PMV and SPMV genome sequences (Omarov et al., 2005). SPMV alone is non-infectious, but when coinfecting with PMV, the satellite virus can induce a synergistic effect on millets (Scholthof, 1999). Generally, this synergism will cause the viral titer for one or both viruses to increase compared to an individual viral infection (Chowda et al., 2019). Because of this up-regulation in viral titer there is the potential for severe symptoms to develop in the host (Scholthof, 1999).

1.3)2. Host range & distribution

A report by the United States Department of Agriculture (USDA) (<https://www.ars.usda.gov/oc/np/pearlmillet/virapm/>) describes the host range of PMV to members of the Panicgrass subfamily in the *Poaceae*, which includes switchgrass and other *Panicum* spp. (*P. capillare* L., *P. decompositum*, *P. dictioniflorum*, *P. hallii* Vasey, *P. miliaceum* L., *P. ramosum*, *P. scribnerianum* Nash, *P. turgidum*, *P. virgatum* L.), centipedegrass (*Eremochloa ophiuroides*), pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica* [L.] Beauv.), barnyardgrass (*Echinochloa crus-galli* [L.] Beauv.), crabgrass (*Digitaria sanguinalis* [L.] Scop.), *Setaria verticillata*, *S.*

lutescens, maize (*Zea mays*), and St. Augustinegrass (*Stenotaphrum secundatum* [Walt.] Kuntze). Additionally, certain experimental model hosts have been reported to be infected with PMV, which include *Brachypodium distachyon* and *Setaria viridis* (Mandadi and Scholthof, 2012; Mandadi et al., 2014). Also, the USDA (<https://www.ars.usda.gov/oc/np/pearlmillet/virapm/>) reported that the geographic distribution of PMV within the USA in switchgrass is limited to Kansas and Nebraska, while PMV occurs on St. Augustinegrass in Arkansas, Louisiana, South Carolina, and Texas; additionally PMV occurs in Mexico. Thomas & Steele (2011) reported PMV in *Stenotaphrum secundatum* in Australia. Because PMV has a wide host range and distribution it is possible that PMV will expand beyond the distribution range described above.

Since SPMV depends on PMV for replication, the host range of SPMV is expected to be similar to PMV. The geographic distribution of SPMV is expected to be more restricted than PMV. Thus far, SPMV has only been reported to occur in field grown switchgrass in Nebraska (Stewart et al, 2015).

1.3)3. Pathogenesis and epidemiology

PMV and SPMV are mechanically transmitted, i.e. they enter the host by plant sap through open wounds, and thus far, there are no known vectors that transmit PMV or SPMV (Niblett et al, 1975). Once inside the host cell, both viruses replicate within the cytoplasm, SPMV depending on the helper virus PMV for replication (Pyle et al., 2018). Scholthof (1999) reported that pearl millet plants infected with PMV alone showed slight stunting and mild chlorotic mottling compared to the severe stunting and severe chlorotic

mottling brought on by the coinfection of PMV and SPMV (Scholthof, 1999). Recently, Chowda et al. (2019) reported that different strains of SPMV can have an impact on synergistic interaction with PMV. In that study, synthesized strains PMV-NE and PMV-85, when inoculated alone into Proso millet, caused mild chlorotic mottling symptoms with slight stunting. When the PMV strains were coinfecting with the SPMV-KS strain, severe leaf chlorosis symptoms developed with severe stunting, whereas, mild leaf chlorosis and mild stunting were observed when either PMV strain was coinfecting with SPMV-Type strain (Chowda et al., 2019). The difference in synergistic interaction from the SPMV strains is due to differences in two amino acids (A35 and R98) within the coat protein (CP) of the SPMV strains (Chowda et al., 2019). These results confirm that not only is there a synergistic effect between PMV and SPMV, but the severity of this synergism can be affected by the type of SPMV strain.

This synergistic effect from co-infection of PMV with SPMV has been studied on millet species, but little is known about the interaction of PMV and SPMV in relation to switchgrass infection. Stewart et al, (2015) reported that field grown switchgrass populations with high disease severity ratings (DSR) of 4 and 5 were often associated with co-infection of PMV and SPMV; and plants that were infected with PMV alone had DSRs of 3 or lower. An unreported greenhouse study, on the other hand, indicated that there was no significant difference of symptom development between PMV alone and the co-infection of PMV and SPMV when examined in four switchgrass populations (Stewart, 2014). This difference between the field study and greenhouse study illustrates the need for a more in-depth study to determine if SPMV has an effect on PMV when co-infecting switchgrass. Additionally, there is currently nothing known about

environmental effects on infection by PMV or the combination of PMV and SPMV. Stewart's (2014) greenhouse study was conducted under a low constant temperature, whereas field grown switchgrass experiences a wide range of temperature changes throughout the growing season. A more comprehensive study has the potential to determine if environmental conditions promote or discourage the infection of PMV and its associated satellite virus.

1.3)4. Host resistance mechanisms & management

When it comes to management of PMV and SPMV, host resistance is the best viable method, since viral diseases in switchgrass are unable to be managed effectively through other conventional strategies such as chemical controls, tillage, or crop rotation. There are two main types of host resistance to viral pathogens that can be developed through breeding, the first is resistance from feeding by vectors that transmit the virus and the second type is resistance to viral replication and spread within the host plant. Since there are no known vectors of PMV and SPMV, resistance to viral replication and spread is the only relevant method of switchgrass resistance to viruses. One well known host resistance mechanism that has been studied in monocot plants is hypersensitive response (HR) which induces programmed cell death (Goldbach et al., 2003). HR resistance is initiated when an interaction between host and pathogen is detected, typically controlled by Avr/R genes (R gene being the host resistance gene and Avr gene being the corresponding avirulence gene in the pathogen), which cause metabolic changes to occur that bring the cell death (Mandadi et al., 2013). Another resistance mechanism is RNA silencing, which the host plant is able to detect and degrade viral

RNA fragments within the cytoplasm of the host cell (Ruiz-Ferrer & Voinnet, 2009). RNA silencing occurs when a ssRNA virus forms dsRNA while replicating within the cytoplasm of the host plant. A plant protein called the dicer that will cleave the viral dsRNA into smaller pieces, which are individually incorporated into an Argonaut protein complex that will target complementary sequences in the viral genome and degrade the viral ssRNA (Ruiz-Ferrer & Voinnet, 2009).

Little is known about what type of host resistance switchgrass may have and which gene(s) would be responsible for viral resistance. Based on research using St. Augustinegrass as the host, there can be tolerance to PMV and SPMV in which the viruses are allowed to replicate and spread throughout the host plant, but the plant expresses minimal to no symptom development (Toler et al., 1983). In regards to switchgrass, certain lowland populations such as Kanlow could potentially have resistance to viral mosaic pathogens such as PMV and SPMV. In the field study, reported by Stewart et al. (2015) the highest disease incidence of 69%, as well as high symptom severity, were found among Summer (upland) populations, while Kanlow (lowland) and Kanlow-derived populations exhibited disease incidence of around 20%, and had low symptom severity (Stewart et al., 2015). Also, this field study reported that Liberty, a KxS cultivar, was intermediate to viral mosaic diseases compared to Kanlow and Summer parents. Stewart (2014) greenhouse study did not support the observations from the previous field study. The greenhouse study observations indicated that Kanlow and Summer were equally susceptible to virus infection from PMV (Stewart, 2014). The difference between these two studies shows the importance for a more in-depth experiment to determine how Kanlow and Summer respond to PMV and its associated satellite virus. Because Liberty

is the only KxS population to have been evaluated for viral mosaic resistance, it is important to investigate additional KxS populations to determine if any express greater resistance to viral mosaic diseases.

1.4) Critical questions

It is important to explore some of the vital knowledge gaps related to viral mosaic diseases such as *Panicum mosaic virus* complex and rust diseases of switchgrass. The research chapters in this thesis and some of the critical questions that are addressed in each chapter are:

Chapter 2: Infection of switchgrass by *Panicum mosaic virus* – effects of co-infection with *Satellite panicum mosaic virus*, host population, and temperature.

- Are there differences among switchgrass populations to infection and systemic spread by PMV?
- Is there a synergistic effect from infection with SPMV on PMV in switchgrass?
- Does temperature effect the infection rate and systemic spread of PMV?

Chapter 3: Field study of switchgrass resistance to rust and viral mosaic diseases

- Is there variation among switchgrass KxS (Kanlow x Summer) hybrid populations as to resistance to rust and viral mosaic diseases?
- Is resistance to rust potentially linked to resistance to viral mosaic diseases in switchgrass populations?
- Are there KxS populations that are resistant to both rust and viral mosaic diseases?
- What is the proportion of rust and viral mosaic resistance that is inherited by the progeny from the parent populations?

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CHAPTER 2:

INFECTION OF SWITCHGRASS BY *PANICUM MOSAIC VIRUS* – EFFECTS OF CO-INFECTION WITH *SATELLITE PANICUM MOSAIC VIRUS*, HOST POPULATION, AND TEMPERATURE

2.1) Introduction

Panicum mosaic virus (PMV) and *Satellite panicum mosaic virus* (SPMV) were the most prevalent viral pathogens found in a survey of field-grown switchgrass (*Panicum virgatum*) in Nebraska (Stewart et al., 2015). In that study, the lowland population ‘Kanlow’ had a lower incidence of virus infection and lower symptom severity compared to the upland population ‘Summer’, suggesting that certain lowland populations have resistance to viral pathogens such as PMV and SPMV. Severe mosaic symptoms and stunting were more frequently associated with co-infection by the two viruses than infection by PMV alone. This finding is consistent with reports of synergism between PMV and SPMV in which the virus combination caused heightened symptom expression over PMV alone when inoculated onto millet species (Chowda et al., 2019; Omarov et al., 2005; Scholthof, 1999). The observations made in the field study, however, were not consistent with findings from an unrepeatable greenhouse experiment examining the response of Kanlow and Summer to inoculation with the two viruses

(Stewart, 2014). In that experiment, the two switchgrass populations were equally susceptible to virus infection, and there was no difference between symptom development with PMV alone versus the two viruses in combination.

The discrepancy between the field observations and the greenhouse experiment could be related to different PMV and SPMV strains infecting the plants. While the origin of the PMV and SPMV isolates used to generate the inoculum in the greenhouse study was not specified (Stewart, 2014), the inoculum isolates were not the PMV and SPMV strains naturally infecting switchgrass in the Nebraska field survey. Chowda et al. (2019) reported that synergism between PMV and SPMV exhibited on Proso millet (*Panicum miliaceum* L.) is dependent on the SPMV isolate; co-infection of two PMV isolates with a Kansas isolate of SPMV (SPMV-KS) caused severe stunting, chlorosis, and led to plant death, whereas co-infection of either PMV isolate with a different SPMV isolate (SPMV-Type) caused only moderate chlorosis and slight stunting. Another factor that may have contributed to different results between the field survey and the greenhouse experiment could be differences in environmental factors, such as temperature. Stewart's greenhouse experiment was conducted at a constant temperature of 21°C, while field temperatures in Nebraska during the growing season reach 35°C or higher.

To provide a better understanding of how PMV and SPMV interact with each other when co-infecting different switchgrass populations, a more extensive greenhouse experiment was conducted with the objectives of verifying whether synergism between PMV and SPMV occurs in switchgrass, and verifying whether Kanlow possesses greater resistance than Summer to the viruses. In this study, the two cultivars were inoculated

with PMV alone and in combination with a SPMV isolate used in Chowda et al. (2019) to produce the highest synergistic effect on Proso millet. Virus-cultivar interactions were assessed on the basis of local infection (infection on the inoculated leaf) and systemic infection (spread to upper non-inoculated leaves). Another objective of this research is to determine if temperature can affect the virus-cultivar interactions. Thus, virus-cultivar interactions were tested under three temperature regimes that together spanned the range of temperatures expected to occur in the field.

2.2) Materials & Methods

Experiment Design

A 2x2x3 factorial experiment was conducted with a randomized complete block split-plot design. The main plot factor was temperature (low, medium, and high) and the split plots factors were switchgrass cultivars (Kanlow and Summer) and viral inoculation treatments (PMV alone, and PMV in combination with SPMV; PMV+SPMV). Three temperature regimens were tested: low = 21-24°C, medium = 26-29°C, and high = 32-35°C. Growth chambers (Convion A2000) were used to regulate temperature regimens and other environmental conditions. Lights were set at 300 micro moles light intensity and maintained at 12 hours on / 12 hours off. Relative humidity was maintained at ~35%. Because only three growth chambers were available and one growth chamber was designated per temperature regimen, temperature treatments were replicated over time. There were three replications, i.e., inoculation of plants with viruses was performed three times, with a growth chamber being assigned a different temperature regimen at random each time.

Within each growth chamber, there were ten (10) plants per switchgrass population inoculated with each of the two virus treatments, and two plants per population given a mock inoculation treatment. These plants were placed in the growth chambers in a randomized array.

Switchgrass Cultivation

Switchgrass plants were grown in plastic conical tubes (Cone-tainers®, Stuewe & Sons, Inc.; 3.8 cm diameter, 21 cm depth, and 164 ml volume) that were filled with a pasteurized greenhouse potting mix (1-part loam soil, 2-part peat moss, 1-part sand, and 1-part vermiculite). Eight seeds were initially planted per Cone-tainer, and as seeds germinated, the seedling numbers were thinned to one per Cone-tainer. Prior to inoculation, plants were grown in a greenhouse with an average temperature of 26°C for about three weeks, or until the third true leaf stage. Cone-tainers were arranged on racks (30 cm W x 61 cm L x 17 cm H) and separated so that plants of different treatments did not touch. Plants were watered uniformly by placing trays (38 cm W x 68 cm L x 7 cm H) under the racks of Cone-tainers and filling trays with water to keep the bottom of the Cone-tainers submerged. Once a week trays were filled with a low dose of fertilizer (20-20-20 NPK) at 250 ppm.

Viral inoculation method

Virus strains used in this study, PMV-NE and SPMV-KS, were provided by Satyanarayana Tatineni's lab, University of Nebraska-Lincoln, USDA-ARS. The genome for PMV-NE was a consensus of PMV-specific sequences identified from multiple

switchgrass samples collected in Nebraska, and SPMV-KS was a Kansas isolate of SPMV (Chowda et al., 2019). PMV-NE alone and the combination of PMV-NE with SPMV-KS (PMV-NE+SPMV-KS) were propagated in Proso millet 'Sunnup'. To prepare inoculum, two grams of virus-infected Proso millet leaf tissue was ground with a pestle and mortar in 10 ml of inoculum buffer (0.02 M sodium phosphate buffer, pH 7.0) that was previously autoclaved, and then sterilized celite was added to the tissue extract to 2% concentration. Switchgrass leaves were rub-inoculated by dipping the pestle into the inoculum mix and rubbed on the top of the third leaf of each plant in a downward motion 4 times with medium pressure. Mock inoculations followed the same procedure except that no Proso millet leaf material was added to the mix. Latex gloves were worn for each inoculation and changed between the mock, PMV-NE alone, and PMV-NE+SPMV-KS inoculations. Inoculation took place at room temperature (23°C) and inoculated plants were then transferred into designated growth chambers.

Sample Collection and Virus Detection

Fourteen days after inoculation, plants were assayed for viral presence based on ELISA and RT-PCR. Plants were not assessed individually for viral symptom development. The inoculated (third) leaf and the non-inoculated top-most (5th) leaf were collected from each plant and assayed separately for the presence of the inoculated virus(es). Presence of the inoculated virus(es) in the inoculated leaf was confirmation of local infection, while viral detection in upper non-inoculated leaf was an indication of systemic spread. Leaves collected were placed in plastic bags (Uline poly bags, 4 x 5 inch, 4MIL, Uline, Co.) and stored in a -80°C freezer until processed for viral detection.

All samples of non-inoculated leaves were analyzed for the presence of PMV using a 2-step strategy similar to that reported in Stewart et al. (2014) in which samples were first analyzed for PMV presence with a commercial PMV-specific DAS ELISA kit (Nano Diagnostics, LLC., Fayetteville, AR), which is an immunoassay using antibodies generated against PMV viral coat proteins. Second, all PMV-negative samples in the immunoassay were then re-assayed for PMV using RT-PCR. When a plant was found to be negative for systemic presence of PMV, the virus-inoculated leaf from that plant was tested for the presence of PMV using the dual assays. Plants that were positive for systemic presence of PMV were assumed to be positive for PMV at the inoculated leaf. All PMV-positive samples from plants inoculated with PMV+SPMV were assayed for SPMV presence via RT-PCR. Samples from mock inoculated plants were tested for PMV presence using RT-PCR.

For the immunoassay, a ratio of 1 gram of leaf tissue was ground in 10 ml of SB1 buffer, prepared according to the DAS ELISA kit protocol, and then the extract was applied in the immunoassay following the manufacturer's protocol. Each assay included two negative controls: an extract from a non-inoculated, greenhouse-grown switchgrass plant and a PMV-free control supplied by the manufacturer. If a sample absorbance reading was twice or higher than the negative controls, that sample was considered positive for PMV. Any PMV-negative sample was then assayed using RT-PCR to confirm if the sample was truly negative.

RT-PCR assays for PMV and SPMV were conducted through a series of steps that included RNA extraction, cDNA synthesis, PCR amplification, and gel electrophoresis. RNA extraction was done by using Direct-Zol RNA MiniPrep (Zymo

Research), which also required the use of TRIzol Reagent (Ambion, Life Technologies). First-strand cDNA was produced by using SuperScript III Reverse Transcriptase (Invitrogen, Thermo Fisher Scientific) and reverse primers that were specific to PMV and SPMV. The primers used for PCR amplification were specific to PMV and SPMV coat proteins (Table 2.1). Primers were synthesized at Integrated DNA Technologies. PCR amplification cycles consisted of 2 minutes at 94°C, 30 seconds at 94°C, 30 seconds at 55°C, and 1 minute at 68°C, which these steps are repeated for 30 cycles, then end the amplification with 5 minutes at 68°C and samples were held at 4°C. PMV and SPMV primers were designed to amplify sequences of 120 base pair (bp). Gel electrophoresis was performed to detect these PCR products. A GeneRuler 1 Kb ladder (Thermo Scientific) was used to indicate DNA size.

Table 2.1. Primers used in RT-PCR detection of PMV and SPMV

Primer	Sequence
PMV-F2	5' – AAG CCC ATT TAC TCG GGA AGT GC – 3'
PMV-R2	5' – CAC TGA ACT CTG GAT TAG TAC – 3'
SPMV-F2	5' – GCG TTC CAG GCG ATC TAA TCG – 3'
SPMV-R2	5' – TAT ATT TCT GGC CGG GTT GGT TG – 3'

Data Analysis

For each replication of a 'treatment' (temperature-virus-cultivar combination), the local infection frequency (LIF), the frequency at which inoculated leaves became infected with PMV, was calculated by dividing the number of plants positive for PMV at the inoculated leaf by the number of inoculated plants. Systemic infection frequency (SIF), the frequency at which local infection by PMV progressed to systemic infection,

was calculated by dividing the number of plants in which PMV was detected in the non-inoculated 5th leaf by the number of plants in which PMV was detected in the inoculated leaf.

Absorbance readings in the immunoassay were used as measures of PMV titer in samples exhibiting PMV systemic infection. PMV titer was not analyzed for local infection at the inoculated leaf due to the lack of such samples that were assayed by ELISA. The absorbance readings for systemic infection was averaged for each treatment combination and analyzed for significant differences. LIF, SIF and systemic PMV titer data were analyzed using the statistical analysis program in RStudio (version 1.1.453). Analysis of variance (ANOVA) for a Randomized Complete Block with a Split-plot design was conducted using treatment means that were computed with the lsmeans package (version 2.27-62). Each of the three inoculation events was treated as a 'block' in the statistical analysis. Least Significant Difference (LSD) was used for mean separation by using the LSD.test package and differences between treatment means at the 95% confidence level were considered to be significant.

2.3) Results

Even though viral symptom expression was not recorded for every plant, it was observed that viral symptom (mosaic) development varied among plants of a given cultivar or viral treatment. No symptoms were observed in mock inoculated plants.

Local infection by PMV

The ANOVA analysis of the local infection (infection of inoculated leaf by PMV) frequency data indicated, there was a significant ($P < 0.05$) virus main effect and a significant temperature X virus interaction (Table 2.2). There was no significant temperature or cultivar main effects, and no other significant interactions. The frequency of local infection by PMV inoculated alone averaged across all cultivars and temperature regimes was 14% higher than that of PMV co-inoculated with SPMV (Figure 2.1). Analysis of the temperature X virus interaction showed that at the high temperature regimen, the PMV+SPMV treatment had a lower infection frequency ($P = 0.0241$) of just over half of that of PMV alone. There was no significant difference between the two virus treatments at either of the lower temperature regimes. For the PMV+SPMV treatment, there was a trend of decreasing frequency of infection by PMV with increasing temperature (Figure 2.2). The presence of SPMV was confirmed in all PMV-positive samples from plants inoculated with PMV+SPMV. All samples from mock-inoculated plants were negative for PMV.

Table 2.2. ANOVA mean local infection frequency
(detection of PMV in inoculated leaves)

Factor	DF	F value	P value
Block	2		
Temperature	2	1.899	0.263
W.P. Error (Block/Temp)	4		
Virus	1	4.870	0.0406
Cultivar	1	0.943	0.3444
Temperature : Virus	2	4.613	0.0241
Temperature : Cultivar	2	0.779	0.4736
Virus : Cultivar	1	0.070	0.7942
Temperature : Virus : Cultivar	2	0.374	0.6932
Residuals	18		

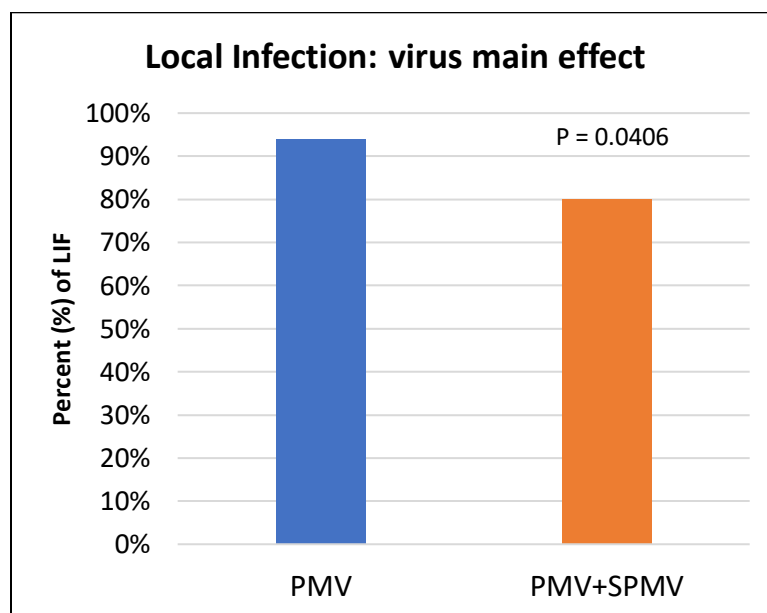


Figure 2.1. Frequencies of local infection on Kanlow and Summer populations by PMV following inoculation with PMV alone or in combination with SPMV (PMV+SPMV).

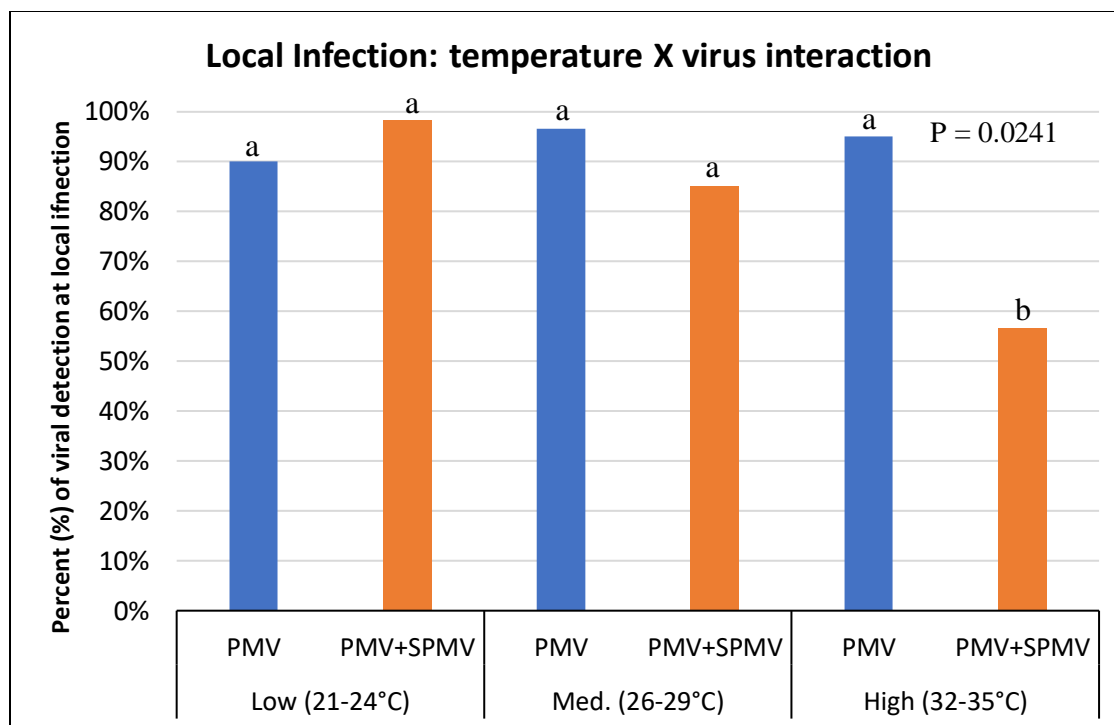


Figure 2.2. Effects of temperature regime and virus treatment on local infection by PMV in Kanlow and Summer populations. Values sharing the same letter are not significantly different at the 95% confidence level.

Systemic infection by PMV

ANOVA analysis of systemic infection frequency showed that there were significant ($P < 0.05$) virus and cultivar main effects, but no significant temperature main effect or significant treatment interactions (Table 2.3). When the virus main effect was examined, the PMV alone treatment exhibited higher frequency of systemic PMV infection than the PMV+SPMV treatment (Figure 2.4), indicating that PMV, when inoculated alone, became systemic more readily than PMV inoculated in combination with SPMV. Analysis of the cultivar main effect revealed a higher frequency of PMV systemic infection occurred in Summer as compared to Kanlow (Figure 2.5).

Table 2.3. ANOVA of mean systemic infection frequency (detection of PMV in upper leaves)

Factor	DF	F value	P value
Block	2		
Temperature	2	1.363	0.354
W.P. Error (Block/Temp)	4		
Virus	1	9.030	0.0076
Cultivar	1	7.606	0.0130
Temperature : Virus	2	2.437	0.1157
Temperature : Cultivar	2	0.746	0.4885
Virus : Cultivar	1	0.550	0.4680
Temperature : Virus : Cultivar	2	0.028	0.9726
Residuals	18		

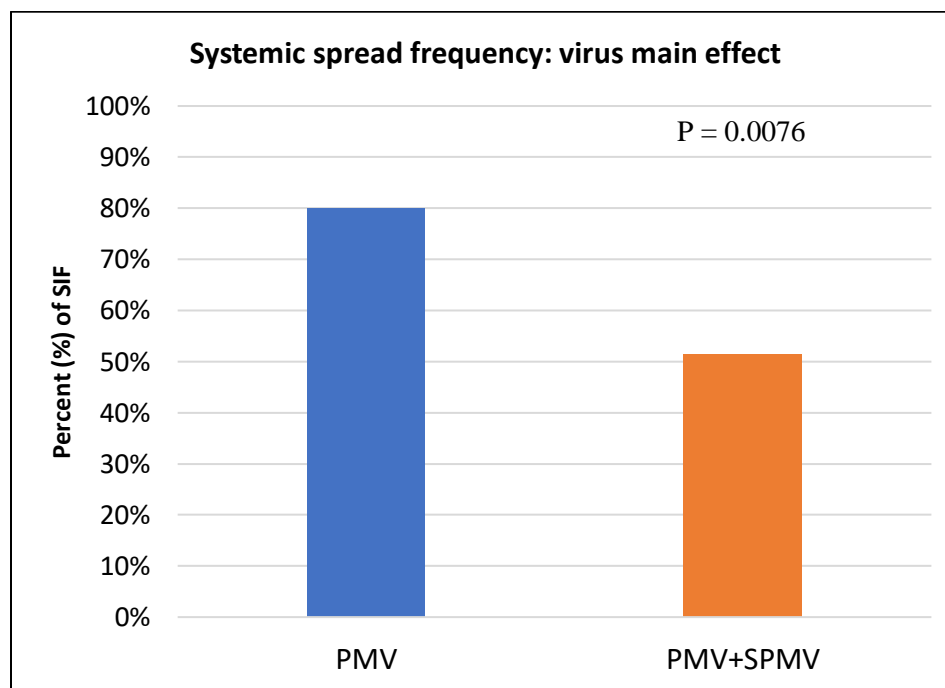


Figure 2.4. Frequencies of systemic infection by PMV following inoculation with PMV alone or in combination with SPMV (PMV+SPMV).

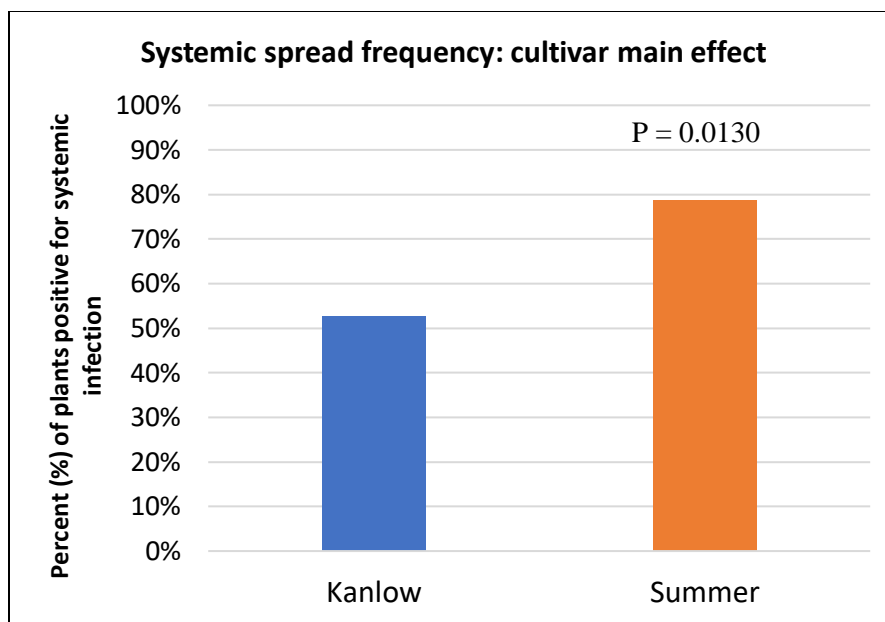


Figure 2.5. Frequencies of systemic spread infection by PMV and SPMV comparing switchgrass cultivars Kanlow and Summer.

The ANOVA analysis of the ELISA absorbance values from the non-inoculated leave, which reflects PMV viral titer, indicated was a significant ($P=0.00218$) cultivar main effect (Table 2.4). There were no significant temperature or viral main effects, and no significant interactions. Switchgrass cultivar Summer had nearly double the PMV viral titer measured in Kanlow (Figure 2.6) at the non-inoculated, leaf.

Table 2.4. ANOVA of mean ELISA systemic absorbance values in upper leaves (PMV titer).

Factor	DF	Fvalue	Pvalue
Block	2		
Temperature	2	1.638	0.3098
W.P. Error (Block/Temp)	4		
Virus	1	0.8431	0.3723
Cultivar	1	14.355	0.00218
Temperature : Virus	2	0.8810	0.4345
Temperature : Cultivar	2	0.4834	0.6271
Virus : Cultivar	1	2.0003	0.1803
Temperature : Virus : Cultivar	2	0.2885	0.7534
Residuals	13		

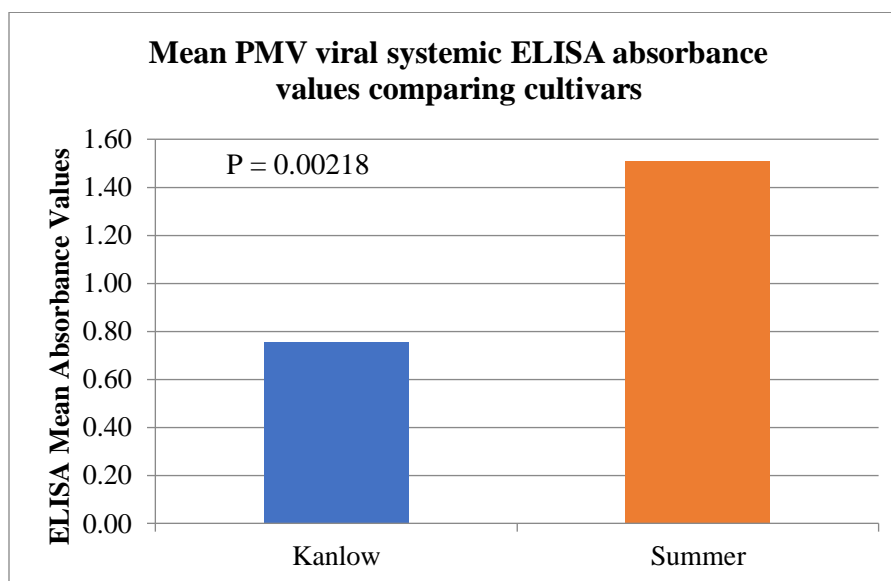


Figure 2.6. PMV titer, measured as ELISA absorbance, in non-inoculated leaves of switchgrass cultivars Kanlow and Summer.

2.4) Discussion

There are several key findings from this study. The first is that while Kanlow and Summer became infected equally at the inoculated leaf by PMV, inoculated alone or in conjunction with SPMV, there clearly is a difference between the two cultivars as to the frequency in which they became systemically infected. The difference in susceptibility to systemic infection was also evident in the reduced PMV titer in Kanlow compared to Summer. These findings would seem to contradict the report by Stewart (2014) of equal susceptibility in Kanlow and Summer to infection by PMV. The difference in findings between the two studies can be explained by the fact that Stewart's assessment of "infection" combined local and systemic infections, i.e., both inoculated and non-inoculated leaves were assayed together for the presence of PMV, and thus any cultivar effects in respects to systemic infection were masked.

In the study by Stewart (2014), all plants within different switchgrass populations eventually became infected by PMV upon repeated inoculation with the virus, suggesting that there is no immunity in switchgrass to PMV, i.e. there is no resistance that completely prevents viral infection. The result from this study provides confirmation that there is greater resistance to systemic spread of the virus in some switchgrass populations than others. The reduced PMV titer in non-inoculated leaves of Kanlow compared to Summer suggests that suppression of systemic spread in Kanlow may be related to inhibition of viral replication. Whether the resistance to systemic spread in Kanlow functions via suppression of cell-to-cell or long-distance virus movement is currently unknown. Regardless of the mechanism, resistance to systemic spread could explain the observation in the field of some switchgrass populations, e.g. Kanlow, exhibiting lower

symptom severity despite being PMV infected (Stewart et al., 2015). Resistance in these populations could be useful to switchgrass breeders for developing new cultivars with greater resistance to PMV and SPMV.

Another key finding is that SPMV appeared to have an antagonistic effect on systemic infection when PMV was co-inoculated with SPMV, which manifested in reduced systemic spread in switchgrass plants compared to inoculation with PMV alone. There also was indication that antagonism occurred during local infection at high temperatures. Scholthof (1999) reported the synergistic effect of PMV+SPMV co-infection in pearl millet to be associated with elevated PMV capsid protein accumulation. In this study, PMV and PMV+SPMV virus treatments yield similar ELISA absorbance readings, which reflect amounts of PMV capsid protein, confirming that synergism from PMV+SPMV co-infection did not occur in switchgrass. Stewart (2014) reported somewhat similar results in that there were no differences in infection frequency, symptom expression, or plant biomass between inoculation of switchgrass with PMV alone compared to PMV+SPMV. These results contradict with previous reports of synergism between PMV and SPMV in millets (Chowda et al., 2019; Scholthof, 1999), as well as the observation that co-infection of switchgrass in the field is associated with higher symptom severity (Stewart et al., 2015). Chowda et al. (2019) found the synergistic effect on Proso millet to be dependent on the SPMV isolate, thus differences among viral isolates could be an explanation for some of the different results among studies. The SPMV isolate used in this study, however, was the same isolate that gave the strongest synergistic effect on Proso millet (Chowda et al., 2019). This suggests that the host plant species is also a determining factor as to whether or not synergism between

PMV and SPMV will occur. Further research is needed to verify the influence of the host species on the PMV+SPMV interaction and to clarify the mechanism involved in the antagonism observed in this study.

The third finding in this study is that temperature does not strongly influence PMV infection of switchgrass. Temperature had no effect on systemic infection. Although local infection resulting from inoculation with PMV+SPMV was reduced at high temperatures, local infection by PMV inoculated alone was consistent across temperature regimes. This might be due to the increased temperature having a negative effect on the SPMV strain. These results would suggest that it is possible for new infections by PMV and systemic spread of the virus to occur in field-grown switchgrass plants throughout the switchgrass growing season.

There is a need for additional research with PMV and SPMV in switchgrass cultivars to confirm the results demonstrated in this study. First, it would be valuable to determine the resistance mechanisms Kanlow may possess. Second, it would be important to determine if the synergistic effect induced by SPMV in millet species and the antagonistic effect that was revealed in this study are indeed species dependent. Experiments should involve multiple switchgrass populations and multiple millet species being inoculated simultaneously with the virus combinations. It would be useful to conduct such experiments using RT-qPCR, which would provide a more accurate and more sensitive measurement of PMV and SPMV viral titer than ELISA. Lastly, the effect of high temperatures on infection by PMV and PMV+SPMV needs to be investigated further to verify that SPMV is more temperature sensitive than PMV.

2.5) References

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CHAPTER 3:

FIELD STUDY ON THE RESPONSE OF HYBRID SWITCHGRASS TO RUST AND VIRAL MOSAIC DISEASE

3.1) Introduction

In Nebraska two surveys were conducted of field grown switchgrass (*Panicum virgatum*) to determine the most prevalent fungal and viral pathogens. One survey reported that *Puccinia emaculata* and *Uromyces graminicola* were the most frequent rust fungi pathogens (Ma, 2015); and the other survey reported that *Panicum mosaic virus* (PMV) and its associated *Satellite panicum mosaic virus* (SPMV) were the most abundant viral pathogens (Stewart et al., 2015). The earlier studies helped establish which diseases the USDA-ARS would need to focus on their breeding efforts for disease resistance. Rust has the potential to become a problem for switchgrass biomass production, since under high disease severity levels rust can reduce downstream conversion of cellulosic ethanol extraction by up to 55% (Sykes et al., 2016). Currently, it is unknown the potential threat that PMV and SPMV could have on switchgrass biomass production.

Past switchgrass breeding efforts in Nebraska, led by USDA-ARS, were focused on the development of hybrid populations from the crosses of parent populations ‘Kanlow’ (lowland) and ‘Summer’ (upland) to develop “hybrid” switchgrass lines that

have increased biomass yield, lower lignin content, along with enhanced winter hardiness. These efforts have resulted in the new biofuel cultivar ‘Liberty’ (Vogel et al., 2014). Resistance to disease such as rust and viral mosaic in these hybrid populations, however, have only recently been addressed. Earlier studies have reported that lowland populations such as Kanlow and Alamo tend to have higher resistance to rust caused by *P. emaculata*, compared to upland populations, such as Summer (Gustafson et al., 2003; Uppalapati et al., 2013). Rust severity levels in Liberty tested in Nebraska were reported by Muhle et al. (2017) to be intermediate between the Summer and Kanlow parents. Stewart et al. (2015) reported that Kanlow grown in the field had lower incidence of infection by PMV and SPMV and lower viral symptom severity compared to Summer, while Liberty was intermediate between the parent populations as to viral mosaic incidence and severity. These studies involving Liberty represent the only investigations on the response of hybrid populations to disease. It is unknown whether other hybrid populations may have improved resistance to rust and viral mosaic compared to Liberty.

As part of the DOE project “Genetics and Genomics of Pathogen Resistance in Switchgrass”, a number of field experiments were established in Nebraska involving large numbers of hybrid populations. These experiments provided opportunities to address these objectives: 1) determine the amount of variation in rust response among switchgrass hybrid populations derived from crosses of Kanlow and Summer, 2) conduct an analysis of heritability of the rust resistance from parent hybrid populations to progeny populations derived from the parent populations, 3) determine the amount of variation in response to viral mosaic among hybrid populations, 4) conduct an analysis of the heritability of viral mosaic resistance from parent to progeny hybrid populations, 5)

determine if there is a linkage between resistance/susceptibility to rust and resistance/susceptibility to viral mosaic, and 6) identify hybrid populations that are resistant to both rust and viral mosaic diseases.

3.2) Materials & Methods

Field experiment description

Three switchgrass fields identified as PV1013-21i, PV1103-70, and PV1609-70 included in this study were located at the University of Nebraska, Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE.

Experiment PV1609-70 was a progeny testing nursery of 31 half-sib families derived from 31 select Kanlow (as the female parent) X Summer (KXS) populations. In addition, Kanlow N1, Summer, Liberty, and Kanlow N3 were included as check populations. The Kanlow N1 population was from one generation of selection from Kanlow, for winter hardiness (Casler et al., 2014), and Kanlow N3 was derived from Kanlow by three generations of selection. Seedlings of each half-sib family and check population were first grown in the greenhouse and then transplanted to the field in 2016. Experiment field design was a randomized complete block design with two replicate plots per population. Plots were made up of single rows of 10 plants, with rows and plants within rows spaced 1.1m apart on center.

Experiment PV1013-21i was a crossing block nursery that was established in 2010. It contained one hundred eleven (111) plants selected from crosses of ‘Summer’, as the female parent, with ‘Kanlow’ (S X K). There were two replicates (ramets, or clones)

for each plant randomly distributed across the field. The experimental design was a completely randomized design, with plants grown on 1.1 m row spacing on center. Plants in PV1013-21i were pollinated by open-pollination. Seed was collected from each of the 2 panicles of each plant and combined to represent one hundred eleven (111) half-sib families that were planted as the test progeny in PV1103-70.

Experiment PV1103-70, planted in 2011, is a progeny test nursery for the one hundred eleven (111) half-sib families derived from the SxK populations planted in PV1013-21i. PV1103-70 also included four check populations Kanlow, Summer, Liberty, and KxS HP C0, which is a hybrid population from the cycle selection 0. Experiment plots were made up of single rows that contain five plants, planted as greenhouse grown seedlings, from the same half-sib family or check population. Plant spacing within rows was 0.5 m. and rows were spaced 1.1 m apart on center. The ends of each plot were separated with 2 m alleys. The experimental design was a randomized complete block design with three replicates per population.

Rust severity rating

Rust severity ratings were obtained from PV1013-21i and PV1103-70 for three years (2016, 2017, and 2018), and from PV1609-70 for only two years (2017 and 2018). Each year, plants were rated for rust during the months of August and September, when plants were entering senescence and rust was in the telial stage. The rust rating scale utilized was reported by Gustafson et al. (2003), and was based on numerical values ranging from 0 (no rust) to 9 (highest rust severity rating) (Table 3.1 and Figure 3.1). For each switchgrass plant, rust ratings were obtained from three randomly-selected leaves at

approximately 130 cm from the ground. The three ratings from a plant were averaged to obtain a single rust rating score.

Table 3.1. Rust rating scale in switchgrass, based on infection from *Puccinia emaculata*, reported by Gustafson et al. (2003).

Rust rating	Sign and Symptom Description
0	No signs of rust or visible symptoms
1	No sporulation, light necrotic and/or chlorotic areas.
2	Trace sporulation, light necrotic and/or chlorotic areas
3	Trace-light sporulation, light necrotic and/or chlorotic areas.
4	Light sporulation, light necrotic and/or chlorotic areas may/may not be present.
5	Moderate sporulation, necrotic and/or chlorotic areas may/may not be present.
6	Moderate-heavy sporulation, necrotic and/or chlorotic areas may/may not be present.
7	Heavy sporulation, necrotic and/or chlorotic areas generally not present.
8	Heavy-abundant sporulation, necrotic and/or chlorotic areas generally not present.
9	Abundant sporulation, no necrotic or chlorotic areas.

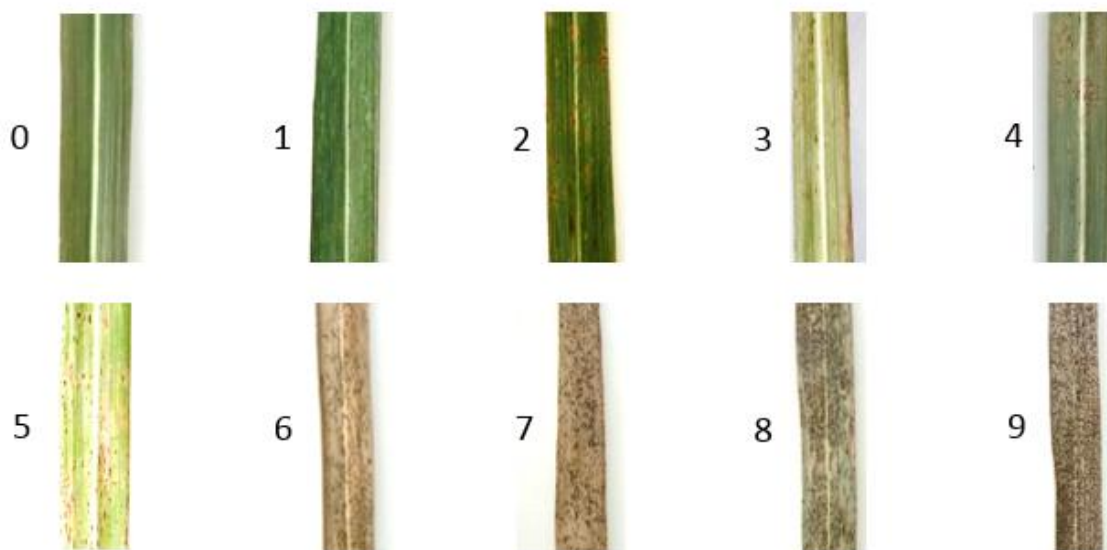


Figure 3.1. Rust severity rating scale used in the Yuen lab that was based on relative numbers of telial pustules, modified from Gustafson et al., 2003.

Viral symptom severity ratings

Two years (2017 and 2018) of viral symptom ratings were obtained from PV1013-21i and PV1609-70. Three years (2013, 2014, and 2015) of viral symptom ratings were obtained from PV1103-70. Viral symptom severity ratings, as described by Stewart et al. (2015) were taken early in the growing season, typically in June. Each plant was first scored visually on a 1 to 4 scale for the extent of chlorotic mottling throughout the plant. An additional point was added to the visual score if the plant was stunted (Table 3.2), giving a potential maximum rating of 5. The rating scale used in this study was a modification of the scale reported in Stewart et al. (2015).

Table 3.2. Viral symptom severity rating scale modified from (Stewart et al, 2014).

Viral symptom rating	Plant symptom description
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1	No symptoms
2	$\leq 25\%$ of plant exhibiting mottling
3	25 – 75% of foliage exhibiting mottling
4	$\geq 75\%$ of foliage exhibiting mottling
+1	Stunting

Data analysis

All data was analyzed using the statistical analysis program in RStudio (version 1.1.453). To address objectives one and three, variation in population responses to rust and viral mosaic, respectively, data from each experimental field was analyzed separately and multiple years of rust or viral severity ratings were applied in an Analysis of Variance (ANOVA) for a Randomized Complete Block Design (experiments PV1609-70 and PV1103-70) and for a Completely Randomized Design (nursery PV1013-21i). The LSmeans for each population across years was computed with the lsmeans package (version 2.27-62), and the Least Significant Difference (LSD) test at 95% confidence level was used for means separation by the LSD.test package. For objectives two and four, heritability of rust and virus resistance, respectively, a heritability correlation was conducted, using cor.test function in RStudio, between LSmeans for the 111 SXX populations planted in PV1013-21i and LSmeans for the 111 progeny populations (half-sib families) planted in PV1103-70. LSmeans were those calculated in objectives 1 and 3. The analysis was performed for each disease separately. For objective 5, rust response-virus response relationship, a correlation analysis, using cor.test function in RStudio, was performed between rust ratings and viral symptom ratings using LSmeans calculated in objectives 1 and 3. The analysis was performed for each experiment separately. In objective six, half-sib populations in PV1609-70 and PV1103-70 that are resistant to both

rust and viral mosaic diseases were identified by identifying those that were statistically similar in rust severity and viral symptom severity ratings to Kanlow (PV1103-70 or KanlowN1 (PV1609-70), based on the LSD test. Kanlow N1 in this study was used as the resistant check, since in a separate study it was shown to be similar to Kanlow in rust resistance (G. Yuen personal communication). Among SXX populations planted in PV1013-21i, those with both the lowest mean rust ratings and the lowest viral symptom ratings were identified.

3.3) Results

Obj. 1: Variation in rust response among hybrid switchgrass populations

The rust disease pressure during the three years (2016 – 2018) in which rust severity ratings were recorded was relatively low, i.e. the mean rust severity ratings in the susceptible check Summer planted in PV1609-70 and PV1103-70 ranged 2.1 to 4.4 on the 0 to 9 scale (Table 3.3).

Table 3.3. Rust severity ratings for Summer populations (Mean \pm SE, 0-9 scale).

Year	PV1609-70	PV1103-70
2016	-	4.4 \pm 0.23
2017	3.7 \pm 0.31	2.2 \pm 0.23
2018	2.1 \pm 0.26	2.5 \pm 0.22

In experiment PV1609, containing 31 KXS half-sib families, the ANOVA of the mean rust ratings indicated a highly significant ($P < 0.05$) Population effect, but no significant Year main effect or Population x Year interaction (Table 3.4).

Table 3.4. ANOVA of rust rating results from experiment PV1609-70

Factor	Df	F value	P value
Block	1		
Population	34	6.279	2.82e-07
Error 1 (B x P)	34		
Year	1	0.493	0.61
Error 2 (B x Y)	1		
Population x Year	34	1.431	0.15
Error 3 (B x P x Y)	34		

Despite the narrow range of mean rust severity ratings in this experiment, different groups could be discerned among the 31 half-sib families based on their response to rust. One population (#11507) was statistically similar in rust response to the resistant check KanlowN1. Ten populations (32%) exhibited statistically similar rust response as the susceptible check Summer. Most of the 31 KXS populations (20, 64%), as well as KanlowN3 and Liberty, exhibited an intermediate response between the resistant and susceptible checks, exhibiting rust ratings statistically different from both KanlowN1 and Summer (Figure 3.2).

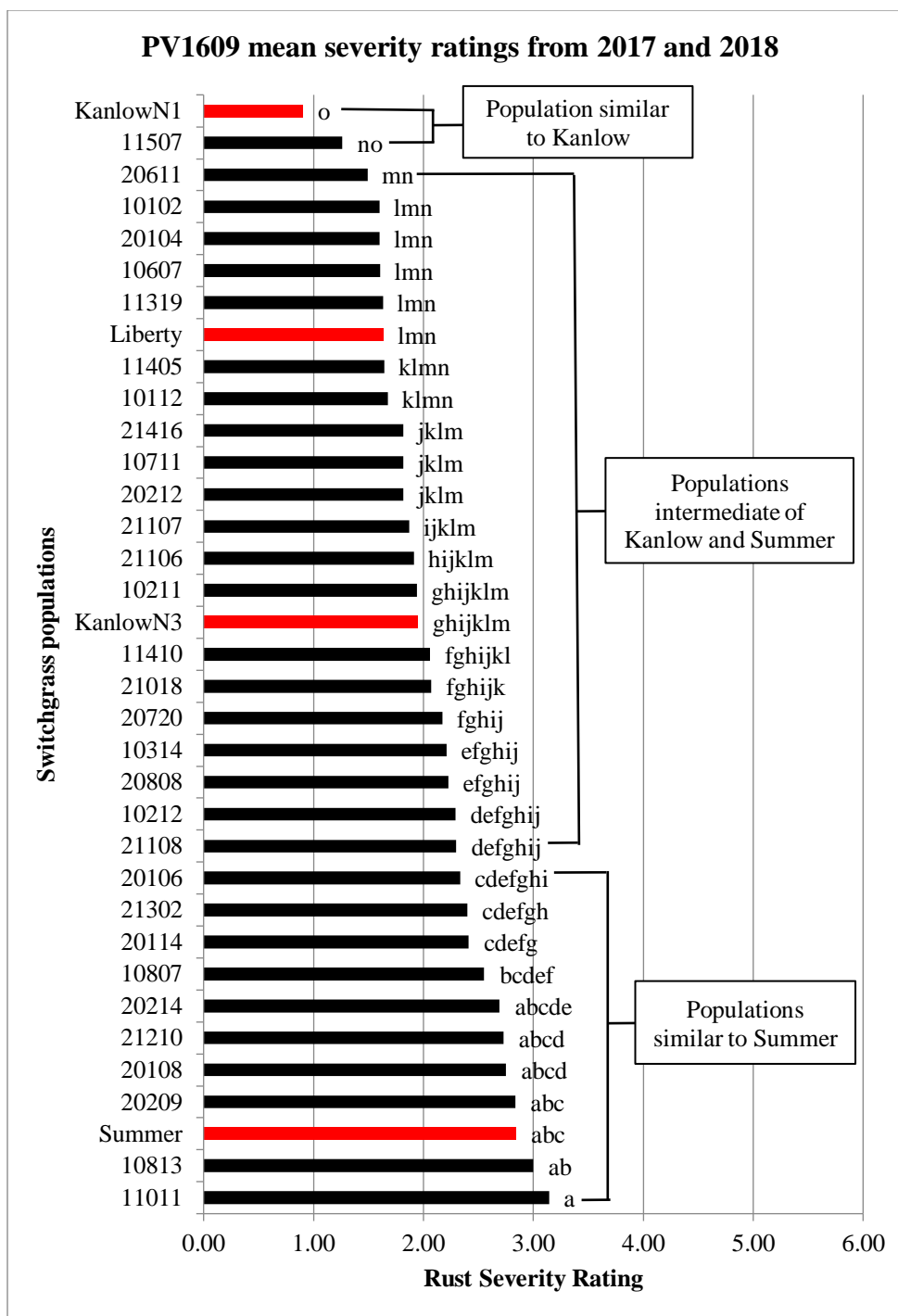


Figure 3.2. Mean rust severity rating (0 to 9 scale) from the years 2017 and 2018, experiment PV1609-70. Values sharing the same letter are not significantly different at the 95% confidence level.

In experimental nursery PV1103-70, the test of 111 SxK half-sib populations, the ANOVA of mean rust ratings indicated highly significant ($P < 0.01$) Population and Year main effects ($P < 0.01$) (Table 3.5). The Population x Year interaction just missed being significant ($P=0.0513$).

Table 3.5. ANOVA of rust rating results from experiment PV1103-70

Factors	Df	F value	P value
Block	2		
Population	115	1.625	0.00101
Error 1 (B x P)	230		
Year	2	38.14	0.00248
Error 2 (B x Y)	4		
Population x Year	230	1.201	0.0513
Error 3 (B x P x Y)	460		

Mean rust rating for only half of the 111 half-sib populations tested in PV1103-70 are shown in Figure 3.3 to illustrate the range of results relative to check populations. The range of mean ratings in this experiment was very narrow (2 to 3.5). Out of the 111 half-sib SxK populations examined in this experiment 7% (8 populations) had rust severity ratings statistically similar to Kanlow, 63% (70 populations) were similar to Summer, 27% (30 populations) had intermediate rust severity ratings compared to the resistant check Kanlow and susceptible check Summer, and 3% (3 populations) had rust severity ratings that were statistically higher than Summer.

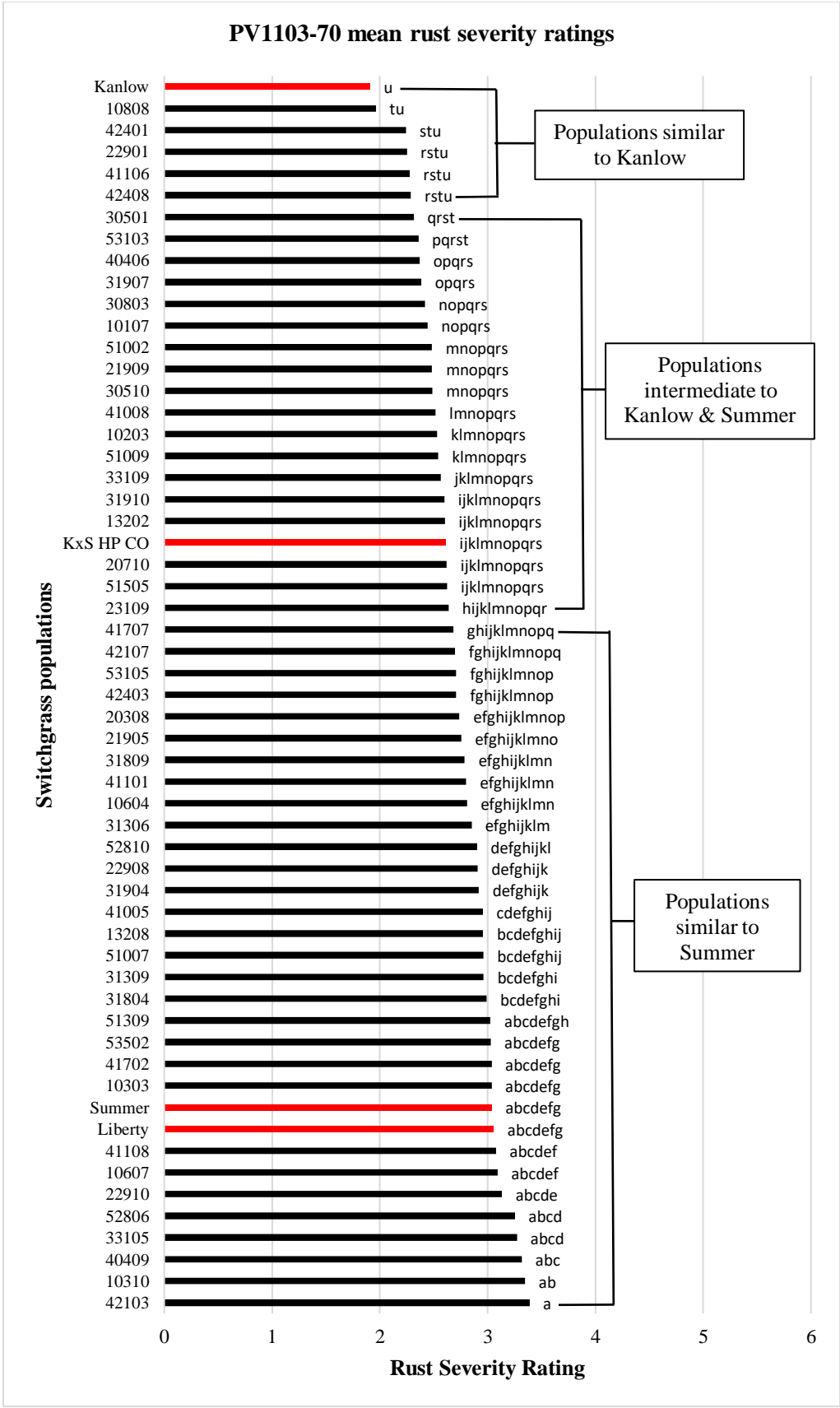


Figure 3.3. Mean rust severity rating (0 to 9 scale) from the years 2016, 2017, and 2018, experiment PV1103-70. Values sharing the same letter are not significantly different at the 95% confidence level. For sake of clarity, only half of the populations are presented to illustrate the variation among populations.

In experiment PV1013-21i, the crossing nursery containing the 111 SxK parent populations, the ANOVA from the mean rust severity ratings of indicated highly significant ($P < 0.01$) Population and Year main effects, as well as a significant ($P=0.0225$) Population X Year interaction (Table 3.6).

Table 3.6. ANOVA of rust rating results from experiment PV1013-21i

Factors	Df	F value	P value
Population	110	2.773	8.6e-08
Rep(Population)	111		
Year	2	500.8	0.00199
Population x Year	220	1.316	0.0225
Residual	222		

Compared to the other experiments, the range of mean rust ratings in PV1013-21i was wider (<1.0 to 4.5). Some population exhibited significantly lower rust rating than other populations (Figure 3.4).

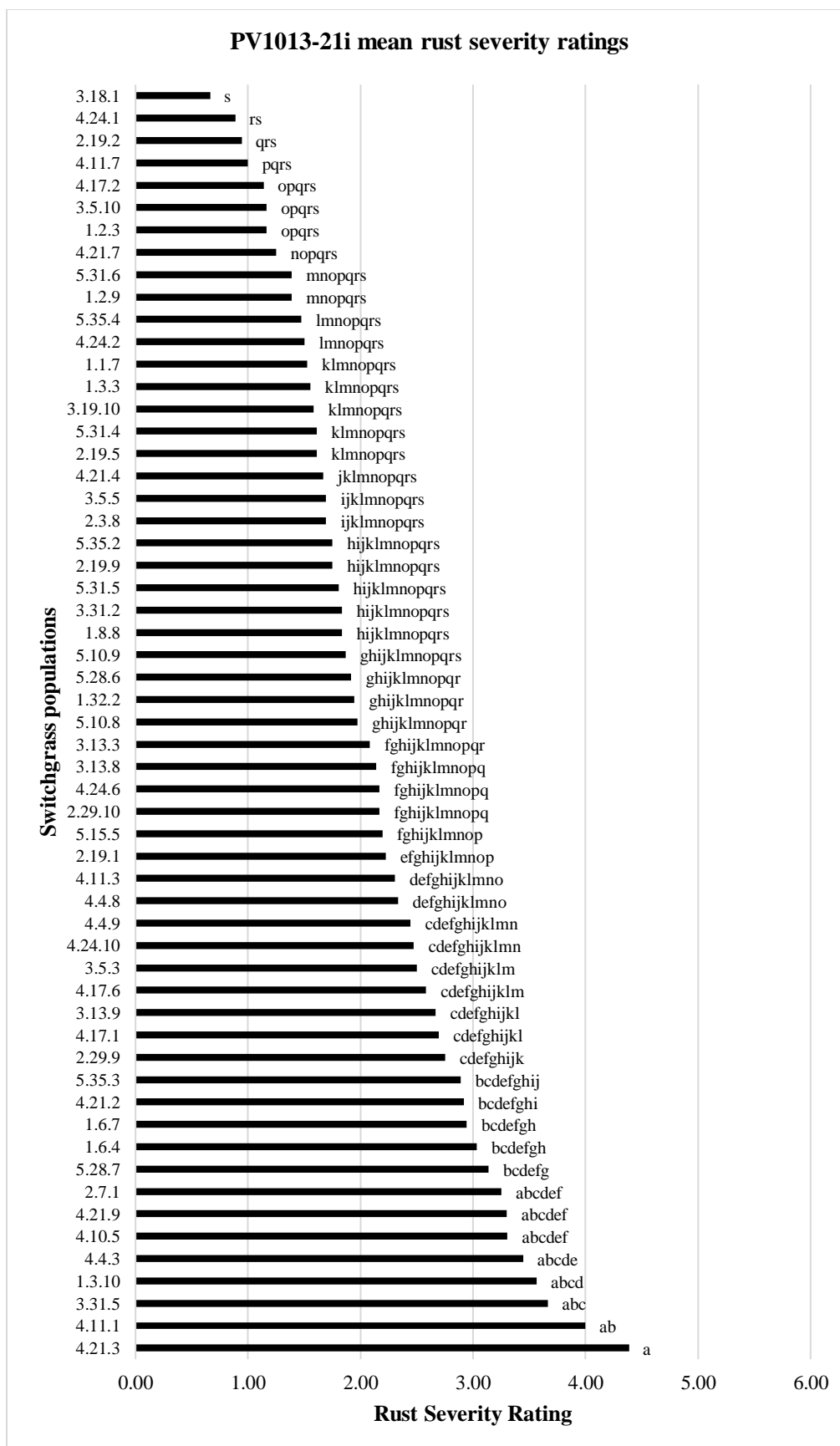


Figure 3.4. Mean rust severity rating (0 to 9 scale) from the years 2016, 2017, and 2018, in experiment PV1013-21i. Values sharing the same letter are not significantly different at the 95% confidence level. For sake of clarity, only half of the populations are presented to illustrate the variation among populations

The results from all three experiments indicated that there is considerable variation of rust severity ratings among hybrid populations. In experiments PV1609-70 and PV1103-70 only a small proportion of the hybrid populations were as resistant as the resistant check population. The remaining hybrid populations were as susceptible to rust as the susceptible check, or had resistance intermediate of the resistant and susceptible checks.

Obj. 2: Rust response heritability

The results from the heritability correlation analysis examining rust severity ratings between the 111 SxK population in crossing nursery PV1013-21i and the corresponding 111 half-sib families in PV1103-70 showed that there is a highly significant ($P < 0.01$) positive correlation (correlation coefficient of 0.5049) between the populations in the two experiments (Figure 3.5). The R^2 value of 0.256 suggests that inheritance from the parent populations in PV1013-21i accounts for approximately 25% of the variation in rust severity among the progeny half-sib families in PV1103-70.

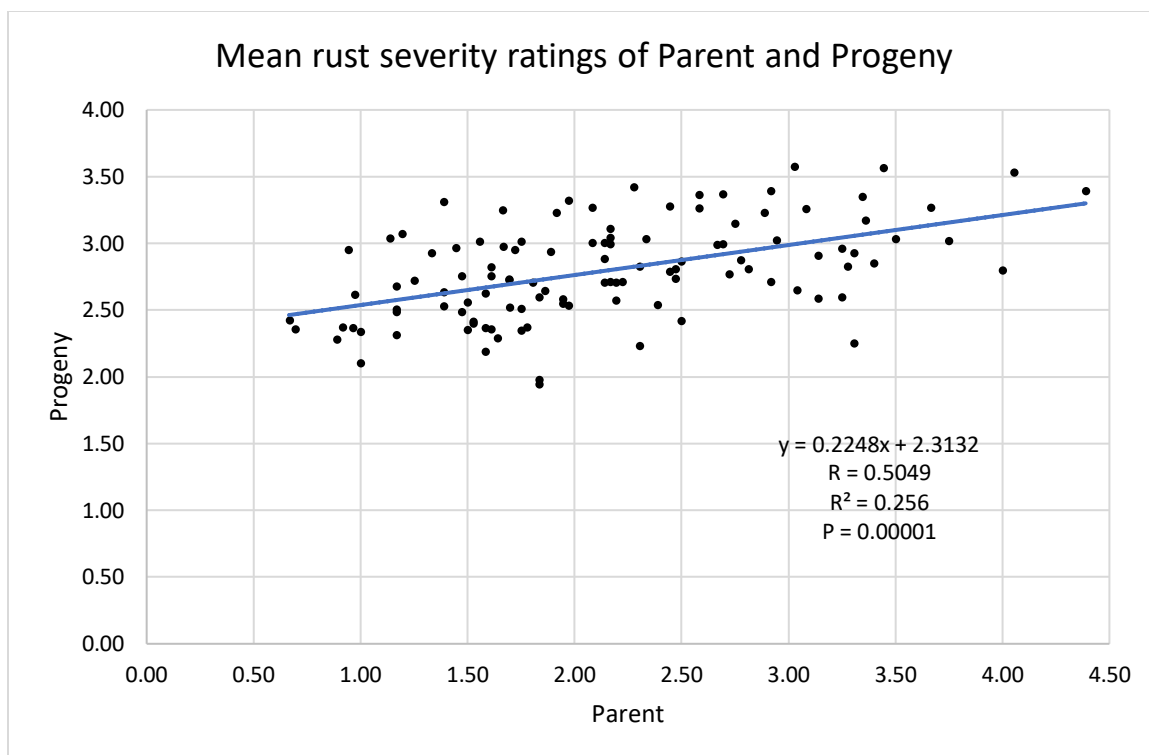


Figure 3.5. Heritability correlation map of parent population (PV1013-21i) and progeny populations (PV1103-70) to rust severity ratings.

Obj. 3: Variation in response to viral mosaic among hybrid switchgrass populations

The mean viral symptom severity ratings in the susceptible check Summer planted in PV1609-70 and PV1103-70 ranged 1.5 to 3.3 on the 1 to 5 scale (Table 3.7).

Table 3.7. Viral symptom severity ratings for Summer populations (Mean \pm SE, 1-5 scale).

Year	PV1609-70	PV1103-70
2013	-	2.5 \pm 0.26
2014	-	3.3 \pm 0.37
2015	-	2.0 \pm 0.32
2017	1.5 \pm 0.20	-
2018	2.4 \pm 0.23	-

In experiment PV1609-70 the ANOVA of the mean viral symptom severity ratings indicated a highly significant ($P < 0.01$) Population main effect as well as a significant ($P=0.032$) Population x Year interaction (Table 3.8).

Table 3.8. ANOVA of viral symptom rating results from experiment PV1609-70

Factors	Df	F value	P value
Block	1		
Population	34	2.991	0.000976
Error 1 (B x P)	34		
Year	1	4.51	0.28
Error 2 (B x Y)	1		
Population x Year	34	1.907	0.032
Error 3 (B x P x Y)	34		

The very low viral mosaic pressure in experiment PV1609-70, with only one population exhibiting a mean virus rating exceeding 2.5, which was significantly higher than the Summer susceptible check (Figure 3.6). Among the remaining 30 KxS populations, seventeen (52%), showed viral symptom severity ratings statistically similar to KanlowN1. Liberty also was in this category. Thirteen (45%) KxS populations, along with KanlowN3, exhibited similar viral symptom severity rating as Summer. There were no populations that were statistically intermediate between Kanlow and Summer.

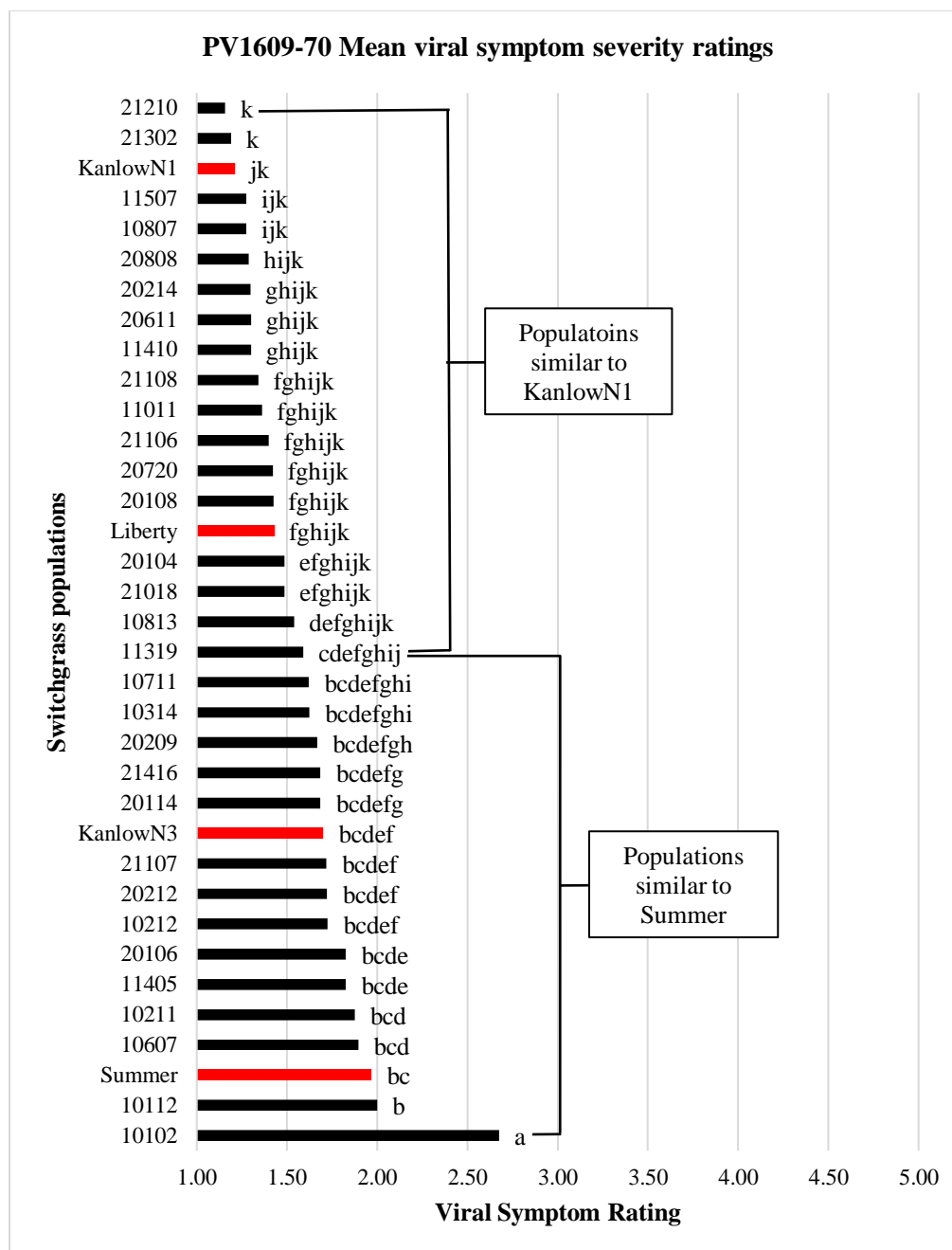


Figure 3.6. Mean viral symptom severity rating (1 to 5 scale) from the years 2017 and 2018, in experiment PV1609-70. Values sharing the same letter are not significantly different at the 95% confidence level.

In experiment PV1103-70, the ANOVA of mean viral symptom severity ratings indicated a highly significant ($P < 0.01$) Population main effect and significant ($P < 0.05$) Year main effect and Population X Year interaction (Table 3.9).

Table 3.9. ANOVA of viral symptom rating results from experiment PV1103-70

Factors	Df	F value	P value
Block	2		
Population	115	1.66	0.000628
Error 1 (B x P)	230		
Year	2	10.87	0.0242
Error 2 (B x Y)	4		
Population x Year	230	1.215	0.0415
Error 3 (B x P x Y)	460		

As in PV1609, there was a narrow range of disease levels (1.5 -2.5) in experiment PV1103-70 (Figure 3.7). Nevertheless, the 111 half-sib SxK populations can be divided into different groups on their viral mosaic severity. The results shown in Figure 3.7 are only half of the population from PV1103-70. 39% (43 populations) exhibited statistically similar viral symptom severity ratings as Kanlow. 52% (58 populations) exhibited statistically similar viral symptom severity ratings as Summer. 8% (9 populations) exhibited statistically different viral symptom severity ratings compared to Kanlow and Summer, and 0.90% (1 population) had statistically higher viral symptom severity ratings compared to Summer.

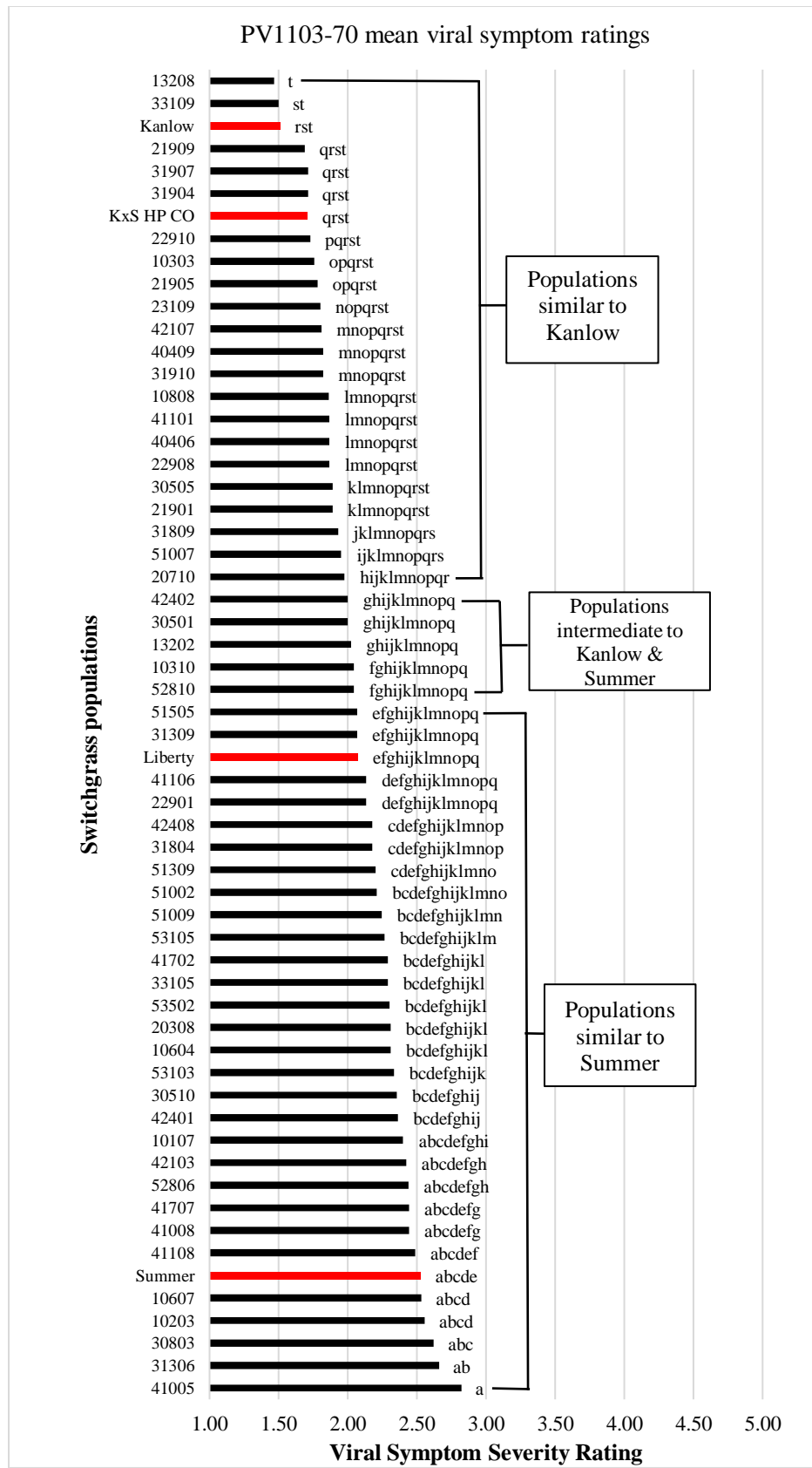


Figure 3.7. Mean viral symptom severity rating (1 to 5 scale) from the years 2013, 2014, and 2015, in experiment PV1103-70. Values sharing the same letter are not significantly different at the 95% confidence level. For sake of clarity, only half of the populations are presented to illustrate the variation among populations.

In experiment PV1013-21i, the ANOVA of the mean viral symptom severity ratings indicated highly significant ($P < 0.01$) Population and Year main effects and Population X Year interaction (Table 3.10).

Table 3.10. ANOVA of viral symptom rating results from experiment PV1013-21i

Factors	Df	F value	P value
Population	110	5.593	< 2e-16
Rep(Population)	111		
Year	2	533.1	0.00187
Population x Year	220	1.509	0.00129
Residual	222		

A wider range of virus ratings and higher virus ratings was found in PV1013-21i compared to the other two experiments (Figure 3.8). Some KxS populations exhibited significantly lower viral symptom ratings than other populations.

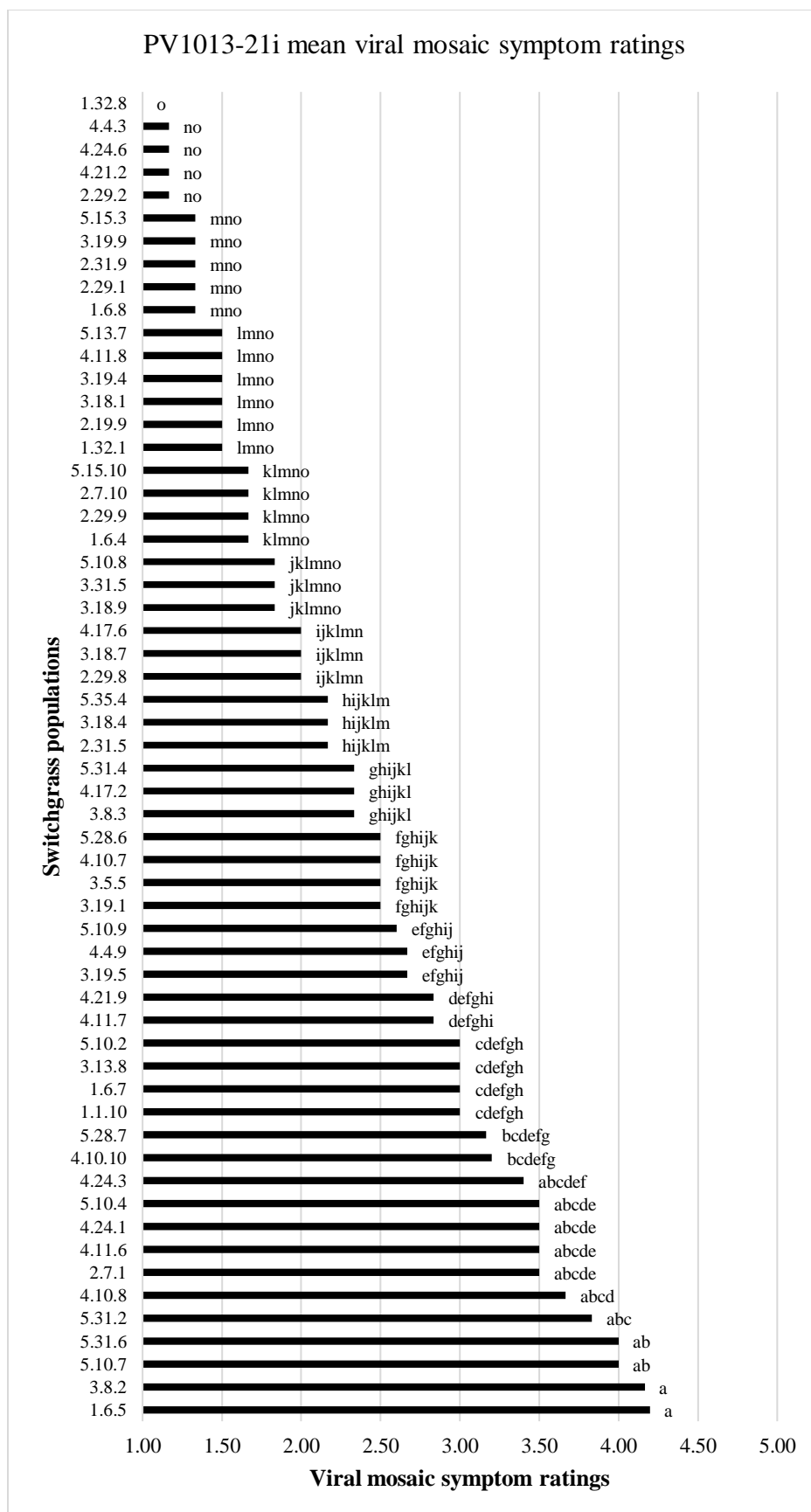


Figure 3.8. Mean viral symptom severity rating (1 to 5 scale) from the years 2016, 2017, and 2018, in experiment PV1013-21i. Values sharing the same letter are not significantly different at the 95% confidence level. For sake of clarity, only half of the populations are presented to illustrate the variation among populations.

The results from all three experiments indicated that there is considerable variation of viral symptom severity ratings among hybrid populations. In experiments PV1609-70 and PV1103-70 nearly half of the hybrid populations were as resistant to the resistant check population. The remaining hybrid populations were as susceptible to rust as the susceptible check, or had resistance intermediate of the resistant and susceptible checks.

Obj. 4: Viral mosaic response heritability

The results from the heritability correlation analysis examining viral symptom severity ratings between the 111 SxK population in crossing nursery PV1013-21i and the corresponding 111 half-sib families in PV1103-70 showed that there is a significant ($P = 0.00001$) positive correlation (correlation coefficient = 0.4786) between the parent populations in PV1013-21i) and progeny half-sib families in PV1103-70 (Figure 3.9). The R^2 value of 0.2281 suggests that the viral mosaic resistance inherited from the parent populations in PV1013-21i accounts for 23% of the variation in viral mosaic rating observed in PV1103-70.

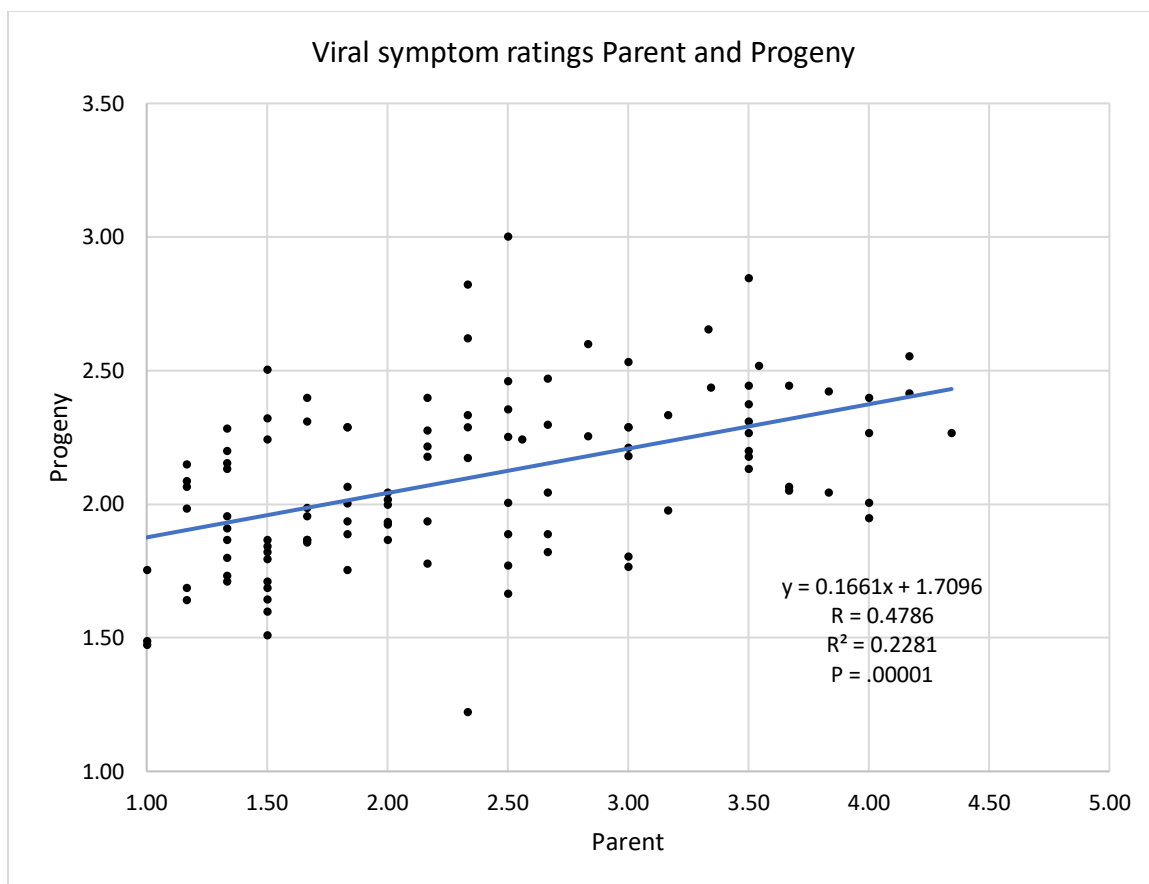


Figure 3.9. Heritability correlation map of parent population (PV1013-21i) and progeny populations (PV1103-70) to rust severity ratings.

Obj. 5: Linkage between rust and viral mosaic diseases

A correlation analysis was performed to determine if there is a relationship between rust severity and viral symptom severity ratings in the each of the three experiments. There was no correlation found in any experiment; correlation coefficients were <0.16 and P exceeded 0.37 in each experiment (Figure 3.10, 3.11, 3.12). These results indicate that the response to these two diseases among hybrid populations is not linked.

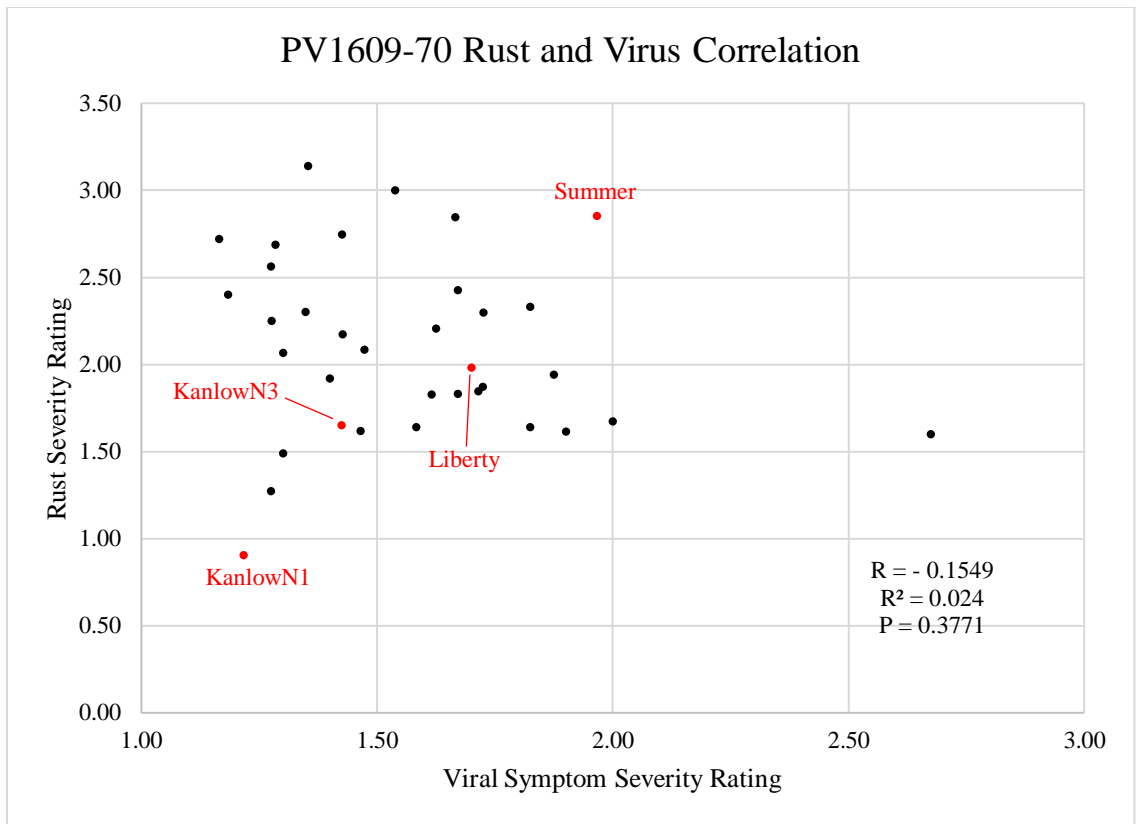


Figure 3.10. PV1609-70 Correlation map of KxS populations comparing rust severity ratings and viral symptoms ratings from 2017 and 2018.

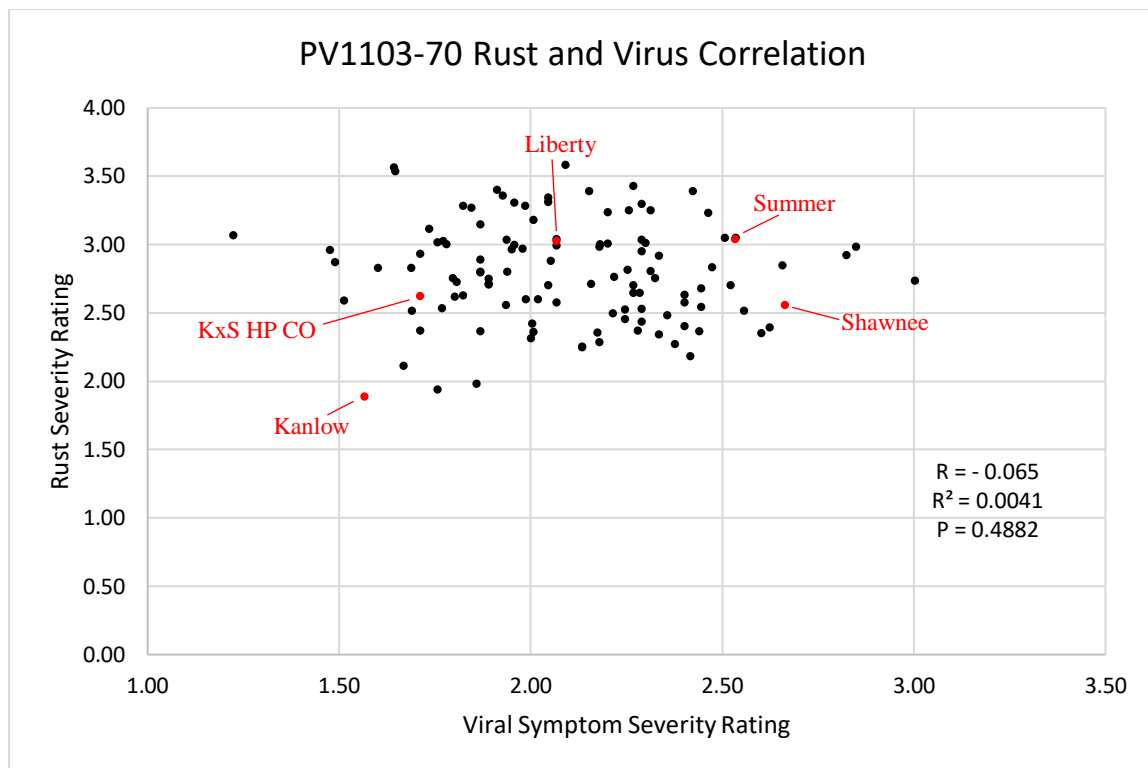


Figure 3.11. PV1103-70 Correlation map of SxK populations comparing rust severity ratings from 2016 – 2018 and viral symptoms ratings from 2013 - 2015.

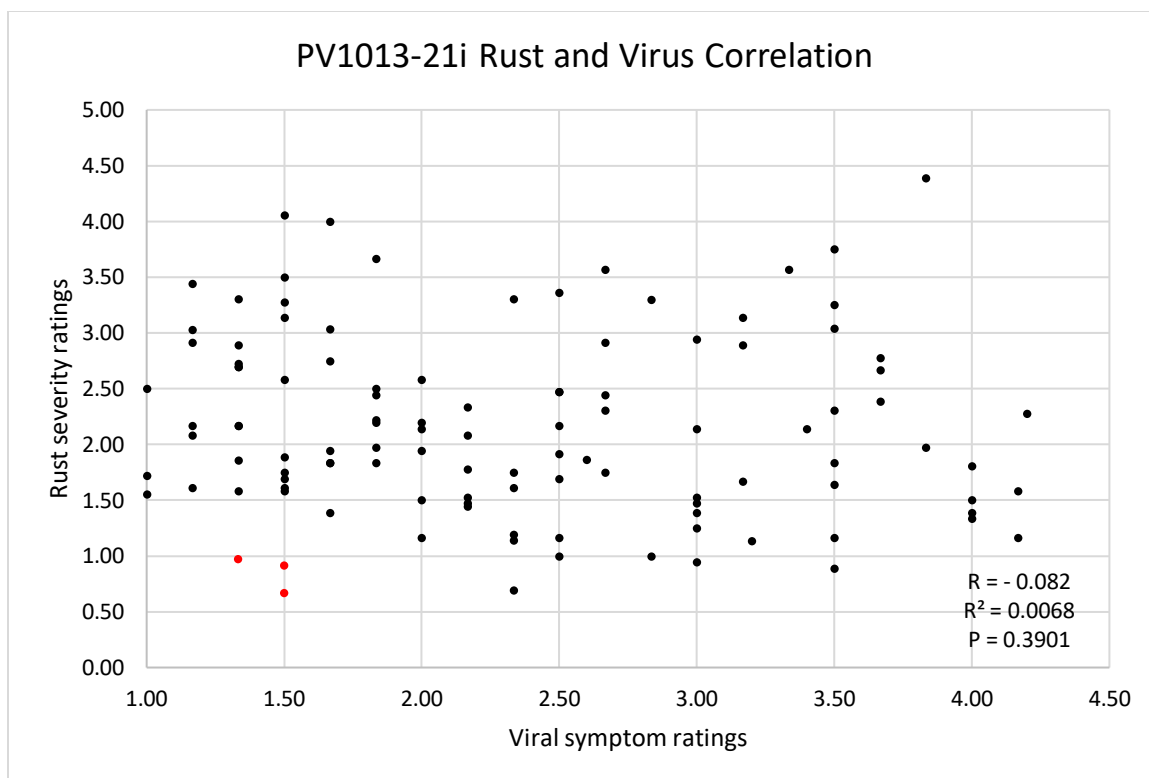


Figure 3.12. PV1013-21i Correlation map of SxK populations comparing rust severity ratings and viral symptoms ratings from 2016 - 2018. The highlighted populations indicate individual populations that have very low rust severity and viral symptom severity ratings.

Obj. 6: Identification of hybrid populations resistant to rust and viral mosaic diseases

In experiment PV1609-70, only one KxS population (11507) was demonstrated to have rust severity and viral symptom severity ratings statistically similar to the resistant check KanlowN1 (Table 3.11). Rust and virus symptom ratings in 11507 were significantly lower than in the susceptible check Summer but did not differ from those found in Liberty.

In experiment PV1103-70, three SxK populations (10808, 30506, and 33102) exhibited rust severity and viral symptom severity ratings that were statistically similar to

the resistant check Kanlow (Table 3.12). Disease levels in these three populations were significantly lower than those in Liberty and the Summer susceptible check.

Table 3.11. Rust severity ratings (left) and Viral symptom ratings (right) KxS populations in PV1609-70 compared to control populations.

Population	Rust Means	Sig. Letter	Population	Viral Means	Sig. Letter
KanlowN1	0.9	c	KanlowN1	1.2	b
11507	1.3	bc	11507	1.3	b
Liberty	1.6	b	Liberty	1.4	b
Summer	2.8	a	Summer	2.0	a

Table 3.12. Rust severity ratings (left) and Viral symptom ratings (right) SxK populations in PV1103-70 compared to control populations.

Population	Rust Means	Sig. Letter	Population	Viral Means	Sig. Letter
Kanlow	1.9	b	Kanlow	1.5	c
10808	2.0	b	10808	1.9	bc
30506	2.1	b	30506	1.7	bc
33102	1.9	b	33102	1.8	bc
Summer	3.0	a	Liberty	2.1	b
Liberty	3.1	a	Summer	2.5	a

In experiment PV1013-21i, three SxK populations (Figure 3.12) exhibited very low mean rust severity (<1 rating on 0 to 9 scale) and very low mean viral mosaic severity (<1.50 on 1 to 5 scale).

These results from these experiments collectively indicate that a small portion of the hybrid populations have resistance to both rust and viral mosaic diseases. Where Kanlow, or a Kanlow derivative, was planted for comparison, these populations exhibit the same degree of resistance to the diseases as the resistant check.

3.4) Discussion

While selection of hybrid (SxK or KxS) populations for increased biomass yield and environmental hardiness is an effective strategy method to improve switchgrass for biofuel production, it is also important to develop hybrid populations that are resistant to diseases such as rust caused by *Puccinia* spp. and viral mosaic disease caused by the *Panicum mosaic virus* complex.

The field study demonstrated the potential to improve rust and viral mosaic disease resistance by selecting hybrid populations with resistance to these diseases from among existing hybrid populations. A proportion of the hybrid populations evaluated in PV1609 and PV1103-70 exhibited similar responses to rust or to viral mosaic compared to the resistant check Kanlow or KanlowN1. In some cases, these populations exhibited lower disease levels than the commercially available hybrid Liberty. Although there were no resistant or susceptible check populations planted in PV PV1013-21i for comparison, some of the hybrid populations in that experiment exhibited very low rust or viral mosaic levels that were significantly different from the other populations in that experiment. Furthermore, hybrid populations were identified from all three experiments appearing to have resistance to both rust and viral mosaic.

It is important to point out that the results relating to rust in these experiments were obtained under relatively low disease pressure. Those populations that appear to be resistant to rust need to be evaluated further in locations with higher rust disease pressure. Populations that appear to be resistant to viral mosaic need to be evaluated further for the same reason. There is also an additional question that must be addressed in regards to

resistance to viral mosaic. Because of logistical difficulties, the plants in these experiments could not be evaluated for the presence of viral pathogens. Thus, it is uncertain whether a plant that was rated 1 for viral mosaic (i.e. virus symptom free) exhibited no symptoms because it is resistant to viral mosaic or because that particular plant escaped inoculation by viral pathogens. Therefore, any population that appears to be virus resistant in the field must be inoculated with viral pathogens under controlled conditions in order to verify resistance.

Once a hybrid population is verified to have resistance to rust and/or viral mosaic, that population would be a good candidate for further selection for other traits and for propagation. Because switchgrass is cross pollinated, care must be taken to cross resistant populations with resistant populations in the propagation process. Results from the heritability analysis comparing rust and virus results from PV1013-21i (111 SXX parent populations) and PV1103-70 (half-sib families derived from the 111 parent populations) indicated that only approximately 25% of the variability in disease levels in PV1103-70 can be accounted for by inheritance from parent populations in PV1013-21i. While differences in microclimate or disease pressure could have contributed to differences in disease levels between the two experiments, open pollination in PV1013-21i between populations with different levels of disease resistance was also a likely cause.

Another key finding from this study is the resistance/susceptibility to rust is unrelated to resistance/susceptibility to viral mosaic. This would suggest that resistance to each type of pathogen involves unique mechanisms controlled by genes that are inherited separately. The significance of this finding is that it shows that selection for resistance to

both rust and viral mosaic cannot be gained by screening for resistance to one type of resistance alone. As shown in objective 6, identification of populations with resistance to both types of disease is possible, but it requires that populations be evaluated for resistance to each pathogen separately.

In summary, this field study found large variation among hybrid switchgrass populations as to their response to rust and viral mosaic disease. The variation provides opportunities to select for populations with improved resistance to these diseases. It is important to continue to investigate switchgrass populations for disease resistance in future breeding programs in the field, as well as in greenhouse studies where environmental conditions and disease pressure can be more rigorously controlled.

3.5) References

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CHAPTER 4:

THESIS CONCLUSION

Switchgrass is considered a model perennial warm-season crop for biofuel and feedstock production. As switchgrass research continues and cultivation increases to meet the demands for renewable biofuel energy, it is vital to develop a management strategy that protects switchgrass production from diseases such as rust caused by *Puccinia* spp., and viral mosaic diseases such as *Panicum mosaic virus* (PMV) and *Satellite panicum mosaic virus* (SPMV). Thus far, the most effective method to manage rust and viral mosaic diseases in a low input system is to utilize host resistance. In order to improve this management method to viral mosaic diseases such as the PMV complex, it is important to understand how these viruses interact during infection and systemic spread within the host plant. In addition, it is important to screen switchgrass populations that express resistance to rust and viral mosaic diseases which then can be used in future breeding programs.

The growth chamber study in this thesis (Chapter Two) serves as an in-depth study examining PMV and effects from the co-infection with SPMV, host populations and temperature. The results indicate that while both switchgrass populations, Kanlow and Summer, are equally able to be infected by PMV and SPMV, Kanlow has the ability to prevent systemic spread from PMV alone or in combination of the co-infection compared to Summer. This could explain why Kanlow, under field conditions, is

observed to have less symptom development than Summer populations. The importance of this result, confirms that the Kanlow population is vital for switchgrass breeders that are trying to improve viral mosaic resistance to viral diseases such as PMV and SPMV. Not only does the population genotype play an important role in pathogen development, but there could be other factors that explain what is observed under field conditions such as extreme temperatures.

Another important finding from this study was that at higher temperature conditions the local infection frequency was significantly lower for the PMV+SPMV treatment compared to the other temperature ranges and viral treatments. This shows that extreme temperatures could have an effect on the SPMV virus. Due to this result, it raises the question if long periods of extreme temperatures could possibly contribute to lower biomass production when switchgrass plants are infected with PMV alone or in combination with the two viruses.

One of the most interesting findings that was observed from this growth chamber study is that there was no synergism between PMV and SPMV in switchgrass. Since this study contradicts what has previous been reported in regards to synergism between PMV and SPMV in millet species, it is important to determine if there is truly no synergism between the two viruses in switchgrass. In future studies to help confirm if synergism between PMV and SPMV in switchgrass truly exists, a more accurate method of quantifying PMV and SPMV viral titer is vital such as utilizing quantitative PCR. This is imperative because it can help switchgrass researchers determine if the SPMV virus is relevant when studying viral mosaic resistance.

The results from the field study in this thesis (Chapter Three) provides extensive information relating to switchgrass hybrid population resistance to rust and viral mosaic diseases. The results indicate that there is significant variation among hybrid populations in response to rust and viral symptom severity ratings. Few hybrid populations had statistically similar disease severity ratings to Kanlow and KanlowN1 check populations, which these hybrid populations could potentially be used to improve rust and viral mosaic disease resistance in future breeding programs.

When rust and viral symptom severity ratings were evaluated in a correlation analysis, the results indicated that there was no linkage between populations response to both diseases. This confirms that the genes responsible for resistance to rust are not linked, or are different genes than those responsible for viral mosaic resistance. In order to obtain a hybrid population that is resistant to both diseases each of the hybrid populations had to be compared to the Kanlow and KanlowN1 populations. The result shows that in PV1609-70 only 1 hybrid population out of 31 was statistically similar to KanlowN1 in response to rust and viral severity ratings, while in PV1103-70 3 hybrid populations out of 111 were statistically similar to the Kanlow check population for both disease severity ratings. This indicates that very few hybrid populations will obtain genes responsible for rust and viral mosaic resistance. These select populations should be cloned and evaluated under controlled conditions where high disease pressure can be applied. If the same hybrid populations remain consistent in their response to rust and viral mosaic diseases, then these populations would be vital in further breeding programs.

This field study also points out how important it is to use only disease resistant populations in breeding and propagating switchgrass. The results from the heritability

analysis comparing parent and progeny populations response to rust and viral mosaic indicated that approximately 25% of the variability in disease resistance observed in PV1103-70 was inherited from parent populations in PV1013-21i. The relatively low heritability analysis could be due to the fact that PV1013-21i contained populations that represented the continuum between resistant and highly susceptible to rust and viral mosaic. Because switchgrass is open pollinated there was genetic mixing among resistant and susceptible populations. More precise pollination methods could result in higher inherited disease resistance.

In summary, the findings from these two studies confirm that Kanlow has the potential to be a superior population for resistance to viral mosaic disease from PMV and SPMV, compared to Summer. In field conditions there is a large variation among switchgrass hybrid populations in their response to rust and viral mosaic diseases. The variation provides opportunities to select for hybrid populations with improved diseases resistance for future breeding programs.