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Distribution and Incidence of Mosaic and Evaluation of Susceptibility in Louisiana's Current Sugarcane Germplasm

Jancee Rice
Louisiana State University and Agricultural and Mechanical College, jrice@agcenter.lsu.edu

DISTRIBUTION AND INCIDENCE OF MOSAIC AND EVALUATION OF SUSCEPTIBILITY IN LOUISIANA'S CURRENT SUGARCANE GERMPLASM

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To my parents, Tricia and Glen, who have always provided their unconditional love, encouragement, and support in everything that I do.

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ABSTRACT

Mosaic is a viral disease of sugarcane caused primarily by Sorghum mosaic virus (SrMV) in Louisiana. Low mosaic incidence has resulted from successful breeding for resistance. However, mosaic was detected in breeding program experimental clones and a new cultivar, HoCP 09-804.Therefore, multiple research approaches were undertaken to assess the current status of mosaic in Louisiana sugarcane and prevent it from re-emerging as an important problem. Field surveys conducted during 2016-2018 of breeding program yield trials and experimental clone seed-cane increases determined disease incidence and distribution. Mosaic was detected in three of five sugarcane production areas and incidence ranged from 0 to 10% in HoCP 09-804. Symptomatic and asymptomatic leaves were tested for SrMV with reverse transcription polymerase chain reaction (RT-PCR). All symptomatic leaves tested positive for SrMV confirming it is the current causal virus species. A low percentage of asymptomatic leaves (0.3%) tested positive for SrMV. Runs analysis detected aggregation of infected plants in rows of surveyed fields. The geographic and within field distribution suggested the source of disease was infected seed-cane. Subsequent surveys of the same locations detected incidence increases and decreases in first ratoon, but incidence decreased for all in second ratoon. The results suggest high rates of disease increase due to aphid transmission are not occurring under current conditions. Recovery from mosaic was evaluated as the emergence of asymptomatic plants from symptomatic stalks. L 10-147 had a higher frequency of recovery (9.4-18.9%) than HoCP 09-804 (0.9-2.3%) across two experiments. RT-PCR failed to detect SrMV in 83% of HoCP 09-804 and 97% of L 10-147 of recovered plant samples. Comparison of asymptomatic and symptomatic stalk plantings found mosaic reduced yield in HoCP 09-804 but not L 10-147. Sources of susceptibility were evaluated in the basic and commercial parent populations by mechanical

inoculations. Mosaic susceptible parents were identified within both populations. The level of susceptibility within the basic parent population was low indicating it will continue to be a resource for continued introgression of mosaic resistance. The results will allow the elimination of sources of susceptibility and informed crossing to continue successful management of mosaic with host plant resistance.

CHAPTER I. LITERATURE REVIEW

1.1. History of Mosaic Disease of Sugarcane

Mosaic is one of the most widely distributed diseases of sugarcane (interspecific hybrids of *Saccharum* L.) (Abbott 1961b; Grisham 2000). The disease was first described by Musschenbroek in 1892, calling it 'gelestrepenziekte' or the yellow stripe disease (Agnihotri 1990). The term "mosaic" was not used until 1918 (Earle 1918). The exact origin of mosaic is unknown, but it was suggested that the virus likely originated from the same location as its host (Agnihotri 1990). The first *Saccharum officinarum* L. cultivars known as the "noble canes" originated from New Guinea where the disease is endemic, but the movement of seed-cane from Java (modern-day Indonesia) likely disseminated mosaic throughout the world before it was recognized as a disease (Agnihotri 1990). Dutch investigators at the time had erroneously concluded the disease to be a bud mutation, and other theories considered the symptoms to have genetic origins due to the inability of inoculation techniques at the time to transmit the disease (Abbott 1961b). Brandes (1919) first provided information on the disease's viral nature comparing it to that of mosaic in tobacco and other crops, along with the first evidence of aphid transmission by *Rhopalosiphum maidis* (syn. *Aphis maidis)* (Brandes 1919, 1920). The desire for new cultivars brought the disease to the United States and other countries, and from 1916 to 1925, outbreaks of mosaic were reported in Louisiana, Cuba, Puerto Rico, and Brazil (Agnihotri 1990). The Louisiana sugarcane industry nearly collapsed in the 1920s due to yield losses from mosaic in combination with Pythium root rot and red rot, a seed-cane rot caused by *Colletotrichum falcatum*, bringing attention to the need to breed for resistance (Koike and Gillaspie 1989). Replacing the noble cane cultivars, D 74, Louisiana Purple, and Louisiana Striped, with tolerant interspecific hybrids, POJ 36, POJ 213, and POJ 234, and then later resistant interspecific hybrids, Co 281 and Co 290, temporarily brought mosaic under control

(Koike and Gillaspie 1989). Incidence of mosaic in Louisiana was low in the 1940s and early 1950s due to destruction of heavily infected fields and rogueing to provide mosaic-free seed-cane. However, mosaic incidence increased with the emergence of a new strain, H, and became widespread with the cultivation of two cultivars with tolerance to the virus, NCo 310 and CP 65-357 (Koike and Gillaspie 1989). The breeding program initiated efforts to introgress resistance to mosaic into the sugarcane germplasm through breeding new interspecific hybrids using wild relatives of sugarcane (Grisham et al. 1992). *Saccharum spontaneum* and *Erianthus* clones were identified to have the highest levels of resistance (Grisham et al. 1992), and the release of a series of cultivars with mosaic resistance reduced mosaic to very low incidence. However, mosaic symptomatic plants were observed in an advanced experimental clone being considered for release, HoCP 09-804, and other advanced experimental clones during 2016. The history of mosaic in Louisiana reveals how the disease continues to remain a potential threat through a continuous cycle of evolution in virus strains and replacement of resistant cultivars (Koike and Gillaspie 1989). There was uncertainty as to whether the current mosaic outbreak was the result of another change in the virus.

1.2. Disease Characteristics of Mosaic

Mosaic is named after the primary symptom it causes which is diffuse mottling of contrasting shades of green and yellow resulting from varying concentrations of chlorophyll in the leaf blade (Figure 1.1). Some cultivar responses include reddening or necrosis on the leaf blade, and midrib reddening discoloration has been reported in certain cultivar and virus strain combinations (Grisham 2000; Koike and Gillaspie 1989). Symptoms are most visible in the basal portion of young, rapidly growing leaves (Grisham 2000). The host range of the virus is restricted to the

Poaceae, including sugarcane, maize, sorghum, and other cultivated and uncultivated grasses (Pirone 1972).

Figure 1.1. Characteristic symptoms of mosaic in a sugarcane leaf.

Mosaic is primarily spread from plant to plant by aphid vectors, as first shown by Brandes using the corn leaf aphid (*Rhopalosiphum maidis*) (Brandes 1920). Several additional aphid species have since been identified as virus vectors, including *Dactynotus ambrosiae*, *Hysteroneura setariae*, *Longiuguis saccari*, and *Toxoptera graminum* (Grisham 2000). The virus is acquired and transmitted by vectors in a non-persistent manner, meaning that the aphid's acquisition of the virus, as well as loss of infectivity, occurs rapidly within seconds to minutes (Hull 2002a). Aphids use a method of short probing with their stylet when testing suitability of a plant host (Hull 2002a). As they sample leaf sap from the epidermal cells of the leaf, virus particles can be acquired or transmitted into the plant (Hull 2002a). In addition, many aphids that vector non-persistent viruses are non-colonizers of the host plant of the virus, favoring spread of

the virus as the aphid searches for a host it can colonize, particularly during seasonal migrations (Hull 2002a).

Mechanical inoculation can be used for effective artificial transmission of the virus, but early attempts often failed (Koike and Gillaspie 1989). There are several conditions that must be favorable in order for an inoculation to be successful which include: infectivity of the leaf sap used, the susceptibility of the host, the method of inoculation used, and the growing conditions for the plants (Koike and Gillaspie 1989). Different extraction buffers can be used to facilitate transmission during the inoculation, and phosphate buffers are commonly used due to their ability to increase the virus' infectivity (Hull 2002b). Phosphate, sulfite, and combinations were found to enhance infectivity, but the donor plant used seemed to have a greater effect on the overall success of the inoculation (Dean 1978). This is supported by additional reports that mosaic is more readily transmitted to or from maize or sorghum than it is to or from sugarcane (Pirone 1972). Because of this, maize or sorghum are often used as the donor plants due to their higher virus titer and ease of cultivation (Koike and Gillaspie 1989). However, the method used to perform the inoculation can also influence the success of the inoculation. There are three different methods commonly used: pinpricking, air brush, and abrasive rubbing (Bird 1961; Brandes 1920; Koike and Gillaspie 1989; Srisink et al. 1994). The condition of the plants is also important since younger sugarcane plants are more susceptible to infection. Good growing conditions are needed to see symptoms quickly and easily and so that stress symptoms do not mask mosaic symptoms (Koike and Gillaspie 1989).

While aphids are mainly responsible for the spread of mosaic from plant to plant, the spread of mosaic from field to field may be caused by planting infected seed-cane (Grisham 2000). Harvesting methods, such as using knives or mechanical harvesters, are not considered to

significantly spread mosaic within fields (Koike and Gillaspie 1989). True seed transmission of mosaic has not been reported in sugarcane (Grisham 2000).

1.3. Virus Species and Strains

Sugarcane mosaic virus (SCMV) and Sorghum mosaic virus (SrMV) are the virus species (genus *Potyvirus*) that are currently considered the primary causal agents of sugarcane mosaic (Grisham 1994, 2000; Grisham and Pan 2007). These viruses are members of the potato virus Y group (Family: Potyviridae), and they consist of particles that are flexuous filaments 750 nm in length and 13 nm in diameter (Pirone 1972). Members of the Potyviridae contain genomes with positive-sense single-stranded RNA that are 8.5-10 kb in size (Hull 2002c). All genera of Potyviridae have a 5' VPg (viral protein genome linked) protein and a polyadenylated 3' end of their genomes (Hull 2002c). Potyviruses contain a single open reading frame which codes for a polyprotein. The polyprotein then self cleaves to produce the proteins necessary for replication (Hull 2002c).

Multiple strains of SCMV were identified, but recent taxonomic studies placed some previously described SCMV strains into the SrMV virus taxon (McKern et al. 1991; Shukla et al. 1989). Virus strains are designated by alphabetical letters, and strains A, B, D, E, H, I, and M have been described in Louisiana (Koike and Gillaspie 1989). Strain E is considered to have been the first strain in Louisiana and was responsible for the devastating infection of the noble cane cultivars (Koike and Gillaspie 1989). Following the introduction of POJ interspecific hybrid cultivars, strain D appeared in 1925, and strain B became common after 1930 (Koike and Gillaspie 1989). These two strains would continue to prevail in the POJ and Co cultivars grown in the 1930s to 1940s (Koike and Gillaspie 1989). Strain A was next discovered and was responsible for isolated outbreaks, but the destruction of infected fields and planting of mosaic

free seed-cane kept mosaic incidence low during this time (Koike and Gillaspie 1989). Strains H, I and M were described in Louisiana in 1956 (Abbott 1961a), 1966 (Tippett and Abbott 1968), and 1973 (Koike and Gillaspie 1976), respectively, and have since remained the predominant virus strains. These strains were considered members of SCMV at the time of their discovery. In 1989, it was proposed that 17 known strains of SCMV be placed into four distinct potyvirus groups (Johnsongrass mosaic virus, maize dwarf mosaic virus, Sorghum mosaic virus, and sugarcane mosaic virus) based on the analysis of the N-termini of the coat protein by electro-bot immunoassay with cross-absorbed polyclonal antibodies (Shukla et al. 1989). Additionally, based on this evidence, it was proposed that the previously described SCMV strains H, I, and M actually represent a distinct group of their own leading to their reclassification as SrMV (Shukla et al. 1989). High performance liquid chromatographic (HPLC) peptide profiling provided confirmation for the designation previously proposed by Shukla et al. (McKern et al. 1991). In recent field surveys, strains A, B, and D were not recovered in field samples, and this was attributed to resistance to these strains, which has been sufficient to eliminate them (Grisham 1994; Grisham and Pan 2007). Work conducted from 1978 to 1995 determined strain H to be the predominant strain in Louisiana by using host differentials to test collected leaf samples (Grisham 1994). However, in follow-up field surveys from 2001-2003, a shift in the strains causing sugarcane mosaic to strain I was confirmed by using reverse transcription polymerase chain reaction (RT-PCR) methods (Grisham and Pan 2007). Additionally, some samples from this study had mosaic symptoms, but did not test positive for either SCMV, SrMV or other identifiable strains (Grisham and Pan 2007). These detections of unidentified viruses associated with mosaic infections in the Louisiana sugarcane breeding program have led to concern that another strain shift might have occurred.

1.4. Detection Methods for Sugarcane Mosaic Virus and Sorghum Mosaic Virus

The visual observation of characteristic symptoms is considered the primary detection method for sugarcane mosaic (Grisham 2000). When inoculated with different strains of SCMV and SrMV, some cultivars were found to have specific reactions to virus strains, making them indicator plants of occurring strains (Summers et al. 1948). Host differentials were based on multiple symptoms, including the severity of mosaic, the presence of necrosis, and the stunting of growth (Summers et al. 1948). As new strains were detected, host differential methods were modified over time, but early molecular methods could not distinguish strains of SCMV and SrMV (Abbott and Tippett 1966; Grisham 1994). The first molecular detection method that could distinguish strains was a reverse transcription polymerase chain reaction with restriction fragment length polymorphism (RT-PCR RFLP) analysis (Yang and Mirkov 1997). The RT-PCR RFLP analysis was developed so that it would be possible to detect each virus species by RT-PCR and then determine strains with enzyme digests from RFLPs. Recent surveys in Louisiana collected samples with strains that were unidentifiable using the described RT-PCR RFLP method. If the presence of a new strain of SCMV or SrMV were to become predominant, a new primer set or method would need to be developed (Grisham and Pan 2007). To remove the time-consuming step of gel electrophoresis, a reverse transcription loop mediated isothermal amplification (RT-LAMP) assay was developed (Keizerweerd et al. 2015). This method was found to be less sensitive than the RT-PCR method developed by Yang and Mirkov, but it may be useful in large scale sampling where the lack of sensitivity is less of an issue (Keizerweerd et al. 2015).

1.5. Yield Loss from Mosaic and Disease Interactions

Yield loss from mosaic is variable and depends on the combination of cultivar and virus strain involved (Grisham 2000). When high levels of infection from SCMV strain D were present, sucrose yield was reduced in the cultivars NCo 376 and N 12 primarily by a reduction in stalk mass and population (Bailey and Fox 1987). Similarly, in the cultivars Co 740 and CoC 671, mosaic reduced bud germination, stalk populations, and sucrose yield (Viswanathan and Balamuralikrishnan 2005).

The yield loss impact of mosaic can be further amplified with synergistic or additive effects from interactions with other sugarcane diseases. The devastating losses from the 1920s mosaic outbreak were due to coinfections with Pythium rot and red rot (Koike and Gillaspie 1989). Later greenhouse studies on the interaction of SrMV strain H and *Pythium graminicola* observed an additive effect on the reduction in stalk height and weight in some cultivars, suggesting the presence of both pathogens could cause a greater reduction in yield under field conditions (Koike and Yang 1971). A combination of mosaic and ratoon stunting disease (RSD) in susceptible cultivars caused a reduction in bud germination, and the combination reduced stand in ratoon crops following freezing conditions (Steib and Chilton 1967). Mosaic and RSD coinfections in other field studies caused greater reductions in yield in the cultivars CP 61-37 and L 62-96, suggesting some cultivars may experience greater yield losses from additive or synergistic effects (Koike 1974).

1.6. Recovery from Mosaic

It was first thought in early work with mosaic that planting an infected stalk would yield only infected, symptomatic plants (Agnihotri 1990). The observation of the loss of symptoms in previously symptomatic plants was reported in early work by Brandes (1920). Research by

Summers et al. (1948) confirmed the observations previously made and even used the occurrence of recovery from mosaic as part of the host differentials (Summers et al. 1948). There are two types of recovery that have been described for mosaic in sugarcane. One termed "germination recovery" is the phenomenon in which an asymptomatic shoot develops from an axillary bud of an infected stalk. In contrast, "foliar recovery" occurs when a previously symptomatic plant loses symptoms in new developing leaves during the growing season (Agnihotri 1990; Benda 1970; Summers et al. 1948). Early research into recovery from mosaic focused on the frequency of its occurrence among cultivars and characteristics of recovery within an individual plant. Incidence of mosaic was found to decrease over crop cycles in cultivars known to recover; however, with the occurrence of secondary infections (due to aphids) in the field, it could be more difficult to determine the extent of recovery (Summers et al. 1948). The extent of recovery was evaluated from cuttings, and it was found that cultivars also varied in the extent of recovery for plants developing from the buds of a stalk (Summers et al. 1948). In older literature, these recovered plants are often described as uninfected, but sensitive molecular assays could provide additional evidence of whether recovered plants are no longer infected by the virus. Bio-assays have been used to test the infectivity of recovered plants, and most of the plants were not capable of producing symptoms in uninfected plants when sap was used as inoculum for mechanical inoculation (Benda 1974). It is still unclear whether the virus is no longer present or if there are undetectable titers of virus in mosaic recovered plants, and the frequency and characteristics of recovery in modern cultivars being grown today is unknown.

Recovery from plant virus symptoms has been reported with a variety of crops and viral diseases. The selection of asymptomatic planting material that is ideally virus-free is important for perennial crops, such as sugarcane and sweetpotato. When sweetpotato cultivars were tested

for the presence of sweet potato feathery mottle virus (SPFMV) over a 10-week-period, some cultivars eventually tested negative for SPFMV by graft inoculation (Gibson et al. 2014). These results show similarity to the characteristics of recovery in sugarcane, in that there is a difference in the frequency of recovery among cultivars. The detection of virus in recovered plants is variable when comparing different plant species. In the perennial crop, cranberry, plants infected with tobacco streak virus (TSV) with symptomatic fruits that tested virus-positive in one year produced asymptomatic fruits in subsequent years. However, the plants still tested virus-positive through ELISA and RT-PCR methods in the second year (Wells-Hansen and McManus 2016). In both the SPFMV and TSV recovery situations, recovered plants can still have detectable amounts of virus.

The underlying mechanisms for recovery from virus infection are likely due to the same mechanisms utilized by the plant defense system against viruses. RNA silencing has become recognized as an adaptive plant defense system that can respond systemically to virus infections (Voinnet 2001). RNA silencing is the process of post-transcriptional control of gene expression that is initiated by double-stranded RNA which is degraded into smaller fragments of RNA (Voinnet 2001). Further justification that RNA silencing is the plant defense system against viruses includes that the majority of plant viruses can also produce proteins capable of suppressing RNA silencing, which would be necessary for the virus to continue to spread throughout the plant and to other plants (Voinnet 2001).

1.7. Objectives

Recent detections of mosaic in cultivars and advanced experimental clones of the Louisiana sugarcane breeding program caused mosaic to become a research priority. Mosaic has been recently been detected in breeding program clones solely through natural infection, but inoculum

pressure has been reduced by the widespread cultivation of resistant cultivars. An evaluation of the status of the disease in Louisiana and resistance frequency and levels in the current breeding parental germplasm would help ensure that mosaic does not re-emerge as an important problem. The objectives of this investigation were as follows:

a) To determine the current distribution and incidence for sugarcane mosaic using field surveys and determine the causal virus species by RT-PCR. Changes in incidence and rates of increase will then be determined in fields with infected plants during subsequent surveys of ratoon crops in the following two seasons.
b) To further investigate the yield impact and frequency of recovery from mosaic in two modern clones and determine if virus is detectable in recovered plants by RT-PCR.
c) To evaluate the current commercial and basic recurrent parents of the sugarcane breeding program for susceptibility to mosaic using mechanical inoculations in a greenhouse setting.

CHAPTER II. SUGARCANE MOSAIC DISTRIBUTION, INCIDENCE, INCREASE, AND SPATIAL PATTERN IN LOUISIANA

2.1. Introduction

Mosaic is a viral disease of sugarcane (interspecific hybrids of *Saccharum* L.) with worldwide distribution (Grisham 2000). The disease is caused by strains of sugarcane mosaic virus (SCMV) and Sorghum mosaic virus (SrMV), both members of the Potyviridae (Grisham 1994; Grisham and Pan 2007). Originally, all virus strains were described as strains of SCMV, but taxonomic analyses of serological and chemical properties reassigned strains H, I, and M to SrMV (McKern et al. 1991; Shukla et al. 1989).

Mosaic has a long history in Louisiana and is known for nearly bankrupting the state's sugar industry in the 1920s (Koike and Gillaspie 1989). Since then, there has been an active effort to manage mosaic through breeding and selection for host plant resistance. The introduction of interspecific hybrids to replace the original *S. officinarum* cultivars resuscitated the industry. However, periodic outbreaks of mosaic have occurred due to virus strain changes associated with the cultivars being grown (Koike and Gillaspie 1989). Virus tolerant cultivars were grown with high incidence of mosaic from the 1950s to the mid-1990s. Annual surveys for mosaic strains were discontinued after it was determined that SrMV strain H had been the predominant strain from 1985-1995 until susceptibility was detected for clones in the later stages of the Louisiana sugarcane breeding program in the early 2000s (Grisham and Pan 2007). Surveys conducted from 2001-2003 concluded another strain shift had occurred and that SrMV strain I had become the predominant strain (Grisham and Pan 2007).

Currently grown commercial cultivars are rated as resistant to mosaic. However, in 2016, symptoms of mosaic were observed in an advanced experimental clone, HoCP 09-804, that was

being considered for commercial release and other advanced experimental clones in later stages of the sugarcane breeding program. Because sugarcane is clonally propagated, it is necessary to vegetatively increase experimental clones prior to release of a new cultivar to farmers. In this process in Louisiana, seed-cane increase fields are planted initially at three Primary Increase Stations and then 42 Secondary Increase Stations on cooperating commercial farms. Experimental clones also are evaluated by the breeding program in multiple-year yield trials at 12 commercial farms in different regions of the industry. The widespread presence of mosaic in experimental clone evaluation and seed-cane increase plots could pose a threat for spread of the virus into commercial plantings and the re-establishment of the disease in the industry. The detection of mosaic symptomatic plants suggested a need to evaluate the current incidence and distribution of mosaic in Louisiana in order to assess the threat to the sugarcane industry and determine an appropriate management strategy.

Field surveys of mosaic incidence in HoCP 09-804 and other experimental clones were needed to determine the incidence and distribution of the disease and then potential rates of increase could be determined in subsequent ratoon crops. Surveys to determine mosaic incidence are based on visual observation of characteristic symptoms consisting of patterns of contrasting shades of green in young leaves (Grisham 2000). The reliability of symptom expression as an indication of virus infection is thought to be high, but the degree of correlation between symptom expression and virus infection is uncertain. A reverse transcription polymerase chain reaction (RT-PCR) method capable of differentially detecting the two viruses associated with mosaic (Yang and Mirkov 1997) could be used to determine the virus species causing the current outbreak and determine the degree of reliability of survey results.

Mosaic is transmitted in a non-persistent manner by aphids and also spread from field to field through the planting of infected seed-cane (Grisham 2000). Determining whether the source of mosaic was due to aphid transmission or from planting infected seed-cane could be pertinent for developing an effective management response to the current outbreak. Sugarcane is planted as whole stalks in Louisiana, so planting of infected seed-cane would result in aggregation of infected plants. Therefore, an evaluation of the degree of within-row aggregation in fields at multiple locations could provide information concerning the relative importance of infection due to virus-infected seed-cane or aphid transmission. Ordinary runs analysis (Madden et al. 1982) would be appropriate to determine the randomness or aggregation of infected plants within sugarcane rows in a field and evaluate the potential role of seed-cane in disease spread.

2.2. Objectives

The objectives of the study were to determine the current distribution and incidence of mosaic in Louisiana sugarcane by field surveys, to determine changes in incidence and rates of increase in the same fields over two subsequent seasons in ratoon crops, to determine the causal virus species and reliability of visual symptoms as an indicator of virus infection using RT-PCR, and to evaluate the degree of within row aggregation of infected plants.

2.3. Materials and Methods

In 2016, fields of experimental clones in plant cane (first year crop) and first ratoon located at three Primary and 34 Secondary Stations managed by the American Sugar Cane League Variety Release Program and nine Outfield yield trials of the Louisiana sugarcane breeding program located on commercial farms were surveyed for incidence of mosaic. Geographic distribution was evaluated by monitoring mosaic incidence in five production areas: Bayou Teche (western), North, Upper Mississippi River, Lower Mississippi, and Bayou Lafourche. HoCP 09-804 was the

only clone surveyed at the Secondary Stations, 35 experimental clones were surveyed at each Primary Station, and 22 experimental clones were surveyed at each Outfield trial. All fields were planted with whole stalks with a two running stalk planting rate.

Surveys were based on the visual observation of characteristic mosaic symptoms in the young leaves of young plants prior to stalk elongation during May. Consistency in the ability to detect symptomatic plants by survey personnel was provided by assessing a common row at locations prior to conducting field surveys. The number of symptomatic plants was recorded for different arbitrarily selected rows at each location in "runs" (single plants and aggregations of more than one symptomatic plant). Total area surveyed was determined, and the overall percentage of infection was calculated for each location by dividing the total number of symptomatic plants recorded by the plant population in fields using an estimate of 6.6 plants per meter of row.

HoCP 09-804 fields at seven Secondary Stations (Raceland, Cedar Grove, Alma, Glendale, Glenwood, Little Texas, and Blackberry) where mosaic was detected were resurveyed in 2017 in first ratoon and 2018 in second ratoon by repeating the survey in the same rows as the first year. Disease rates of increase were determined by calculating the percent change in initial incidence from plant cane to first ratoon and from plant cane to second ratoon.

Symptomatic plant runs within rows were evaluated for aggregation using ordinary runs analysis (Madden et al. 1982). Z aggregation statistic values were calculated for each row at a location. Significant aggregation was considered at Z aggregation values less than -1.64 ($p = 0.05$). The percentage of rows exhibiting aggregation was then calculated for each location, and the frequency of runs for categories of increasing numbers of symptomatic plants per run was compared (Campbell and Madden 1990).

Single young leaf samples were arbitrarily collected from individual mosaic symptomatic and asymptomatic plants at each location for SrMV and SCMV detection by RT-PCR (Yang and Mirkov 1997). Samples were placed in a plastic bag on ice to return to the lab and stored at -70°C until RNA extraction was performed. Total RNA was extracted using the Plant Total RNA Kit (Spectrum™, Sigma Aldrich) with modifications to the tissue homogenization steps. Approximately 300 mg of tissue was homogenized in a BIOREBA extraction bag (BIOREBA AG, Switzerland) with 2 ml prepared lysis buffer from the Plant Total RNA Kit (Spectrum™, Sigma Aldrich) using a BIOREBA standard rack tissue homogenizer. RT-PCR was carried out in two steps using a modification of the RT-PCR method described by Yang and Mirkov (1997). Complementary DNA synthesis was performed using the SuperScript™ First-Strand Synthesis System (Invitrogen™, Thermo Fisher Scientific), and RNA was primed with 1 µl (2 µM stock) of SrMV-R3 or SCMV-R3. The 2 µl of diluted cDNA product in nuclease free water (1:50) was added to a 25 µl PCR solution. The PCR solution consisted of 12.5 µl of GoTaq® Green Master Mix, 2X (Promega), 11.86 µl of nuclease free water, 0.25 µl (10 µM stock) of SrMV-F3 and SrMV-R3 or SCMV-F3 and SCMV-R3, 0.14 µl of bovine serum albumin V (100 µg/µl). The PCR program used for SrMV was 95°C for 2 min; 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1 min; and final extension at 72°C for 5 min. The PCR program used for SCMV was 95°C for 2 min; 35 cycles of 95°C for 30 s, 51°C for 30 s, 72°C for 1 min, and final extension at 72°C for 5 min. RT-PCR products were electrophoresed in 2.0% agarose gel containing ethidium bromide (final concentration 0.35 µg/ml) for 1 h, and bands were visualized using a UV transilluminator. Presence of a visible band in an electrophoresis gel at 871 bp for SrMV and either 873, 885, or 897 bp for SCMV was considered a positive test result.

Symptomatic samples were tested for SrMV from nine clones: HoCP 09-804 (193 total samples), L 10-147 (24), L 11-183 (15), Ho 11-532 (21), Ho 11-573 (1), Ho 12-626 (1), Ho 12-671 (3), L 13-242 (2), and L 13-269 (4). Asymptomatic samples were tested for SrMV from eight clones: HoCP 09-804 (250 total samples), L 10-147 (14), L 11-183 (13), Ho 11-532 (23), Ho 11-573 (1), Ho 12-626 (1), Ho 12-671 (4), and L 13-242 (2). The assay for SCMV was performed for a subsample of symptomatic HoCP 09-804 (84) and a subsample of asymptomatic samples from all clones (306) (two asymptomatic samples from 2018 were not tested for SCMV).

2.4. Results

Incidence of mosaic in the 2016 survey for HoCP 09-804 in Secondary Station increase fields ranged from 0 to 3.5%, except for two fields at Little Texas that had incidences of 9.0 and 10.4% (Table 2.1). In the Bayou Teche and North areas, no mosaic was detected, whereas three areas along the Mississippi River and Bayou Lafourche had locations with and without mosaic incidence (Table 2.1). In fields at the three Primary Stations, mosaic was detected in a total of six of the 35 (17.1%) experimental clones. Percent symptomatic plants was calculated for each clone when mosaic was detected: L 10-147 (10.1% at one of three locations), L 11-183 (0.4% at one location), Ho 11-512 (0.5% at one location), Ho 11-532 (1.4% at one location and 0.6% at a second location), Ho 12-626 (0.1% at two locations), and HoCP 12-671 (0.2% at one location). In the breeding program yield trials, mosaic was detected in four of the 22 (18.2%) experimental clones across nine locations, and the percent infected plants was calculated in each case: L 10-147 (2.2, 11.6, 11.6, 23.9, 27.2, 28.2, and 31.9%), L 13-263 (3.6%), L 13-242 (1.4%), and Ho 13-769 (4.3%).

Table 2.1. Mosaic incidence detected by field surveys of HoCP 09-804 in Secondary Station seed-cane increase fields for the American Sugarcane League Variety Release Program during 2016.

Area/parish	Crop cycle year	Location	Field area (ha)	Area surveyed	Symptomatic plants	Estimated incidence
Bayou Teche and North						
Iberia Parish	First ratoon	Hebert	0.1	100%	0	0%
Rapides Parish	Plant cane	Harper	2.0	30%	0	0%
St. Martin Parish	First ratoon	Berard	0.1	100%	0	0%
St. Martin Parish	First ratoon	Levert St. John	0.2	100%	0	0%
St. Mary Parish	First ratoon	Adeline	0.6	56%	0	0%
St. Mary Parish	First ratoon	Breaux Brothers	0.2	90%	0	0%
St. Mary Parish	First ratoon	Judice	0.1	100%	0	0%
St. Mary Parish	First ratoon	North Side	0.1	100%	0	0%
St. Mary Parish	First ratoon	Sterling	0.2	100%	0	0%
Vermilion Parish	First ratoon	Domingues	0.2	67%	0	0%
Vermilion Parish	First ratoon	Duplantis	0.2	100%	0	0%
Upper Mississippi River						
Pointe Coupee Parish	Plant cane	Alma	0.6	23%	569	2.5%
Pointe Coupee Parish	Plant cane	LaCour	0.8	44%	55	0.2%
West Baton Rouge Parish	Plant cane	Morris	1.0	50%	74	0.2%
Pointe Coupee Parish	First ratoon	Beaud	0.3	100%	15	<0.1%
Iberville Parish	First ratoon	Landry	0.3	67%	0	0%
Iberville Parish	First ratoon	Pearce	0.4	52%	0	0%
Iberville Parish	First ratoon	St. Louis	0.3	100%	0	0%
Lower Mississippi River and Bayou Lafourche						
Lafourche Parish	Plant cane	Little Texas 1	0.4	24%	1,627	10.4%
Lafourche Parish	Plant cane	Little Texas 2	0.4	100%	1,231	9%
Assumption Parish	Plant cane	Glenwood 1	1.6	100%	411	1.4%
Assumption Parish	Plant cane	Cedar Grove	0.5	27%	228	1.3%

(table cont'd)

Area/parish	Crop cycle year	Location	Field area (ha)	Area surveyed	Symptomatic plants	Estimated incidence
Lafourche Parish	Plant cane	Raceland	0.6	44%	264	1.2%
Assumption Parish	Plant cane	Thibodaux Brothers Goldmine	0.6	48%	214	0.9%
St. John Parish	Plant cane	Glendale	0.6	28%	188	0.9%
Lafourche Parish	Plant cane	McCloud	1.8	33%	334	0.6%
St. James Parish	Plant cane	Blackberry	1.0	30%	171	0.5%
Assumption Parish	Plant cane	Glenwood 2	0.2	30%	21	0.3%
Terrebonne Parish	Plant cane	Naquin	0.6	29%	54	0.2%
Ascension Parish	Plant cane	Palo Alto	0.6	29%	34	<0.1%
Lafourche Parish	Plant cane	Knight	0.7	33%	21	<0.1%
Assumption Parish	Plant cane	Belle Alliance	1.2	-	12	<0.1%
St. James Parish	Plant cane	Martin and Poche	0.6	25%	8	<0.1%
Assumption Parish	Plant cane	Glenwood	0.2	57%	4	<0.1%
St. James Parish	First ratoon	Bon Secour	0.2	100%	0	0%

Leaf samples from HoCP 09-804, L 10-147, L 11-183, Ho 11-532, Ho 11-573, Ho 12-626, Ho 12-671, L 13-242, and L 13-269 were tested for SrMV by RT-PCR. The number of samples collected and results from each surveyed location is provided in Appendix A.1. All 264 symptomatic leaf samples from all experimental clones tested across three seasons were positive for SrMV. Only one of 250 (0.4%) asymptomatic samples from HoCP 09-804 tested positive; all other asymptomatic samples were negative for SrMV (Table 2.2). A total of 306 asymptomatic samples from multiple experimental clones and 84 symptomatic samples from HoCP 09-804 tested negative for SCMV by RT-PCR.

The runs analysis of 17 fields of HoCP 09-804 detected aggregation of symptomatic plants within at least 70% of the rows for 16 out of the 17 (94.1%) locations, and the mean number of plants in a run ranged from 1.2 to 4.7 (Table 2.3). Six categories of numbers of plants per run (1, 2, 3-6, 7-12, 13-24, and >24 symptomatic plants per run) were used to evaluate the occurrence of runs with increasing numbers of plants (Table 2.3). The percentages for single plants ranged from 11 to 79%, two plants per run ranged from 16 to 40%, 3-6 plants per run ranged from 5 to 68%, 7-12 plants per run ranged from 0 to 23%, 13-24 plants per run ranged from 0 to 10%, and more than 24 plants ranged from 0 to 2% (Table 2.4). Overall, symptomatic plants occurred in runs of more than one plant 64% of the time, but only 12% of runs consisted of more than six plants and 4% more than 12 plants (Table 2.4).

Table 2.2. RT-PCR results for SrMV detection in mosaic symptomatic and asymptomatic leaves collected from advanced experimental sugarcane clones during field surveys conducted from 2016 - 2018.

Year	Clone	Number of asymptomatic samples	Number SrMV positive (%)	Number of symptomatic samples	Number SrMV positive (%)
2016	HoCP 09-804	168	0 (0)	115	115 (100)
2016	L 10-147	14	0 (0)	24	24 (100)
2016	L 11-183	5	0(0)	5	5 (100)
2016	Ho 11-532	12	0 (0)	11	11 (100)
2016	Ho 12-626	1	0 (0)	1	1 (100)
2016	Ho 12-671	4	0 (0)	3	3 (100)
2016	L 13-242	2	0 (0)	2	2 (100)
2016	L 13-269	-	-	4	4 (100)
2017	HoCP 09-804	82	1 (1.2)	78	78 (100)
2017	L 11-183	6	0 (0)	6	6 (100)
2017	Ho 11-532	11	0 (0)	10	10 (100)
2017	Ho 11-573	1	0 (0)	1	1 (100)
2018	L 11-183	2	0 (0)	4	4 (100)
Total	HoCP 09-804	250	1 (0.4)	193	193 (100)
Total	All clones	308	1 (0.3)	264	264 (100)

Table 2.3. Results of runs analysis to evaluate aggregation of mosaic symptomatic plants within rows of HoCP 09-804 in fields surveyed during 2016.

Parish	Location	Cultivar	Mean plants per run	Rows with aggregation	Z[a] range
Assumption	Little Texas	L 11-532	4.7	6/7 (86%)	-38.4 to 1.5
Pointe Coupee	Alma 1	HoCP 09-804	4	5/5 (100%)	-29.4 to -10.2
Pointe Coupee	LaCour	HoCP 09-804	4	7/7 (100%)	-35.4 to -14.2
Assumption	Thibodaux Brothers French	HoCP 09-804	3.9	8/8 (100%)	-37.5 to -11.8
St. John	Glendale	HoCP 09-804	3.3	8/8 (100%)	-34.8 to -14.9
Assumption	Little Texas 1	HoCP 09-804	3.2	7/7 (100%)	-33.9 to -14.1
Assumption	Little Texas 2	HoCP 09-804	3.1	8/8 (100%)	-33.4 to -23.0
Pointe Coupee	Alma 2	HoCP 09-804	3	13/15 (87%)	-31.6 to 1.3
Terrebonne	Naquin	HoCP 09-804	2.8	8/8 (100%)	-32.7 to -21.9
Assumption	Little Texas	L 11-183	2.5	7/7 (100%)	-30.1 to -15.1
Lafourche	McCloud	HoCP 09-804	2.4	23/28 (82%)	-35.6 to 1.6
St. James	Blackberry	HoCP 09-804	2.2	12/14 (86%)	-31.9 to 1.6
West Baton Rouge	Morris	HoCP 09-804	2.2	6/6 (100%)	-45.2 to -15.6
Lafourche	Raceland	HoCP 09-804	2.1	10/11 (91%)	-24.8 to 1.2
Assumption	Glenwood 1	HoCP 09-804	1.8	25/33 (76%)	-22.4 to 1.2
Assumption	Cedar Grove	HoCP 09-804	1.5	7/10 (70%)	-27.6 to 0.50
Assumption	Glenwood 2	HoCP 09-804	1.2	3/9 (33%)	-14.4 to 1.1

[a] Z aggregation statistic values were calculated for each row at a location; values less than -1.64 ($p = 0.05$) were considered to exhibit significant aggregation.

Table 2.4. Runs of mosaic symptomatic plants recorded by categories with increasing numbers of plants per run in HoCP 09-804 field surveys conducted during 2016.

Parish	Location	Clone	Runs grouped by categories for number of symptomatic plants per run (%)					
			1	2	3-6	7-12	13-24	>24
Assumption	Cedar Grove	HoCP 09-804	43 (64)	14 (21)	9 (13)	1 (2)	0 (0)	0 (0)
Assumption	Glenwood 1	HoCP 09-804	74 (58)	25 (20)	27 (21)	1 (1)	0 (0)	0 (0)
Assumption	Glenwood 2	HoCP 09-804	15 (79)	3 (16)	1 (5)	0 (0)	0 (0)	0 (0)
Assumption	Little Texas	HoCP 09-804	157 (34)	104 (23)	140 (30)	46 (10)	16 (4)	0 (0)
Assumption	Little Texas	HoCP 09-804	99 (35)	58 (20)	73 (25)	34 (12)	19 (7)	4 (1)
Assumption	Thibodaux Brothers French	HoCP 09-804	42 (27)	31 (20)	58 (37)	19 (12)	5 (3)	0 (0)
Lafourche	McCloud	HoCP 09-804	48 (36)	33 (25)	45 (34)	7 (5)	0 (0)	1 (1)
Lafourche	Raceland	HoCP 09-804	63 (54)	31 (27)	16 (14)	5 (4)	1 (1)	0 (0)
Pointe Coupee	Alma 1	HoCP 09-804	15 (16)	23 (25)	39 (42)	11 (12)	4 (4)	2 (2)
Pointe Coupee	Alma 2	HoCP 09-804	31 (26)	26 (22)	39 (33)	16 (13)	6 (5)	2 (2)
Pointe Coupee	LaCour	HoCP 09-804	3 (23)	3 (23)	4 (31)	3 (23)	0 (0)	0 (0)
St. James	Blackberry	HoCP 09-804	33 (41)	23 (28)	23 (28)	2 (3)	0 (0)	0 (0)
St. John	Glendale	HoCP 09-804	21 (38)	14 (26)	16 (29)	3 (6)	1 (2)	0 (0)
Terrebonne	Naquin	HoCP 09-804	2 (11)	4 (21)	13 (68)	0 (0)	0 (0)	0 (0)
West Baton Rouge	Morris	HoCP 09-804	7 (25)	11 (40)	9 (32)	0 (0)	1 (4)	0 (0)
Assumption	Little Texas	L 11-183	7 (26)	8 (30)	12 (44)	0 (0)	0 (0)	0 (0)
Assumption	Little Texas	L 11-532	10 (20)	5 (10)	20 (41)	9 (18)	5 (10)	0 (0)
	Total		669 (36)	416 (23)	544(29)	157(8)	58 (3)	9 (1)

In first ratoon during 2017, disease incidence in different fields both increased and decreased, but in second ratoon during 2018, all locations experienced a decrease in disease levels from the 2016 initial infection incidence (Table 2.5 and Figure 2.1). Changes in mosaic incidence from plant cane to first ratoon for the seven locations ranged from -8.8% to +98% and from -44% to -100% in second ratoon (Table 2.5). The greatest increase of disease was observed in 2017 at Raceland, where the initial incidence increased from 4.0 to 7.9%.

Table 2.5. Change in incidence of mosaic symptomatic plants in fields of HoCP 09-804 from plant cane to first and second ratoon.

Parish (location)	2016 Initial infection incidence in plant cane[a]	2017 Infection incidence and percent change in first ratoon	2018 Infection incidence and percent change in second ratoon[b]
Assumption (Cedar Grove)	1.0%	1.6% (+60%)	0.3% (-70%)
Assumption (Glenwood)	1.0%	0.7% (-30%)	0.0% (-100%)
Assumption (Little Texas)	6.8%	4.1% (-39%)	1.0% (-85%)
Lafourche (Raceland)	4.0%	7.9% (+98%)	2.0% (-50%)
Pointe Coupee (Alma)	6.8%	6.2% (-8.8%)	2.6% (-62%)
St. James (Blackberry)	0.9%	0.4% (-56%)	0.5% (-44%)
St. John (Glendale)	1.6%	1.2% (-25%)	0.2% (-88%)

[a] Initial infection percentages calculated only from rows that were resurveyed.
[b] Percent change is calculated from the change in incidence from plant cane to second ratoon.

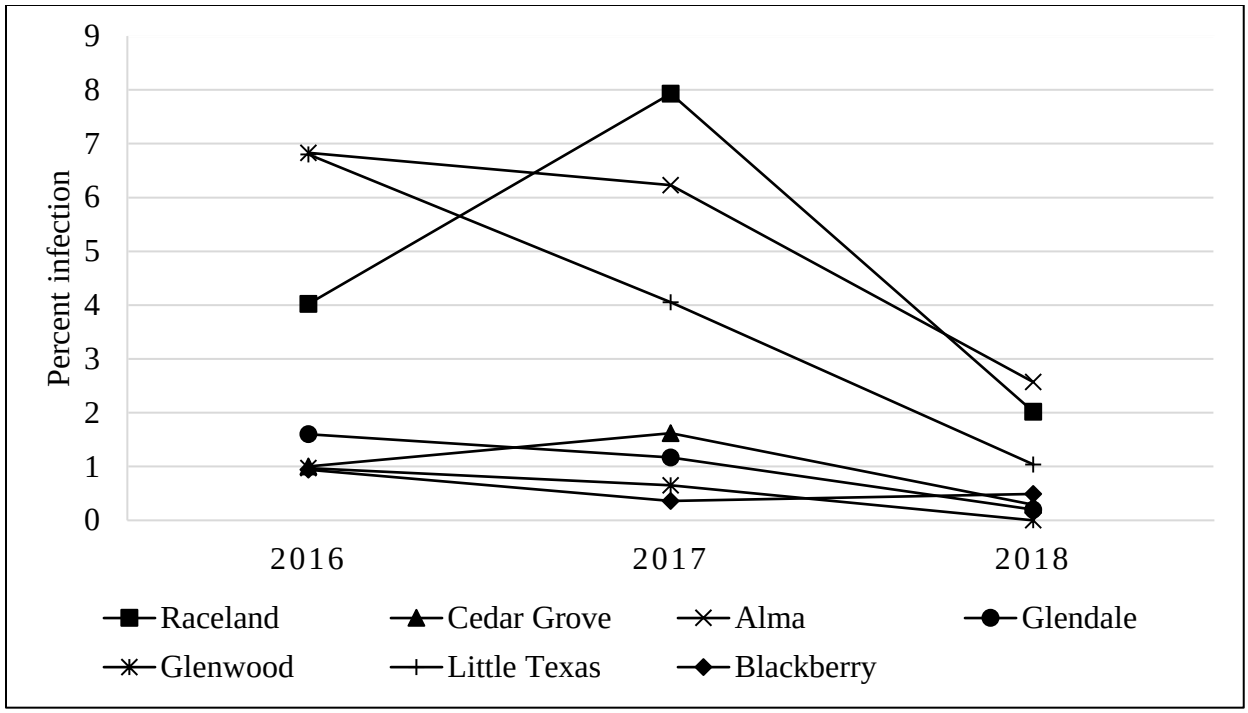

Figure 2.2. Changes in mosaic incidence in fields of HoCP 09-804 from plant cane (2016) to first ratoon (2017) to second ratoon (2018) at seven locations.

2.5. Discussion

Field observations of mosaic symptomatic plants in advanced experimental clones in the sugarcane breeding program during 2016 prompted concern about a possible re-emergence of the historically important disease in Louisiana. Field surveys successfully determined the current distribution and incidence of mosaic in different areas of the industry. Mosaic incidence was either low or absent in the locations that were surveyed during 2016, except for two fields of HoCP 09-804 at the Little Texas Primary Station with incidences of 9.0 and 10.4%. The distribution of mosaic incidence was variable across the geographic areas of the industry. Mosaic symptomatic plants were not detected in the Bayou Teche (western) and North areas, whereas mosaic was detected in some locations but not others in the Upper Mississippi River, Lower Mississippi River, and Bayou Lafourche areas.

The Louisiana Cooperative Sugarcane Breeding Program is conducted at facilities associated with the Louisiana State University Agricultural Center and the USDA-ARS Sugarcane Research Unit and on cooperating commercial farms. Seed-cane from experimental clones is obtained from the respective agency research farms and planted at three Primary Stations and 42 Secondary Stations of the American Sugar Cane League Variety Release Program located on commercial farms. Chance distribution of virus-free or systemically infected seed-cane to these locations could be the cause of the pattern of geographic distribution and incidences detected in the surveys. Mosaic was not detected at Secondary Stations in the Bayou Teche and North areas that were supplied with seed-cane increased at the Primary Station in the Bayou Teche area in contrast to the detection of mosaic at the Secondary Stations in other areas planted with seed-cane from the two Primary Stations in the Bayou Lafourche area, in particular the Little Texas Primary Station that had the highest disease incidence. These results support the hypothesis that infected seed-cane was the origin for the new disease outbreak, rather than spread by migrating aphids.

All symptomatic leaf samples tested positive for SrMV using the RT-PCR detection method developed by Yang and Mirkov (1997). These results indicate that SrMV is the virus species responsible for the current outbreak. Additional confirmation is needed to determine whether the strain occurring in recent mosaic infections is SrMV strain H, I, or M (Yang and Mirkov 1997). There were no samples for which the causal virus was unidentifiable using the Yang and Mirkov primers in contrast to a previous survey (Grisham and Pan 2007). In addition, there were no symptomatic samples that tested positive for SCMV, so it is unlikely that there are frequent occurrences of SrMV and SCMV co-infections. The lack of samples that tested positive for SCMV also suggests a continuance of the absence of this virus species that was indicated by

the field surveys conducted by Grisham and Pan (2007). The low percentage (0.3%) of asymptomatic samples that tested positive for SrMV suggests a rarity of asymptomatic infections and provides support for the reliability of field surveys based on observation of visual symptoms.

Runs analysis of the incidence results from surveyed fields in plant cane showed that most of the fields exhibited extensive aggregation of symptomatic plants within rows. The occurrence of single symptomatic plants could suggest aphid spread of mosaic into and within fields, and local aphid movement to adjacent plants could then result in runs of infected plants. The planting of whole infected stalks would result in multiple symptomatic plants occurring together. The consistent aggregated disease spatial pattern detected within rows planted with whole stalks across locations considered along with the geographic distribution pattern detected supports the hypothesis that the initial occurrence of mosaic was due to the planting of infected seed-cane. The spatial pattern of other non-persistent virus species has differed within a crop. A spatial pattern analysis of plum pox virus strain M concluded that a range from no aggregation to high levels of aggregation could be observed in symptomatic peach trees (*Prunus persica*) (Dallot et al. 2003). Studies in narrow-leafed lupin (*Lupinus angustifolius*) observed different types of spatial patterns due to aphid transmission for cucumber mosaic virus (CMV) and bean yellow mosaic virus (BYMV) from primary inoculum sources, in which CMV spread occurred in large aggregations while BYMV spread exhibited diffuse patterns (Jones 2005).

Repeat surveys of multiple locations in first and second ratoon provided information on potential rates of disease increase due to aphid transmission. Mosaic incidence increased at some locations and decreased at others in first ratoon then incidence decreased at all locations in second ratoon. The greatest increase in incidence occurred in first ratoon at Raceland where the number of infected plants increased 97%, but with an initial incidence of 4%, the change in

infection did not result in a high level of disease. A higher rate of initial incidence, 6.8% at Little Texas, did not result in a higher rate of increase, as incidence was progressively lower at this location in two subsequent ratoon crops. The low rates of disease increase and decreases in incidence observed in the majority of cases, suggest that disease spread by migrating aphids was not effective during the two additional survey years. The low initial incidence in plant cane and lack of other inoculum sources were likely additional contributing factors. Informal surveys conducted in commercial fields surrounding the surveyed fields did not detect any mosaic symptomatic plants. The lack of disease increase at multiple locations suggested that the potential for rapid rates of mosaic increase are currently unlikely in Louisiana. However, the explanation for disease decreases from one crop year to the next is uncertain. The occurrence of recovery from mosaic has been documented in sugarcane in Louisiana (Benda 1974; Summers et al. 1948), and this may have contributed to the decreases in the number of symptomatic plants observed in ratoon crops. It also is possible that mosaic-infected plants are less able to survive winter freezes than healthy plants as has been documented for smut-infected plants (Hoy et al. 1987).

In summary, mosaic incidence was found to be generally low or absent in the surveyed locations of the Louisiana sugarcane breeding program. RT-PCR results for field-collected leaf samples indicated that SrMV continues to be the virus species causing mosaic and confirmed the accuracy of using the visual observation of symptoms in plants for determining disease incidence. The geographic distribution, consistent aggregation, and numbers of plants in runs suggest that the source of the initial infection in the current outbreak was the planting of infected seed-cane. The lack of disease increase over two seasons and the continued failure to detect

mosaic in fields of current commercial cultivars suggest mosaic is not likely to rapidly re-emerge under the current conditions in Louisiana.

CHAPTER III. RECOVERY FROM MOSAIC IN SUGARCANE AND IMPACT OF THE DISEASE ON YIELD

3.1. Introduction

Mosaic is a viral disease of sugarcane (interspecific hybrids of *Saccharum*) that is named after the symptoms it causes. Symptoms consist of diffuse intermixing of light and dark green tissue most readily seen in the basal portion of young, rapidly growing leaves of young plants prior to stalk development (Agnihotri 1990; Grisham 2000). The causal viruses, sugarcane mosaic virus (SCMV) and Sorghum mosaic virus (SrMV) of the Potyviridae, are vectored by multiple species of aphids in a non-persistent manner (Brandes 1920; Grisham 2000). Strains of SrMV were previously considered to be strains of SCMV, but taxonomic studies reclassified these strains as a separate virus species based on serological and molecular characteristics (McKern et al. 1991; Shukla et al. 1989). Field surveys conducted in Louisiana during the early 2000s concluded that SrMV is currently the causal species of mosaic (Grisham and Pan 2007).

Mosaic caused a near collapse of the sugar industry in Louisiana during the 1920s. Fortunately, resistance was obtained through the importation of the first interspecific hybrids between *S. officinarum* (susceptible) and *S. spontaneum* (resistant). However, occasional outbreaks have continued to occur due to changes in the virus strain. This necessitated an on-going effort to breed for resistance to mosaic, and sugarcane breeding efforts have focused on additional introgression of resistance to SrMV strains from the wild relatives of sugarcane, in particular *S. spontaneum* (Grisham et al. 1992). Susceptibility considered as the severity of yield loss is variable and dependent on the sugarcane cultivar and virus strain combination (Bailey and Fox 1987; Grisham 2000; Viswanathan and Balamuralikrishnan 2005).

Sugarcane clones can have variable responses to mosaic, and one additional trait that has been noted in addition to yield loss is the recovery from symptom expression and possibly virus

infection (Benda 1974; Summers et al. 1948). Recovery can be broadly defined as the loss of disease symptoms and virus accumulation within an infected plant, and this phenomenon has been studied in other virus-crop pathosystems, such as sweetpotato feathery mottle virus (SPFMV) in sweetpotato and tobacco streak virus (TSV) in cranberry (Gibson et al. 2014; Wells-Hansen and McManus 2016). Observations of the loss of mosaic symptoms in early work contradicted the previous notion that planting an infected stalk would yield only infected, symptomatic plants (Agnihotri 1990; Bailey and Fox 1987; Brandes 1920; Summers et al. 1948). Two types of recovery from mosaic have been described: germination and foliar recovery. Germination recovery refers to recovery that occurs when an asymptomatic shoot develops from the axillary bud of an infected stalk, while foliar recovery occurs when a previously symptomatic plant loses symptoms over time during the growing season (Benda 1974; Summers et al. 1948).

Sugarcane is a perennial crop that is vegetatively propagated by planting stalks or stalk sections. In Louisiana, sugarcane is grown with multiple annual crops obtained from a single planting: a plant cane crop (first year) and 2-3 ratoon crops. Early research on recovery from mosaic in sugarcane primarily focused on determining the frequency of its occurrence over crop cycles, characteristics in individual plants, and how it can influence mosaic incidence (Benda 1974; Summers et al. 1948). Cultivars that displayed traits of recovery were compared across crop cycles, and mosaic incidence decreased when symptomatic stalks were planted. However, secondary spread of the virus by aphid transmission can make it difficult to determine levels of recovery in field experiments (Summers et al. 1948). The extent of recovery assessed as whether buds on a stalk produced either a portion or all asymptomatic plants was found to be variable when comparing cultivars (Summers et al. 1948). Bio-assays testing the infectivity of recovered

sugarcane plants usually failed to produce symptoms in inoculated plants (Benda 1974). The frequency of recovery occurring in modern sugarcane cultivars is unknown.

At times, cultivation of mosaic susceptible cultivars for other desirable traits is attempted, so any factor that can limit disease increase, such as recovery, could play a role in disease management considerations (Summers et al. 1948). A mosaic susceptible cultivar, HoCP 09-804, was released for commercial production in 2016. It was released despite some level of mosaic susceptibility indicated by a low incidence of disease detected in some seed-cane increase fields. Mosaic was observed at the same time in other high yielding, advanced experimental clones in the breeding program. Repeated field surveys of HoCP 09-804 detected decreases in disease incidence in ratoon crops of HoCP 09-804 (Chapter 2). Therefore, further evaluation of recovery in modern cultivars was initiated.

A reverse transcription polymerase chain reaction (RT-PCR) method capable of the specific detection of SrMV was developed (Yang and Mirkov 1997), and a comparative study determined it is the most sensitive test available (Keizerweerd et al. 2015; Yang and Mirkov 1997). Thus, a molecular assay could provide additional evidence concerning whether virus is present in recovered plants. Finally, recovery experiments would offer the opportunity to further evaluate the impact of mosaic on bud germination success and sugarcane yield.

3.2. Objectives

To further investigate recovery from mosaic in two modern sugarcane clones, to determine if virus is detectable in recovered plants by RT-PCR, and to evaluate the impact of disease on bud germination and crop yield.

3.3. Materials and Methods

Field experiments were conducted to evaluate recovery from mosaic at the Louisiana State University Agricultural Center Sugar Research Station (St. Gabriel, LA) from 2016 to 2018. In May 2016, individual plants were identified and tagged as mosaic symptomatic or asymptomatic for one cultivar HoCP 09-804 and one experimental clone L 10-147. Stalks were collected from previously identified symptomatic plants during September, and each stalk was assessed for foliar recovery, defined as loss of visible symptoms of mosaic in the leaves. Stalks were then planted as individual stalks in single-row plots. Separate rows were planted with either previously asymptomatic or symptomatic stalks, with two rows planted in between with a mosaic resistant cultivar, HoCP 96-540, to minimize natural spread by aphids. In the first experiment, 46 stalks from asymptomatic plants and 63 stalks from symptomatic plants of HoCP 09-804 were planted, and 67 stalks from asymptomatic plants and 82 stalks from symptomatic plants of L 10-147 were planted. Stalks from previously identified asymptomatic and symptomatic plants were taken from plots in the first experiment and used to plant a second experiment during September 2017. Seventy stalks from symptomatic plants and 70 stalks from asymptomatic plants were planted of each cultivar. Each stalk collected from a previously symptomatic plant was assessed for foliar recovery before planting.

Prior to planting during September, the number of buds on each stalk was recorded to determine bud germination success assessed as primary shoots that emerged from each stalk after planting. The number of emerged primary shoots was recorded for each plot in October 2016 for the plant cane year of experiment one and in November 2017 for plant cane in experiment two. Percent germination was estimated by dividing the number of emerged primary shoots by the recorded number of buds for each planted stalk. Spring shoot counts were then taken in March 2016 and 2017 of the plant cane crop for each experiment to evaluate re-emergence after winter.

Stalk counts were taken for each single-stalk plot in August 2017 for the first experiment and in August 2018 for the first experiment in first ratoon and second experiment in plant cane. To estimate additional yield components, eight 10-stalk samples were taken for each clone and symptom combination in November 2017 for the first experiment. Stalk weight (kg) was determined for each sample. The stalks were shredded, and sucrose content was determined by fourier transformed near-infrared spectral reflectance (SpectraCane, Bruker). Sucrose content (kg per metric ton of cane), cane yield (kg per metric ton), and sucrose yield (kg per ha) were then calculated.

To assess germination recovery from mosaic, defined as an asymptomatic plant emerged from a bud on a symptomatic stalk, a visual evaluation of mosaic symptoms was conducted for each single-stalk plot of each clone prior to stalk elongation during May 2017 for the first experiment in plant cane and during June 2018 for the first experiment in first ratoon and plant cane of the second experiment. The number of asymptomatic plants and total number of plants was recorded for each plot, and the mean percent recovery per plot was calculated for each clone. The total number of plots exhibiting germination recovery was recorded, and it was determined whether recovery was partial or complete for affected stalks. Partial recovery was considered to be single stalk plots that contained both symptomatic and asymptomatic plants. The total number of recovered plants also was recorded for each clone, and the overall percent recovery was calculated for each clone in each crop. In addition, plots of both cultivars planted with asymptomatic stalks were visually assessed for mosaic symptomatic plants to evaluate the extent of aphid spread of mosaic.

During the symptom evaluations in 2017 and 2018, a single asymptomatic leaf sample was taken from each germination recovered plant to evaluate SrMV infection by RT-PCR. In

2017, three asymptomatic samples were collected from HoCP 09-804 and 55 asymptomatic samples were collected from L 10-147. Symptomatic leaf samples were also collected from plants exhibiting symptoms in rows with plots planted with asymptomatic stalks. Five symptomatic samples were collected from HoCP 09-804 and 10 samples were collected from L 10-147 to confirm infection by SrMV by RT-PCR. In 2018, leaf samples were taken from germination-recovered plants developing from planted symptomatic stalks and from symptomatic plants developing from planted asymptomatic stalks (one sample collected per asymptomatic plot) for the first experiment in first ratoon and second experiment in plant cane. In 2018, samples were collected from one apparently recovered asymptomatic plant for HoCP 09-804 and 48 L 10-147 plants in first ratoon, and asymptomatic leaf samples were also collected from the second experiment in plant cane for HoCP 09-804 (2 samples) and L 10-147 (38). Three symptomatic samples were collected for previously asymptomatic HoCP 09-804 and 11 samples were collected for L 10-147 in first ratoon, and two HoCP 09-804 and one L 10-147 samples were collected from the second experiment plant cane to confirm aphid spread of SrMV.

Leaf samples were tested for SrMV by RT-PCR as follows. Samples were stored at -70°C until RNA extraction was performed. Total RNA was extracted using the Plant Total RNA Kit (Spectrum™, Sigma Aldrich) with modifications to the tissue homogenization steps. Approximately 300 mg of tissue was homogenized in a BIOREBA extraction bag (BIOREBA AG, Switzerland) with 2 ml prepared lysis buffer from the Plant Total RNA Kit (Spectrum™, Sigma Aldrich) using a BIOREBA standard rack tissue homogenizer. RT-PCR was carried out in two steps and using a modification of the RT-PCR method described by Yang and Mirkov (1997). Complementary DNA synthesis was performed using the SuperScript™ First-Strand Synthesis System (Invitrogen™, Thermo Fisher Scientific), and RNA was primed with 1 µl (2

µM stock) of SrMV-R3. The 2 µl of diluted cDNA product (1:50) in nuclease free water was added to a 25 µl PCR solution. The PCR solution consisted of 12.5 µl of GoTaq® Green Master Mix, 2X (Promega), 11.86 µl of nuclease free water, 0.25 µl (10 µM stock) of SrMV-F3 and SrMV-R3, 0.14 µl of bovine serum albumin V (100 µg/µl). The PCR program used was 95°C for 2 min; 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1 min; and final extension at 72°C for 5 min. RT-PCR products were electrophoresed in 2.0% agarose gel containing ethidium bromide (final concentration 0.35 µg/ml) for 1 h, and bands were visualized using a UV transilluminator. Positive results were concluded when a visible band was present at 871 bp.

Recovery and yield data were analyzed using Proc GLM (SAS 9.4). Interactions determined how treatment results should be compared for the dependent variables. Arcsin transformations of percentage means were analyzed to determine significance ($p<0.05$). Mean separations were determined by least significant difference (LSD).

3.4. Results

The number of recovered plants assessed as asymptomatic plants that emerged from planted stalks of symptomatic plants varied between the two clones for experiment one in plant cane and first ratoon and plant cane in experiment two (Table 3.1). Recovery assessed as the number of plots with asymptomatic plants, total number of asymptomatic plants, and mean percentage of asymptomatic plants per plot were all higher for L 10-147 than for HoCP 09-804 in plant cane and first ratoon in experiment one and plant cane in experiment two (Table 3.1). The mean percentages of asymptomatic plants per plot of HoCP 09-804 were 2.3, 0.9, and 1.2% compared to 19.8, 15.9, and 9.4% for L 10-147 in experiment one plant cane and first ratoon and experiment two plant cane, respectively (Table 3.1). The rate of recovery was numerically higher for both clones in experiment one than experiment two (Table 3.1).

The total numbers and percentages of symptomatic plants that were observed in the asymptomatic stalk planted plots in plant cane and first ratoon of experiment one and plant cane of experiment two were generally low ranging from 0.2-6.3% (Table 3.2). Incidence did not increase in first ratoon of experiment one and was lower in the plant cane of the second experiment. The numbers and percentages of plots with symptomatic plants observed for the asymptomatic stalk planted plots were higher ranging from 1.4-16.4% (Table 3.2). The same pattern was evident with little or no increase in first ratoon and lower incidence in plant cane of the second experiment.

Table 3.1. Comparison of mosaic recovery evaluated as asymptomatic plants developing from single stalk plots of HoCP 09-804 and L 10-147 that were planted with individual mosaic symptomatic stalks.

Crop year and experiment [a]	Clone	Plots with asymptomatic plants	Total number of asymptomatic plants	Percent asymptomatic plants per plot[b]
Plant cane exp 1	HoCP 09-804	2/58 (3.4%)	3/152 (2.0%)	2.3 b
	L 10-147	26/81 (32.1%)	55/291 (18.9%)	19.8 a
First ratoon exp 1	HoCP 09-804	1/54 (1.9%)	1/160 (0.6%)	0.9 b
	L 10-147	24/80 (30%)	48/294 (16.3%)	15.9 a
Plant cane exp 2	HoCP 09-804	2/70 (2.9%)	2/291 (0.7%)	1.2 b
	L 10-147	11/70 (15.7%)	38/384 (9.9%)	9.4 a

[a] Two experiments (exp) were conducted. Results in experiment one were determined in both plant cane and first ratoon. In experiment two, results were determined only in plant cane.
[b] Mean percentages of asymptomatic plants were transformed with arcsin for statistical analyses separately comparing clones within each individual crop year and experiment. Means followed by different letters were significant at $p<0.05$.

Table 3.2. Comparison of mosaic symptomatic plants that occurred due to aphid transmission in single stalk plots of HoCP 09-804 and L 10-147 that were planted with individual asymptomatic stalks.

Crop year and experiment [a]	Clone	Total plants	Number of symptomatic plants (%)	Total plots	Number of symptomatic plots (%)
Plant cane exp 1	HoCP 09-804	204	7 (3.4)	46	5 (10.9)
	L 10-147	286	18 (6.3)	67	10 (14.9)
First ratoon exp 1	HoCP 09-804	230	1 (1.3)	46	3 (6.5)
	L 10-147	338	21 (6.3)	67	11 (16.4)
Plant cane exp 2	HoCP 09-804	400	5 (1.3)	70	2 (2.9)
	L 10-147	442	1 (0.2)	70	1 (1.4)

[a] Two experiments (exp) were conducted. Results in experiment one were determined in both plant cane and first ratoon. In experiment two, results were determined only in plant cane.

A comparison of the extent of recovery within single stalk plots, where complete recovery was defined as all plants produced from a single stalk being asymptomatic, did not detect large differences between the two clones and between crop years. Across experiments, partial and complete recovery were both observed for single-stalk plots of both clones (Table 3.3). No plots of HoCP 09-804 exhibited complete recovery in first ratoon of experiment one and plant cane of experiment two and, the number of plots with recovered plants was low (Table 3.3). The average percentage of recovery within plots that exhibited recovery across both experiments ranged from 42 to 67% in HoCP 09-804 and 53 to 62% in L 10-147 (Table 3.3). The range for percent asymptomatic plants within plots was variable for both clones (Table 3.3).

Table 3.3. Comparison of the extent of mosaic recovery within single stalk plots of HoCP 09-804 and L 10-147 that were planted with individual symptomatic stalks.

Crop year and experiment [a]	Clone	Plots exhibiting 100% recovery from mosaic	Plots exhibiting less than 100% mosaic recovery	Mean percent recovery within plots	Range in percent recovery within plots
Plant cane exp 1	HoCP 09-804	1	1	67%	33-100%
	L 10-147	10	16	62%	14-100%
First ratoon exp 1	HoCP 09-804	0	1	50%	50%
	L 10-147	5	19	53%	20-100%
Plant cane exp 2	HoCP 09-804	0	2	42%	33-50%
	L 10-147	2	9	60%	14-100%
Total	HoCP 09-804	1	4	53%	33-100%
	L10-147	17	44	58%	14-100%

[a] Two experiments (exp) were conducted. Results in experiment one were determined in both plant cane and first ratoon. In experiment two, results were determined only in plant cane.

The frequency of detection of SrMV by RT-PCR was generally low for leaf samples collected from each plant considered recovered (an asymptomatic plant that emerged from a symptomatic stalk). Six total plants were tested for HoCP 09-804 and 143 total plants were tested for L 10-147 across both experiments, and most samples tested negative for SrMV: 83 and 97% for HoCP 09-804 and L 10-147, respectively (Table 3.4). No samples collected from first ratoon of experiment one tested positive for SrMV for either clone (Table 3.4).

Stalks collected from mosaic symptomatic and asymptomatic plants varied in the amount of foliar recovery observed for each cultivar in both experiments. In the first experiment, no foliar recovery was observed for any stalk collected from multiple symptomatic plants of HoCP 09-804. In contrast, extensive foliar recovery was observed for plants and collected stalks of L 10-147. For the first experiment, mosaic symptoms were only observed for stalks collected from one of six previously symptomatic plants. For the second experiment, similar differences were

detected between the two clones. Only four of 70 (5.7%) stalks of HoCP 09-804 exhibited foliar recovery compared to 44/70 (62.9%) stalks of L 10-147.

The subsequent determination of germination recovery during experiment two revealed an association between foliar and germination recovery for L 10-147. No germination recovery was detected in the four plots planted with stalks of HoCP 09-804 exhibiting foliar recovery. However, for L 10-147, 37/38 (97.4%) of the plants exhibiting germination recovery were from plots planted with stalks exhibiting foliar recovery. Ten of the 44 (22.7%) plots planted with stalks exhibiting foliar recovery produced plants with germination recovery compared to 1/26 (3.8%) of plots planted with symptomatic stalks. For the 10 plots planted with foliar recovery stalks that produced plants exhibiting germination recovery, two plots (20%) exhibited complete recovery and eight (80%) had partial recovery.

Table 3.4. Detection of Sorghum mosaic virus (SrMV) by RT-PCR in recovered (asymptomatic) plants developing from single stalk plots of HoCP 09-804 and L 10-147 planted with individual mosaic symptomatic stalks.

Crop year and experiment [a]	Clone	Total plants tested	Number of plants positive for SrMV
Plant cane exp 1	HoCP 09-804	3	1 (33.3%)
	L 10-147	55	3 (5.5%)
First ratoon exp 1	HoCP 09-804	1	0 (0%)
	L 10-147	49	0 (0%)
Plant cane exp 2	HoCP 09-804	2	0 (0%)
	L 10-147	39	2 (5.1%)

[a] Two experiments (exp) were conducted. Results in experiment one were determined in both plant cane and first ratoon, but results in experiment two were determined only in plant cane.

The impact of mosaic on bud germination and yield was different for HoCP 09-804 and L 10-147 in two experiments from 2016 to 2018 (Table 3.5). The germination and yield impact results for L 10-147 differed between years for some components, so the results are reported separately for years and crops. The mean numbers of buds per stalk determined prior to planting

were similar for the two clones and for symptomatic and asymptomatic stalks. In experiment one, bud number per stalk was 12 (range = 5-16 buds) in asymptomatic HoCP 09-804, 12 (4-17) in symptomatic HoCP 09-804, 11 (5-15) in asymptomatic L 10-147, and 11 (7-18) in symptomatic L 10-147. In experiment two, the mean number of buds per stalk recorded was 14 (range = 10-17 buds) for asymptomatic HoCP 09-804, 13 (8-16) for symptomatic HoCP 09-804, 13 (9-18) for asymptomatic L 10-147, and 14 (8-18) for symptomatic L 10-147. Bud germination for mosaic asymptomatic stalks of HoCP 09-804 (28%) and L 10-147 (33%) was similar in experiment one, but in experiment two, buds on asymptomatic stalks of HoCP 09-804 (38%) had lower germination than buds on asymptomatic stalks of L 10-147 (46%) (Table 3.5). Bud germination was adversely affected by mosaic for HoCP 09-804 in both experiments, whereas germination was unaffected for L 10-147 in both experiments (Table 3.5).

Spring shoot populations following winter were similar for plots planted with asymptomatic stalks of both cultivars in both experiments (Table 3.5). Shoot populations in plant cane were lower in plots planted with symptomatic compared to asymptomatic stalks for both clones in experiment one; however, the reduction in population (61%) was greater in HoCP 09-804 than in L 10-147 (29%) (Table 3.5). In experiment two, the spring shoot population in HoCP 09-804 was 42% lower in the symptomatic plots than in asymptomatic plots, but the L 10-147 spring shoot population was higher in the symptomatic plots than in asymptomatic plots by 11% (Table 3.5).

Stalk populations were similar in plots planted with asymptomatic stalks for both cultivars in plant cane and first ratoon (Table 3.5). In the plant cane and first ratoon crops of the first experiment and plant cane of the second experiment, HoCP 09-804 had a lower population of stalks in plots planted with previously symptomatic than asymptomatic stalks, whereas L 10-

147 only had a lower stalk population in the first ratoon crop of experiment one (Table 3.5). The reduction in stalk population for symptomatic compared to asymptomatic plots was greater for HoCP 09-804 (36%) compared to L 10-147 (8%) in plant cane of experiment one.

Table 3.5. Comparison of bud germination, spring shoot populations, and stalk populations for single stalk plots of HoCP 09-804 and L 10-147 that were planted with individual mosaic asymptomatic or symptomatic stalks.

Crop cycle and experiment [a]	Clone [b]	Bud germination [c]	Spring shoots per hectare [d]	Stalks per hectare [e]
Plant cane exp1	HoCP 09-804 A	28% a	57,363 a	83,630 a
	HoCP 09-804 S	15% b	22,346 c	53,822 c
	L 10-147 A	33% a	63,430 a	80,956 ab
	L 10-147 S	33% a	45,101 b	74,748 b
First ratoon exp 1	HoCP 09-804 A	na	-	90,699 a
	HoCP 09-804 S	na	-	61,728 b
	L 10-147 A	na	-	82,319 a
	L 10-147 S	na	-	66,146 b
Plant cane exp 2	HoCP 09-804 A	38% b	31,607 ab	86,385 a
	HoCP 09-804 S	30% c	18,489 c	75,773 c
	L 10-147 A	46% a	29,304 b	83,010 ab
	L 10-147 S	46% a	33,040 a	79,047 bc

[a] Two experiments (exp) were conducted. Results in experiment one were determined in both plant cane and first ratoon. In experiment two, results were determined only in plant cane.
[b] Asymptomatic (A) and symptomatic (S) stalks were planted for each clone.
[c] The percentage of buds that germinated out of the total number of buds on a single stalk was estimated from primary shoot emergence. Means within an experiment followed by different letters were significantly different at $p<0.05$. Na = not applicable.
[d] Shoot populations were counted during the following spring. Means within an experiment followed by different letters were significantly different at $p<0.05$. Spring shoot counts were not determined in first ratoon of experiment one.
[e] Stalk populations were determined during late summer. Means within an experiment and crop year followed by different letters were significantly different at $p<0.05$.

The yield components of stalk weight, sucrose content, cane yield, and sucrose yield varied among treatments in experiment one for the plant cane crop (Table 3.6). All four yield components were similar for plots planted with asymptomatic stalks of both clones (Table 3.6).

Individual stalk weight was similar in plots planted with asymptomatic and symptomatic stalks for both clones, but stalk weight was lower for symptomatic stalk plots of HoCP 09-804 compared to L 10-147 (Table 3.6). Sucrose content in stalks of cane was less in asymptomatic stalk plots of HoCP 09-804 (90.5 kg/ton) than symptomatic plots of HoCP 09-804 (94.8 kg/ton), while sucrose content was similar in asymptomatic (88.5 kg/ton) and symptomatic (87.4 kg/ton) plots of L 10-147 (Table 3.6). Cane yield and sucrose yield were lower in symptomatic compared to asymptomatic plots of HoCP 09-804 but were similar in asymptomatic and symptomatic plots of L 10-147 (Table 3.6).

Table 3.6. Comparison of stalk weight, sucrose content, cane yield, and total sucrose yield estimated for plant cane from single stalk plots of HoCP 09-804 and L 10-147 of experiment one that were planted with individual mosaic symptomatic or symptomatic

Clone [a]	Stalk weight (kg)[b]	Sucrose per ton of cane (kg) [b]	Cane yield (tons/ha) [b]	Sucrose yield (kg per ha)[b]
HoCP 09-804 A	1.03 ab	90.5 b	103.1 a	9,368.8 a
HoCP 09-804 S	0.96 b	94.8 a	55.8 b	5,335.7 b
L 10-147 A	1.18 a	88.5 b	116.5 a	10,325.3 a
L 10-147 S	1.16 a	87.4 b	107.2 a	9,347.5 a

[a] Mosaic asymptomatic (A) and symptomatic (S) stalks were planted for each clone.
[b] Means within a column followed by different letters were significantly different at $p < 0.05$.

3.5. Discussion

The frequency of recovery from mosaic differed for the two sugarcane clones included in this study. Previous research on the frequency of recovery in sugarcane also found differences among cultivars (Summers et al. 1948). The frequencies of foliar and germination recovery varied between HoCP 09-804 and L 10-147 in a similar manner. L 10-147 exhibited higher frequencies of both foliar and germination recovery than HoCP 09-804. HoCP 09-804 exhibited a lower frequency of germination recovery for plants developing from previously symptomatic planted stalks, with recovery ranging from 0.9%-2.3% compared to 9.4-19.8% for L 10-147 in the two plant cane and one first ratoon crops. Most germination recovery (97%) occurred with stalks that

exhibited foliar recovery for L 10-147. However, additional research is needed to verify this association.

Variation in the rate of germination recovery was detected between two crops in different seasons, particularly for L 10-147 that exhibited a lower rate of recovery in the plant cane crop of experiment two compared to experiment one. For a perennial crop like sugarcane with multiple annual cuttings of stalks obtained from initial planting, there are opportunities for additional changes in recovery in ratoon growth. In this study, a decreased frequency of recovery was detected in the first ratoon growth of L 10-147 and HoCP 09-804. Results where the frequency of recovery increased in ratoon growth was reported by Summers et al. (1948) in three cultivars, POJ 234, POJ 36-M, and POJ 213. However, secondary infections due to aphid transmission increased mosaic incidence in one location of the study, so in this event, they were unable to determine an increase in the frequency of recovery (Summers et al. 1948). The percentage of symptomatic plants in plots planted with asymptomatic stalks that occurred due to aphid transmission was low in the current study, so it is likely that secondary spread did not affect the recovery results.

It is uncertain what other conditions would affect the extent of recovery for different clones and variability in the rate of recovery between year and crop. Environmental factors, such as temperature, might influence the occurrence and rate of recovery from year to year. Sugarcane in Louisiana is planted in late summer. Primary shoots begin to grow but then die back when freezing temperatures occur during the winter months. Severe freezing temperatures with multiple nights with temperatures below -8^{o}C occurred during January 2018, and a lower frequency of recovery occurred during that season. Summers et al. (1948) compared the frequency of recovery in different cultivars following a freeze that killed shoots to the ground

surface. The cultivar POJ 36-M exhibited similar levels of recovery as previous experiments, and POJ 234 exhibited less recovery than reported in previous experiments. Cryotherapy in liquid nitrogen is capable of producing virus-free rootstock planting materials in different fruit crops (Brison et al. 1997; Wang et al. 2003). Temperature dependent recovery from virus symptoms has been demonstrated in tomato ringspot virus infected plants, where higher temperatures were associated with an increased amount of symptom recovery (Ghoshal and Sanfaçon 2015). Thermotherapy, a method where plant material in tissue culture is subjected to elevated temperatures, has been demonstrated to at least partially eliminate viruses in different virus-crop combinations (Cieslinska 2000, 2007; Conci and Nome 1991; Nascimento et al. 2003). Serial hot water treatments (57°C) of mosaic infected sugarcane stalks resulted in plants that bio-assayed negative for SCMV (Benda 1971).

The extent of recovery from mosaic determined as partial or complete recovery of plants produced from a previously symptomatic stalk varied similarly for both clones with both exhibiting more partial than complete recovery. The overall percentage of plots exhibiting partial recovery was 80% for HoCP 09-804 and 72% for L 10-147. Summers et al. (1948) reported that cultivars can differ in the extent of recovery exhibited; POJ 36-M exhibited either no recovery or complete recovery while POJ 234 exhibited partial recovery.

When testing recovered sugarcane plants for detectable levels of SrMV by RT-PCR, most samples tested negative for the virus. The SrMV detection rates were 17% for HoCP 09-804 and only 3% for L 10-147. It is possible that there is a low virus titer that was undetectable by RT-PCR in recovered plants, despite the sensitivity of this assay. It was not possible to precisely identify the same plants from plant cane into first ratoon. However, the frequency of recovered plants did not increase in first ratoon, so it is likely that the same recovered plants tested negative

for SrMV in two subsequent crops. Similar results were found in bio-assays of mosaic, where the titer of virus present was shown to be incapable of mechanical transmission (Benda 1974). Testing for virus detection in recovered plants has been documented in other plant-virus pathosystems of vegetatively propagated crops, including sweetpotato and cranberry (Gibson et al. 2014; Wells-Hansen and McManus 2016). In sweetpotato, recovery responses to SPFMV were reported in graft inoculated cultivars that were tested for 10 weeks using quantitative polymerase chain reaction (qPCR) (Gibson et al. 2014). East African cultivars were reported to assay negative for SPFMV 10 weeks after graft inoculation, but some cultivars from North and South American still assayed positive for SPFMV (Gibson et al. 2014). In the perennial crop cranberry, plants exhibiting recovery from TSV can produce asymptomatic fruit in subsequent years that still test positive for TSV (Wells-Hansen and McManus 2016). When different parts of recovered plants were tested, TSV was additionally detected in leaves, roots, stems, and terminal buds (Wells-Hansen and McManus 2016).

The failure to detect SrMV using the sensitive RT-PCR assay suggests recovered sugarcane plants may no longer be virus infected. The mechanism by which this could occur is uncertain, but this phenomenon might be explained by an RNA silencing mechanism that results in the degradation of viral RNA (Ghoshal and Sanfaçon 2015; Voinnet 2001).

Mosaic was found to have varying effects on yield components in HoCP 09-804 and L 10-147. In general, mosaic had fewer adverse effects on L 10-147 than on HoCP 09-804. Mosaic reduced shoot emergence resulting from bud germination for HoCP 09-804 in two subsequent plant cane crops but did not affect bud germination and stand establishment for L 10-147 in either year. Mosaic also resulted in reduced shoot populations the following spring for HoCP 09-804 during both plant cane crops, while spring shoot population was lower in L 10-147 during

experiment one but not experiment two. Similarly, mosaic adversely affected stalk populations for HoCP 09-804 in plant cane and first ratoon in experiment one and plant cane of experiment two, while stalk population was only lower for L 10-147 in first ratoon. In addition, mosaic had no effect on L 10-147 plant cane yield components of stalk weight, sucrose content, cane yield, and sucrose yield, while HoCP 09-804 exhibited variable effects of mosaic with lower cane yield and sucrose yield but higher sucrose content. These results are consistent with previously reported variable effects of mosaic on yield in different cultivars (Bailey and Fox 1987; Grisham 2000; Viswanathan and Balamuralikrishnan 2005). Mosaic can cause significant yield loss in the cultivar HoCP 09-804 due to the reduction of initial stand establishment leading to reduced stalk populations. In contrast, L 10-147 exhibited tolerance to the disease, and its aggregate yield components, cane yield and sucrose yield, were not affected which was similar to two cultivars NCo 310 and CP 65-357 cultivated extensively in Louisiana from the mid-1950s to the 1990s (Breaux and Koike 1978; Grisham 1994; Koike and Gillaspie 1989).

The results from the single stalk plot experiments comparing mosaic recovery and impact of the disease on yield confirmed that these traits vary by clones. Rates of recovery and yield impact had a different but similar pattern for each of the two clones included in the study with low recovery and higher yield loss in HoCP 09-804 and higher recovery with little yield loss in L 10-147. Additional research is needed to determine if there is an association between recovery potential and yield loss across multiple sugarcane genotypes and seasons. Similarly, the potential association between foliar and germination recovery requires further study. Low rates of disease increase and decreases in incidence in ratoon crops were observed in field surveys of plantings of HoCP 09-804 conducted in plant cane and two subsequent ratoon crops during the same seasons as this study (Chapter 2). Considered altogether, the results suggest that recovery could affect

mosaic incidence in commercial sugarcane plantings and the rate of disease increase over the crop cycle for some sugarcane cultivars.

CHAPTER IV. EVALUATION OF SUSCEPTIBILITY TO MOSAIC IN LOUISIANA'S SUGARCANE BREEDING GERMPLASM

4.1. Introduction

Mosaic is a widely distributed, economically important disease of sugarcane (interspecific hybrids of *Saccharum*) controlled primarily through cultivation of resistant cultivars (Grisham 2000). Resistance breeding in sugarcane has remained important for the management of mosaic in Louisiana since the disease nearly bankrupted the sugar industry in the 1920s. Mosaic is a viral disease caused by either sugarcane mosaic virus (SCMV) or Sorghum mosaic virus (SrMV), both members of the Potyviridae. Strains of SrMV were previously described as SCMV strains, but taxonomic studies reclassified them as representatives of a distinct virus species (McKern et al. 1991). Reverse transcription polymerase chain reaction (RT-PCR) assays were developed for the specific detection of SCMV and SrMV (Yang and Mirkov 1997). Testing of samples collected during field surveys determined that SrMV is now the primary causal agent of mosaic in Louisiana, and the current predominant strain is I (Grisham 1994; Grisham and Pan 2007). Mosaic is spread from plant to plant by migratory aphids in a non-persistent manner but also can be spread from field to field by planting infected seed-cane (Grisham 2000).Yield loss from mosaic is cultivar and virus strain dependent (Grisham 2000).

Historic yield losses from mosaic in the 'noble cane' (*S. officinarum*) cultivars were alleviated by the introduction of the first interspecific hybrids that provided resistance in the sugarcane germplasm through the introgression of genes from wild relatives, *S. spontaneum* and *S. barberi*. Since that time, periodic outbreaks have occurred due to virus strain changes. Cultivar responses to mosaic, in addition to susceptibility or resistance, can include tolerance where plants are susceptible to infection but yield losses are not significant (Grisham 2000). Two

tolerant cultivars, NCo 310 and CP 65-357, were grown during the mid-1950s until the early 1990s due to their ability to yield more than resistant cultivars grown at the time (Grisham 1994). After the appearance of SrMV strain H, sugarcane breeders undertook a sustained basic breeding effort to introgress additional sources of resistance from *S. spontaneum* (Grisham et al. 1992). Due to success in breeding for resistance to mosaic caused by SrMV and widespread cultivation of resistant cultivars, incidence of the disease has decreased to undetectable levels in commercial fields.

Sugarcane is vegetatively propagated and has a multiple year crop cycle with a plant cane (first year crop) and 2-3 ratoon crops obtained in Louisiana. As a result, the commercial sugarcane breeding program requires 12 years to produce a new cultivar. The commercial breeding program utilizes primarily recurrent selection with a continuous infusion of accessions used as parents from the basic breeding program. Early stage selection occurs on two experimental farms where mosaic is endemic and concludes with three multiple-crop-year yield trials located on 12 commercial farms in different regions of the industry. Identification of mosaic susceptibility currently relies on the observation of symptoms resulting from natural infection by aphids during the course of cultivar development. Natural infection requires available inoculum in the form of virus-infected plants and the presence of aphid vectors to successfully transmit mosaic to other susceptible plants. Because of a high frequency of resistance in the parents and selection population and cultivation of resistant commercial on farms where yield trials are conducted, inoculum pressure may be insufficient to result in infection and detection of susceptible clones during the selection process. In addition, mosaic symptoms are most obvious in young developing leaves at the shoot apex, and these leaves are most easily observed in young plants prior to stalk elongation. Field surveys to detect plants with

mosaic symptoms have not been conducted recently at times of the year when plant growth is most favorable for observation of symptoms. These factors could result in escape of mosaic susceptible experimental clones from infection or failure to detect low levels of infection, and mosaic susceptible clones could potentially continue to advance in the program undetected.

During 2016, symptoms of mosaic were detected in multiple experimental clones that were well advanced in the breeding program, including one being considered for commercial release, HoCP 09-804. These observations caused recognition of the uncertainty about current levels of resistance in the commercial and basic breeding parent populations and made it apparent that research was urgently needed to assess the degree of mosaic incursion in the breeding program and the threat of re-emergence of this important disease in the industry.

Both SrMV and SCMV can be mechanically transmitted, and previously, greenhouse inoculation experiments were used to screen for disease resistance (Grisham 2000). Renewed mechanical inoculations were needed to evaluate mosaic resistance levels in the current basic and commercial breeding parent populations to enable breeders to eliminate susceptible parents and make informed crosses.

4.2. Objectives

To evaluate the current basic and commercial sugarcane selection parent populations for resistance to mosaic using mechanical inoculations in a greenhouse setting and to verify the association of virus infection with symptom expression by reverse transcription polymerase chain reaction (RT-PCR).

4.3. Materials and Methods

Four mechanical inoculations were performed during 2017: two at the USDA-ARS Sugarcane Research Unit (Houma, LA) and two at Louisiana State University (Baton Rouge, LA). Clones

of current and potential parents were tested from the USDA basic and commercial breeding programs (211 in experiment one and 153 in experiment two) and from the LSU Agricultural Center commercial breeding program (109 in experiment one and 9 in experiment two). To test for repeatability of the results, 20 clones were inoculated twice (two replicates of six plants) in the second USDA experiment. Five of the 20 repeat clones in the second USDA experiment were also inoculated in experiment one, and nine clones (the entire experiment) were repeated in the second LSU inoculation. Experimental clones/cultivars with known susceptibility levels were included as checks in each inoculation, including mosaic resistant HoCP 96-540, highly susceptible L 08-088, and moderately susceptible HoCP 09-804, and a highly susceptible *Sorghum bicolor* cultivar 'Rio' was included as an additional susceptible check. At least three replicates of six plants were inoculated and a single replicate was left uninoculated for the checks in each experiment.

Single-node stalk cuttings were heat-treated in water at 50^{o}C for 45 min, and six single-node cuttings were planted per clone in 18-cell styrofoam trays (Speedling Inc., Ruskin, FL) in soil-less potting mix. Plants were maintained throughout the experiment in greenhouses located either at the USDA research station or LSU campus greenhouses. Plants were watered daily and 24-8-16 N-P-K fertilizer (Scotts Miracle Gro, Marysville, OH) was applied once a week. Inoculations were performed once plants had approximately 5-6 leaves.

Inoculum preparation followed modified instructions from Bureau of Sugarcane Experiment Stations mosaic trial guidelines. Inoculation stock buffer consisted of 0.1 M potassium phosphate buffer (pH 7) that was diluted to 0.01 M buffer for use. Mosaic-infected plants were cultivated at the LSU AgCenter Sugar Research Station to provide a source of inoculum consisting of young symptomatic leaves. One kg of leaf tissue was combined with 4 L

of 0.01 M buffer in a food processor. The homogenized mixture was then incubated for 1 h at 4°C to allow virions to diffuse out of the leaf tissue. Leaves were strained from the buffer mixture using cheese-cloth or insect-netting. The filtered inoculum was kept on ice throughout the inoculation. Carborundum (600 grit) was applied as an abrasive onto the leaves. Inoculum was applied using a Scotch-Brite™ scouring pad (3M, Maplewood, MN) in an upward motion maintaining contact with both sides of the leaf. The three youngest leaves were inoculated on each plant. Plants were rinsed with tap water briefly after inoculum was applied, kept in the headhouse of the greenhouse overnight, and then placed in the greenhouse the next morning. The prior fertilizer and watering schedules were maintained until plants were evaluated 5 wk after inoculation.

Plants were evaluated for mosaic by visual observation of symptoms, and the number of plants with symptoms out of the total number of plants that germinated for each clone was recorded. The recorded numbers were then converted to percent infection and grouped into percentage intervals of 0, 1-24, 25-49, 50-74, and 75-100%. These infection percentage intervals were then assigned ratings as 0% = highly resistant, 1-24% = moderately resistant, 25-49% = moderately susceptible, 50-74% = susceptible, and 75-100% = highly susceptible. Due to variability in the success of germination from the single-node cuttings, results are only reported for those clones that had at least four plants to evaluate, so 203 of 211 clones are reported for the first USDA inoculation, 117 of 153 clones for the second USDA inoculation, 73 of 109 clones from the first LSU inoculation, and 8 of 9 clones from the second LSU inoculation. In addition, chlorosis due to nutritional deficiency was extensive in the first LSU experiment, and it was necessary to remove plants that were too chlorotic to accurately evaluate for mosaic from the total number of plants evaluated for a clone.

A total subsample of 55 leaves of three types (12 from USDA experiment one, 15 from USDA experiment two, 17 from LSU experiment one, and 11 from LSU experiment two) was collected arbitrarily to test for SrMV using reverse transcription polymerase chain reaction (RT-PCR). The types of collected samples included asymptomatic (19 total), symptomatic (22), and uncertain leaf samples for which symptom expression was difficult to evaluate (14). Leaves were collected in plastic bags and placed on ice until return to the lab and stored at -70°C until RNA extraction.

Total RNA was extracted using the Plant Total RNA Kit (Spectrum™, Sigma Aldrich) with modifications to the tissue homogenization steps. Approximately 300 mg of tissue was homogenized in a BIOREBA extraction bag (BIOREBA AG, Switzerland) with 2 ml prepared lysis buffer from the Plant Total RNA Kit (Spectrum™, Sigma Aldrich) using a BIOREBA standard rack tissue homogenizer. RT-PCR was carried out in two steps and using a modification of the RT-PCR method described by Yang and Mirkov (1997). Complementary DNA synthesis was performed using the SuperScript™ First-Strand Synthesis System (Invitrogen™, Thermo Fisher Scientific), and RNA was primed with 1 µl (2 µM stock) of SrMV-R3. Then 2 µl of diluted cDNA product (1:50) in nuclease free water was added to a 25 µl PCR solution. The PCR solution consisted of 12.5 µl of GoTaq® Green Master Mix, 2X (Promega), 11.86 µl of nuclease free water, 0.25 µl (10 µM stock) of SrMV-F3 and SrMV-R3, 0.14 µl of bovine serum albumin V (100 µg/µl). The PCR program used was 95°C for 2 min; 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1 min; and final extension at 72°C for 5 min. RT-PCR products were electrophoresed in 2.0% agarose gel containing ethidium bromide (final concentration 0.35 µg/ml) for 1 h, and bands were visualized using a UV transilluminator. Positive results were concluded when a visible band was present at 871 bp.

4.4. Results

Mechanical inoculations detected variable mosaic resistance levels for the sugarcane breeding program basic and commercial parent populations (USDA and LSU programs). Six single-node cuttings were planted per clone, but not all cuttings germinated for each. The results are reported for clones with a total of at least four plants. All clone responses, including those with less than four plants and check cultivars are provided in Appendix B.1. The inoculation of the checks L 08-088, Rio sorghum, HoCP 09-804, and HoCP 96-540 gave expected results of 67-100, 100, 0-40, and 0% infection for each, respectively, across the two USDA experiments indicating the inoculations were successful in mechanically transmitting the virus to susceptible clones. In both LSU experiments, the inoculated HoCP 09-804 plants did not express symptoms, and some repetitions of L 08-88 did not express over 75% symptomatic plants. Nutritional deficiency symptoms in the first LSU experiment made it difficult to evaluate symptom expression in some parents and check sugarcane cultivars, and the Rio sorghum check plants could not be evaluated.

No symptoms were observed in 69.5, 70.9, and 79.5% of the clones following the first and second USDA inoculations and first LSU inoculation, respectively, and were rated as highly resistant (Table 4.1). Inclusion of clones rated as moderately resistant increased the total percentage of resistant clones in each experiment to 73.9, 78.6, and 86.3%, respectively. In each of the experiments, varying levels of susceptibility indicated as different proportions of symptomatic plants were observed for the other clones (Table 4.1). When comparing the basic versus the commercial breeding program parents in the first and second USDA experiments and LSU experiment one overall, susceptibility was detected in 13.5, 18.7, and 0%, respectively, of the basic parents compared to 32.3, 24.2, and 14.4%, respectively, of the commercial parents (Table 4.1). Variability in frequency of susceptibility was detected among the different year-of-assignment series for USDA commercial parents. For the different series in the two USDA

experiments, the frequency of susceptibility for all the clones in the more advanced 2011 -2013 series (in experiment two) combined was 33.3%, and the frequencies for the 2014, 2015, 2016 series from experiment one and the 2017 series from experiment two were 31.0, 21.2, 40.0, and 15.8%, respectively (Table 4.1).

Repeatability of mosaic infection reactions resulting from mechanical inoculation was evaluated for five clones that were included in both USDA experiments, two replicates of 20 clones inoculated in the second USDA experiments, and nine clones repeated in the second LSU experiment. The results reported included only clones with four or more plants. For the five clones included in both USDA experiments, three had no infection in both, while the other two had infected plants in both but were rated as moderately susceptible and susceptible in experiment one and moderately resistant in experiment two (Table 4.2). For the clones repeated as two replicates in USDA experiment two, 4 of 13 (30.8%) received a different rating for the two replicates; however, only 2 (15.4%) varied enough to change from a resistant to susceptible rating (Table 4.2). Both of these clones developed no infected plants in one replicate and 40-50% infection in the second replicate. For the clones repeated in the two LSU experiments, 3 of 9 (33%) had infection percentage changes sufficient to change from a susceptible to resistant rating (Table 4.2). Two of the clones had infected plants in the first experiment but none in the second.

Table 4.1. Mosaic mechanical inoculation results for experiments conducted at the USDA-ARS Sugarcane Research Unit and LSU Agricultural Center during 2017.

Experiment and parent population [a]	Series [b]	Number of clones	Number of clones (percentage) categorized by resistance rating [c]				
			HR	MR	MS	S	HS
USDA 1							
Basic	2016	67	56	2	1	3	5
	B total	67	56 (83.6)	2 (3)	1 (1.5)	3 (4.5)	5 (7.5)
Commercial	2014	29	19	1	1	3	5
	2015	52	37	2	2	7	2
	2016	55	29	4	1	9	12
	C total	136	85 (62.5)	7 (5.1)	4 (2.9)	19 (14)	21 (15.4)
	Total	203	141 (69.5)	9(4.4)	5 (2.5)	22 (10.8)	26 (12.8)
USDA 2							
Basic	2017	59	48	0	8	1	2
	B total	59	48 (81.4)	0 (0)	8 (13.6)	1 (1.7)	2 (3.4)
Commercial	2011	2	0	1	0	1	0
	2012	3	3	0	0	0	0
	2013	4	2	0	1	0	1
	2014	7	4	2	0	1	0
	2015	4	0	0	2	1	1
	2017	38	26	6	1	0	5
	C total	58	35 (41.2)	9 (15.5)	4 (6.9)	3 (5.2)	7 (12.1)
	Total	117	83 (70.9)	9 (7.7)	12 (10.3)	4 (3.4)	9 (7.7)

(table cont'd)

Experiment and parent population [a]	Series [b]	Number of clones	Number of clones (percentage) categorized by resistance rating [c]				
			HR	MR	MS	S	HS
LSU 1							
Basic	2009	2	2	0	0	0	0
	2011	1	1	0	0	0	0
	2012	1	1	0	0	0	0
	B total	4	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Commercial	1981	1	1	0	0	0	0
	1983	1	0	0	0	1	0
	1985	1	1	0	0	0	0
	1986	1	0	1	0	0	0
	1992	2	2	0	0	0	0
	1994	1	1	0	0	0	0
	1995	2	2	0	0	0	0
	1996	1	1	0	0	0	0
	1997	2	2	0	0	0	0
	1998	2	1	0	0	0	1
	1999	2	1	1	0	0	0
	2001	4	3	1	0	0	0
	2002	1	1	0	0	0	0
	2004	1	1	0	0	0	0
	2005	3	3	0	0	0	0
	2006	6	4	1	1	0	0
	2008	3	2	0	0	0	1
	2009	8	7	0	1	0	0
	2011	4	4	0	0	0	0
	2012	3	3	0	0	0	0

(table cont'd)

Experiment and parent population [a]	Series [b]	Number of clones	Number of clones (percentage) categorized by resistance rating [c]				
			HR	MR	MS	S	HS
	2013	7	5	0	1	1	0
	2014	8	6	1	0	0	1
	2015	5	3	0	2	0	0
	C total	69	54 (78.3)	5 (7.2)	5 (7.2)	2 (2.9)	3 (4.3)
	Total	73 (100)	58 (79.5)	5 (6.8)	5 (6.8)	2 (2.7)	3 (4.1)

[a] Three experiments were conducted: two with clones from the USDA-ARS basic breeding (Basic) and commercial (Com.) parent populations and one from the LSU AgCenter commercial parent population.
[b] Series refers to clone groupings by year of assignment of permanent identification number. B = basic parent population and C = commercial parent population.
[c] Results are reported for clones that had at least four inoculated plants. Assigned ratings were HR = highly resistant (0% mosaic infection), MR = moderately resistant (1-24%), MS = moderately susceptible (25-49%), S = susceptible (50-74%), and HS = highly susceptible (75-100%).

Table 4.2. Repeated clone inoculation results from greenhouse mosaic mechanical inoculation experiments conducted at the USDA-ARS Sugarcane Research Unit and LSU during 2017.

	Number of symptomatic plants (percent infection) [a]		
Experiment and clone	Experiment one	Experiment two	
USDA experiments		Replicate one	Replicate two
Ho 12-615	-	0 (0)	0 (0)
Ho 13-739	-	0 (0)	0 (0)
HoL 14-841	-	4 (67)	4 (80)
HoCP 13-740	-	0 (0)	0 (0)
Ho 11-573	-	0 (0)	2 (40)
HoCP 14-801	4 (67)	1 (17)	-
HoCP 15-510	-	0 (0)	3 (50)
Ho 15-921	-	5 (100)	6 (100)
HoCP 14-826	0 (0)	0 (0)	0 (0)
Ho 14-864	2 (40)	1 (17)	0 (0)
HoCP 14-885	-	0 (0)	0 (0)
HoL 15-508	-	1 (25)	1 (25)
HoCP 14-802	0(0)	0 (0)	0 (0)
HoCP 14-867	0(0)	0 (0)	0 (0)
LSU experiments			
Ho 08-730	4 (100)	0 (0)	-
Ho 09-832	2 (33)	1 (20)	-
L 12-201	0 (0)	0 (0)	-
L 13-251	0 (0)	0 (0)	-
L 14-267	0 (0)	0 (0)	-
L 14-282	0 (0)	0 (0)	-
L 15-305	2 (33)	0 (0)	-

[a] Percent infection of the plants with mosaic symptoms in the inoculation. - = clone not included in the inoculation.

Leaf samples from 55 total clones were tested for SrMV by RT-PCR when the inoculations were evaluated. Across experiments, all 22 symptomatic samples were positive for SrMV, whereas 2 of 19 (10.5%) asymptomatic samples (both from the first LSU experiment) and 8 of 14 (57.1%) uncertain samples tested positive for SrMV (Table 4.3).

Table 4.3. Detection of SrMV by RT-PCR in leaf samples collected from greenhouse mosaic mechanical inoculation experiments conducted at the USDA-ARS Sugarcane Research Unit and LSU during 2017.

Clones in four experiments	Mosaic symptoms (A,S,?)[a]	SrMV result (+,-)[b]
USDA experiment one		
HoL 15-511	A	-
HoCP 15-548	A	-
HoH15-927	A	-
Ho 15-943	A	-
Ho 15-954	A	-
Ho 16-663	?	-
Ho 16-9013	?	+
L 08-88	S	+
Ho 14-863	S	+
HoCP 15-525	S	+
HoCP 15-543	S	+
Ho 15-921	S	+
USDA experiment two		
HoCP 96-540	A	-
HoCP 17-703	A	-
Ho 17-724	A	-
Ho 17-738	A	-
Ho 17-9113	A	-
HoCP 09-804	?	+
HoL 15-508	?	+
Ho 17-159	?	-
HoCP 17-702	?	+
Ho 17-727	?	-
L 08-88	S	+
L 11-183	S	+
HoCP 13-758	S	+
Ho 17-723	S	+
Ho 17-732	S	+
LSU experiment one		
HoCP 95-951	A	+
HoCP 96-540	A	-
L 06-038	A	-
L 12-201	A	-
L 14-282	A	+
US 01-040	?	-
Ho 07-617	?	+

(table cont'd)

Clones in four experiments	Mosaic symptoms (A,S,?)[a]	SrMV result (+,-)[b]
L 08-88	?	+
L 11-183	?	-
L 13-234	?	+
L 14-265	?	-
L 14-266	?	+
L 98-209	S	+
Ho 06-563	S	+
L 08-88	S	+
Ho 08-730	S	+
L 14-275	S	+
LSU experiment two		
HoCP 96-540	A	-
L 08-88	A	-
HoCP 09-804	A	-
L 11-183	A	-
L 08-88 #1	S	+
L 08-88 #2	S	+
Ho 09-832	S	+
HoCP 13-723 #1	S	+
HoCP13-723 #2	S	+
Sorghum 'Rio' #1	S	+
Sorghum 'Rio' #2	S	+

[a] Leaf samples were recorded as asymptomatic (A), symptomatic (S), or uncertain (?) for mosaic symptoms.
[b] Leaf samples were tested for SrMV using RT-PCR. A positive result = + and a negative result = -. Presence of a visible band in electrophoresis gel at 871 bp size = +; absence of expected band = -.

4.5. Discussion

The observation of mosaic symptoms in advanced experimental clones of the sugarcane breeding program indicated that susceptibility in some cases had not been detected during early stages of selection. This finding further suggested that there could be some level of undetected susceptibility in the current parent populations in the breeding program. The long-term absence or low percentage of mosaic in experimental clones has made detection more difficult,

particularly as new personnel work in the breeding program. The disease is difficult to detect at low levels, and symptoms are readily seen only in young leaves of plants at immature stages of growth. The current outbreak indicated a need for greater scrutiny of mosaic resistance in parents and subsequent evaluation of progeny in selection.

Mechanical inoculations were conducted in the greenhouse to obtain mosaic resistance reactions for the commercial and basic breeding recurrent parents in order to determine whether mosaic susceptibility was unknowingly permeating these populations. The inoculations of the USDA commercial and basic populations were successful and detected varying levels of mosaic susceptibility in both populations. The check clones (resistant HoCP 96-540, moderately susceptible HoCP 09-804, and highly susceptible L 08-088 and *S. bicolor* 'Rio') included in the inoculations responded as expected, indicating that the mechanical inoculations were capable of accurately detecting different levels of susceptibility in the clones with unknown levels of susceptibility.

Clones with varying levels of susceptibility were detected in all series of both basic and commercial breeding populations. The frequency of susceptibility varied some among different series of the commercial parent population but occurred at undesirably high levels throughout. An evaluation of mosaic susceptibility in the parentage of susceptible clones indicated that approximately 40% of these clones had two of the same parents, HoCP 01-517 and Ho 09-831, in common. Mosaic susceptibility was detected in the basic breeding population, but the frequency of susceptibility was lower than for the commercial parent population. This result indicates that the basic breeding program will continue to be a valuable resource for the continued incorporation of resistance to mosaic.

Because mechanical inoculations had not been conducted in years, it was necessary to evaluate the reliability of the method and repeatability of the results. Further optimization of the methodology related to plant production, source of inoculum, virus extraction, and inoculation method may be possible. Due to a low bud germination rate, a few clones could not be assigned a rating due to an inadequate number of plants to provide confidence in the results. The evaluation of infection in six plants per clone allowed enough infection percentage intervals to assign resistance reaction types capable of distinguishing a range of ratings from highly resistant to highly susceptible. The degree of correlation of these ratings with responses in the field due to natural infection will need to be determined over time.

Repeatability of clone resistance reactions is also an important concern. In this study, repeatability was only evaluated in a small number of clones, but it was shown to be variable for some clones. Twenty of 27 (74%) clones did not vary between a resistant and susceptible rating in the repeat inoculations, but 5 of 27 (18.5%) of the clones developed some level of mosaic infection following one inoculation but not the other demonstrating that escapes are possible. Therefore, clone resistance ratings will need to come from multiple inoculations to ensure the assignment of accurate ratings, particularly highly resistant ratings.

A final consideration potentially affecting accurate determination of clonal resistance levels by inoculation would be how reliably virus-infected plants develop visible mosaic symptoms. To evaluate this, leaf samples were collected from a subsample of clones to determine SrMV infection association with visible symptom expression by RT-PCR. All samples classified as symptomatic tested positive for SrMV. All samples classified as asymptomatic from the USDA experiments tested negative. In the LSU experiments, 2 of 9 (22.2%) asymptomatic samples tested positive, but leaf symptoms of nutrient deficiency made it difficult to evaluate

mosaic symptoms. Overall, a small proportion of plants were considered uncertain as to whether they were symptomatic or asymptomatic (1.4% across the USDA experiments and 5.9% across the LSU experiments that exhibited nutrient deficiency symptoms). Testing of leaf samples from these plants found that 1 of 2 (50%), 3 of 5 (60%), and 4 of 7 (57%) of these samples tested positive for SrMV within the first and second USDA and first LSU experiments, respectively. These results indicate confusion is possible in visual evaluation of mild mosaic symptoms for some clones, and the possibility of a low level of error exists in the evaluations. Providing optimal growing conditions for the inoculated plants to allow symptoms to develop is therefore very important for insuring the most accurate visual evaluation possible.

The results from the mechanical inoculations to screen for mosaic susceptibility in the commercial and basic breeding programs' recurrent parents of the sugarcane breeding program indicate that this method is needed to accurately monitor and maintain mosaic resistance when natural disease pressure is low during the selection program. Resistance ratings determined by mechanical inoculation do not take into account host interactions with aphid vectors that could affect disease in the field. However, the mechanical transmissibility of the virus provides the opportunity to obtain valuable information concerning host resistance to the virus. Information gained from these inoculations provided a current status of mosaic susceptibility in the breeding germplasm. This information will allow breeders to eliminate some obvious sources of susceptibility then make informed crosses with agronomically desirable parents with moderate susceptibility that will minimize the impact of mosaic on the program. Many clones in the breeding germplasm exhibited resistance, so successful control of mosaic through the deployment of host plant resistance should be maintainable in the future.

CHAPTER V. CONCLUSIONS

Following the detection of mosaic infections in advanced experimental clones of the breeding program and a recently released cultivar, HoCP 09-804, multiple research objectives were undertaken to address the extent and causes of the outbreak and prevent it from re-emerging as an important problem in the Louisiana sugarcane industry. The research undertaken led to the following conclusions:

- Field surveys determined that mosaic was irregularly distributed in the Primary and Secondary Stations of the American Sugar Cane League Variety Release Program and breeding program Outfield trials, occurring in only three of five production areas, and that the incidence of mosaic at the locations where it was detected was generally low.
- The reverse transcription polymerase chain reaction (RT-PCR) assay demonstrated that there is an association between visual mosaic symptoms and virus infection, and this finding provided confidence in the survey incidence results that were based on visual observation of symptomatic plants. The RT-PCR results also determined that Sorghum mosaic virus (SrMV) is the current causal virus species of mosaic in Louisiana.
- A runs analysis of infected plants within rows at surveyed locations detected frequent aggregation of infection, and these results along with the geographic distribution of mosaic suggest that the mosaic infections detected during the surveys resulted from planting infected seed-cane.
- Disease incidence did not increase across three crop years, so high rates of disease increase due to aphid transmission of the virus are not likely under current conditions.
- Foliar and germination recovery from mosaic was determined to be variable in modern clones. L 10-147 exhibited a higher frequency of both foliar and germination recovery than

HoCP 09-804. The extent of recovery observed was more often partial than complete for both clones. The frequency of germination recovery decreased in one ratoon crop for both HoCP 09-804 and L 10-147, but the expression of recovery in ratoon crops needs further study.

- L 10-147 exhibited more germination recovery in plants produced from buds on planted stalks that exhibited foliar recovery, but additional research is needed to determine if there is a consistent association between foliar and germination recovery.
- Negative RT-PCR assay results suggested that most recovered plants may no longer be infected by SrMV, suggesting that recovery from mosaic is not only a loss of symptoms.
- Mosaic impact on yield varied between the two study clones, negatively affecting bud germination and yield components in the cultivar HoCP 09-804 but not L 10-147.
- L 10-147 exhibited both a higher frequency of recovery and a lower yield loss than HoCP 09-804. However, additional research is needed to determine if there is an association between recovery potential and yield loss.
- Recovery could be a factor affecting disease management since it may decrease mosaic incidence in commercial plantings.
- Mechanical inoculations revealed variable levels of susceptibility to mosaic within the basic and commercial breeding program's parent populations and allowed detection of sources of resistance and susceptibility in the sugarcane breeding program.
- Limited inoculation repeatability results suggested infection escapes are possible and additional optimization of the mechanical inoculations is needed to improve the repeatability of results and to determine the correlation between resistance ratings assigned from inoculations and field responses.

- A considerable level of resistance to mosaic is present in the current breeding germplasm, particularly the basic breeding parent population, so it should be possible to continue the management of mosaic through host plant resistance. The information gained from these inoculations will allow sugarcane breeders to remove stays in the program and make informed crosses when using susceptible parents.

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APPENDIX A. SRMV RT-PCR RESULTS FROM FIELD SURVEY LOCATIONS

APPENDIX A.1: Locations from which leaf samples were collected and tested for Sorghum mosaic virus (SrMV) by RT-PCR, the number of samples collected, and the percentage of virus positive samples. [a]

Year	Parish	Location	Crop cycle year	Clone	Number of asymptomatic samples	Percent positive SrMV	Number of symptomatic samples	Percent positive SrMV
2016	Assumption	Cedar Grove	First ratoon	HoCP 09-804	-	-	4	100
2016	Assumption	Lula	First ratoon	HoCP 09-804	8	0	-	-
2016	St. Martin	Huey Dugas	First ratoon	HoCP 09-804	5	0	-	-
2016	Assumption	Carmouche	Plant cane	HoCP 09-804	5	0	-	-
2016	Assumption	Cedar Grove	Plant cane	HoCP 09-804	-	-	8	100
2016	Assumption	Glenwood	Plant cane	HoCP 09-804	27	0	9	100
2016	Assumption	Little Texas	Plant cane	HoCP 09-804	10	0	11	100
2016	Assumption	Thibodaux Brothers	Plant cane	HoCP 09-804	14	0	10	100
2016	Assumption	Thibodaux Brothers	Plant cane	HoCP 09-804	10	0	13	100
2016	Lafourche	McCloud	Plant cane	HoCP 09-804	10	0	10	100
2016	Pointe Coupee	John Good	Plant cane	HoCP 09-804	12	0	7	100
2016	St. James	Bon Secour	Plant cane	HoCP 09-804	10	0	9	100
2016	St. James	Martin & Poche	Plant cane	HoCP 09-804	9	0	8	100
2016	St. John	Glendale	Plant cane	HoCP 09-804	18	0	-	-
2016	Terrebonne	Naquin	Plant cane	HoCP 09-804	13	0	11	100
2016	West Baton Rouge	Robert Morris	Plant cane	HoCP 09-804	17	0	15	100
2016	Assumption	Landry	Introduction	L 10-147	5	0	5	100
2016	Assumption	Little Texas	Introduction	L 10-147	3	0	-	-
2016	Assumption	Little Texas	Introduction	L 10-147	-	-	3	100
2016	Pointe Coupee	Beaud	Introduction	L 10-147	3	0	3	100
2016	Rapides	Harper	Introduction	L 10-147	-	-	5	100
2016	St. Martin	Levert St. John	Introduction	L 10-147	-	-	5	100
2016	Terrebonne	Naquin	Introduction	L 10-147	3	0	3	100
2016	Assumption	Little Texas	Plant cane	L 11-183	5	0	5	100

2016	Assumption	Little Texas	Plant cane	L 11-532	12	0	11	100
2016	Assumption	Little Texas	Plant cane	Ho 12-626	1	0	1	100
2016	Assumption	Little Texas	Plant cane	Ho 12-671	4	0	3	100
2016	Assumption	Landry	Introduction	L 13-242	2	0	2	100
2016	Assumption	Glenwood	Introduction	L 13-269	-	-	4	100
2017	Assumption	Cedar Grove	First ratoon	HoCP 09-804	8	0	8	100
2017	Assumption	Glenwood	First ratoon	HoCP 09-804	4	0	4	100
2017	Assumption	Little Texas	First ratoon	HoCP 09-804	8	0	7	100
2017	Assumption	Thibodaux Brothers French	First ratoon	HoCP 09-804	12	8	6	100
2017	Lafourche	McCloud	First ratoon	HoCP 09-804	5	0	5	100
2017	Lafourche	Raceland	First ratoon	HoCP 09-804	7	0	10	100
2017	Pointe Coupee	Alma	First ratoon	HoCP 09-804	10	0	10	100
2017	St. James	Blackberry	First ratoon	HoCP 09-804	5	0	5	100
2017	St. John	Glendale	First ratoon	HoCP 09-804	5	0	6	100
2017	Assumption	Cedar Grove	Plant cane	HoCP 09-804	5	0	5	100
2017	Pointe Coupee	Alma	Plant cane	HoCP 09-804	8	0	8	100
2017	St. James	Blackberry	Plant cane	HoCP 09-804	5	0	4	100
2017	Assumption	Little Texas	First ratoon	L 11-183	5	0	5	100
2017	Pointe Coupee	Alma	Plant cane	L 11-183	1	0	1	100
2017	Ascension	Palo Alto	First ratoon	L 11-532	5	0	5	100
2017	Assumption	Little Texas	First ratoon	L 11-532	6	0	5	100
2017	Assumption	Little Texas	Plant cane	L 11-573	1	0	1	100
2018	Assumption	Little Texas	Plant cane	L 11-183	-	-	1	100
2018	Pointe Coupee	Alma	Plant cane	L 11-183	2	0	2	100
2018	Assumption	Little Texas	Second ratoon	L 11-183	-	-	1	100

[a] - = no samples collected

APPENDIX B. MOSAIC MECHANICAL INOCULATION RESULTS FOR ALL CLONES

APPENDIX B.1. Mosaic infection results from four greenhouse mechanical inoculation experiments of the basic and commercial recurrent parents for the sugarcane breeding program conducted in cooperation with the USDA-ARS Sugarcane Research Unit and the LSU Agricultural Center Sugar Research Station.

Experiment and cultivar/experimental clone [a]	Total plants [b]	Symptomatic plants [b]	Percent symptomatic plants [b]
USDA experiment one			
HoL 14-841	4	4	100
HoCP 14-843	6	5	83
HoCP 14-801	6	4	67
Ho 14-863	6	4	67
HoCP 14-890	5	3	60
HoCP 14-865	6	3	50
Ho 14-864	5	2	40
HoCP 14-876	3	1	33
Ho 14-827	5	1	20
HoCP 14-901	5	1	20
HoCP 14-853	6	1	17
HoCP 14-802	4	0	0
HoCP 14-803	6	0	0
HoCP 14-814	5	0	0
Ho 14-819	6	0	0
HoCP 14-823	6	0	0
HoCP 14-826	4	0	0
HoCP 14-828	5	0	0
HoCP 14-829	6	0	0
HoCP 14-830	6	0	0
HoCP 14-831	5	0	0
Ho 14-832	5	0	0
Ho 14-836	4	0	0
HoCP 14-844	6	0	0
HoCP 14-855	5	0	0
HoCP 14-867	6	0	0
HoCP 14-878	4	0	0
HoCP 14-885	5	0	0
HoCP 14-892	6	0	0
HoCP 14-897	5	0	0

HoCP 14-902	2	0	0
HoCP 15-915	6	6	100
Ho 15-921	6	6	100
Ho 16-607	6	6	100
HoCP 15-510	6	5	83
Ho 16-605	5	4	80
HoL 15-547	6	4	67
HoCP 15-543	6	3	50
Ho 15-918	6	3	50
HoCP 15-991	6	3	50
HoCP 15-525	5	2	40
Ho 15-975	5	2	40
Ho 15-962	6	2	33
HoCP 15-987	6	2	33
HoCP 15-519	5	1	20
Ho 15-963	5	1	20
Ho 16-609	5	1	20
HoL 15-501	6	1	17
Ho 15-971	6	1	17
HoL 15-502	5	0	0
HoCP 15-503	6	0	0
HoCP 15-504	6	0	0
HoCP 15-506	5	0	0
HoL 15-508	6	0	0
HoL 15-511	6	0	0
HoL 15-513	6	0	0
Ho 15-531	5	0	0
HoL 15-534	3	0	0
HoCP 15-537	6	0	0
Ho 15-538	6	0	0
HoL 15-539	6	0	0
HoCP 15-548	6	0	0
Ho 15-916	6	0	0
HoH 15-926	5	0	0
HoH 15-927	6	0	0
Ho 15-930	6	0	0
Ho 15-936	4	0	0
Ho 15-938	4	0	0
Ho 15-943	5	0	0
Ho 15-944	6	0	0
Ho 15-945	6	0	0

Ho 15-954	5	0	0
Ho 15-957	6	0	0
Ho 15-958	5	0	0
Ho 15-959	3	0	0
Ho 15-960	6	0	0
Ho 15-964	6	0	0
Ho 15-965	4	0	0
Ho 15-970	6	0	0
Ho 15-972	5	0	0
Ho 15-979	6	0	0
Ho 15-984	6	0	0
Ho 15-985	5	0	0
HoCP 15-986	5	0	0
HoCP 15-990	5	0	0
HoL 15-993	5	0	0
HoL 15-994	6	0	0
HoCP 15-996	6	0	0
Ho 16-619	5	5	100
Ho 16-621	6	6	100
Ho 16-623	5	5	100
Ho 16-631	6	6	100
Ho 16-651	5	5	100
Ho 16-656	6	6	100
Ho 16-677	5	5	100
Ho 16-9033	5	5	100
Ho 16-9070	6	6	100
Ho 16-9013	6	5	83
Ho 16-9018	6	5	83
Ho 16-9020	5	4	80
Ho 16-638	4	3	75
Ho 16-634	6	4	67
Ho 16-636	5	3	60
Ho 16-628	6	3	50
Ho 16-641	6	3	50
Ho 16-645	2	1	50
Ho 16-649	6	3	50
Ho 16-654	6	3	50
HoCP 16-672	6	3	50
Ho 16-9014	6	3	50
Ho 16-9054	6	3	50
Ho 16-648	5	2	40

Ho 16-666	5	2	40
Ho 16-9049	5	2	40
Ho 16-632	6	2	33
Ho 16-652	6	2	33
Ho 16-9061	4	1	25
Ho 16-650	6	1	17
Ho 16-653	6	1	17
Ho 16-678	6	1	17
Ho 16-680	6	1	17
Ho 16-9034	6	1	17
Ho 16-9042	6	1	17
Ho 16-600	6	0	0
Ho 16-601	6	0	0
Ho 16-603	5	0	0
Ho 16-604	5	0	0
Ho 16-606	4	0	0
Ho 16-608	6	0	0
Ho 16-610	6	0	0
Ho 16-612	3	0	0
Ho 16-617	6	0	0
Ho 16-618	5	0	0
Ho 16-622	6	0	0
Ho 16-624	6	0	0
Ho 16-625	6	0	0
Ho 16-626	6	0	0
Ho 16-627	6	0	0
Ho 16-635	6	0	0
Ho 16-639	6	0	0
Ho 16-642	6	0	0
Ho 16-644	6	0	0
Ho 16-646	5	0	0
Ho 16-647	6	0	0
Ho 16-657	3	0	0
Ho 16-658	4	0	0
Ho 16-662	5	0	0
Ho 16-663	5	0	0
Ho 16-664	6	0	0
Ho 16-667	6	0	0
HoCP 16-669	5	0	0
HoCP 16-670	6	0	0
HoCP 16-674	4	0	0

HoCP 16-675	5	0	0
Ho 16-9003	5	0	0
Ho 16-9004	6	0	0
Ho 16-9005	6	0	0
Ho 16-9006	6	0	0
Ho 16-9007	6	0	0
Ho 16-9008	5	0	0
Ho 16-9009	6	0	0
Ho 16-9010	6	0	0
Ho 16-9011	6	0	0
Ho 16-9012	6	0	0
Ho 16-9015	4	0	0
Ho 16-9016	4	0	0
Ho 16-9017	5	0	0
Ho 16-9019	6	0	0
Ho 16-9021	6	0	0
Ho 16-9022	2	0	0
Ho 16-9023	5	0	0
Ho 16-9024	6	0	0
Ho 16-9025	6	0	0
Ho 16-9026	6	0	0
Ho 16-9027	6	0	0
Ho 16-9028	5	0	0
Ho 16-9029	6	0	0
Ho 16-9030	5	0	0
Ho 16-9031	6	0	0
Ho 16-9032	5	0	0
Ho 16-9035	6	0	0
Ho 16-9036	6	0	0
Ho 16-9037	6	0	0
Ho 16-9038	6	0	0
Ho 16-9039	5	0	0
Ho 16-9040	5	0	0
Ho 16-9041	6	0	0
Ho 16-9043	6	0	0
Ho 16-9044	6	0	0
Ho 16-9045	6	0	0
Ho 16-9046	5	0	0
Ho 16-9047	6	0	0
Ho 16-9048	4	0	0
Ho 16-9050	6	0	0

Ho 16-9051	5	0	0
Ho 16-9052	4	0	0
Ho 16-9053	6	0	0
Ho 16-9055	4	0	0
Ho 16-9056	6	0	0
Ho 16-9057	6	0	0
Ho 16-9058	5	0	0
Ho 16-9059	6	0	0
Ho 16-9060	6	0	0
Ho 16-9062	6	0	0
Ho 16-9063	5	0	0
Ho 16-9064	6	0	0
Ho 16-9065	6	0	0
Ho 16-9066	6	0	0
Ho 16-9067	5	0	0
Ho 16-9068	6	0	0
Ho 16-9069	6	0	0
USDA experiment two			
L 11-183	4	3	75
L 11-183	1	0	0
Ho 11-573	5	2	40
Ho 11-573	6	0	0
L 12-201	4	0	0
L 12-201	3	0	0
Ho 12-615	6	0	0
Ho 12-615	5	0	0
Ho 12-630	3	0	0
Ho 12-630	4	0	0
Ho 13-708	2	2	100
Ho 13-708	4	3	75
HoCP 13-758	3	1	33
HoCP 13-758	4	1	25
Ho 13-739	5	0	0
Ho 13-739	6	0	0
HoCP 13-740	6	0	0
HoCP 13-740	5	0	0
HoL 14-841	5	4	80
HoL 14-841	6	4	67
Ho 14-864	6	1	17
Ho 14-864	6	0	0

HoCP 14-801	6	1	17
HoCP 14-801	3	0	0
HoCP 14-802	4	0	0
HoCP 14-802	4	0	0
HoCP 14-826	6	0	0
HoCP 14-826	6	0	0
HoCP 14-867	6	0	0
HoCP 14-867	5	0	0
HoCP 14-885	6	0	0
HoCP 14-885	5	0	0
HoCP 15-915	2	2	100
HoCP 15-915	5	3	60
Ho 15-921	5	5	100
Ho 15-921	6	6	100
HoL 15-508	4	1	25
HoL 15-508	4	1	25
HoCP 15-510	5	0	0
HoCP 15-510	6	3	50
HoCP 17-709	5	5	100
Ho 17-725	1	1	100
Ho 17-734	1	1	100
Ho 17-756	3	3	100
Ho 17-775	5	5	100
Ho 17-717	5	4	80
Ho 17-764	5	4	80
Ho 17-9122	5	4	80
Ho 17-9143	4	3	75
Ho 17-768	2	1	50
Ho 17-9150	2	1	50
Ho 17-9155	4	2	50
Ho 17-9161	6	3	50
Ho 17-9160	5	2	40
Ho 17-726	3	1	33
Ho 17-727	3	1	33
HoCP 17-767	3	1	33
Ho 17-9135	3	1	33
HoCP 17-702	4	1	25
Ho 17-723	4	1	25
Ho 17-748	4	1	25
Ho 17-9157	4	1	25
HoCP 17-715	5	1	20

Ho 17-743	5	1	20
Ho 17-9114	5	1	20
Ho 17-9149	5	1	20
Ho 17-731	6	1	17
Ho 17-732	6	1	17
Ho 17-9146	6	1	17
Ho 17-9159	6	1	17
HoCP 17-700	5	0	0
HoCP 17-701	2	0	0
HoCP 17-703	5	0	0
HoCP 17-704	6	0	0
HoCP 17-705	6	0	0
HoCP 17-706	2	0	0
HoCP 17-707	3	0	0
HoCP 17-710	4	0	0
HoCP 17-711	6	0	0
HoCP 17-712	6	0	0
HoCP 17-713	5	0	0
HoCP 17-714	4	0	0
HoCP 17-716	4	0	0
Ho 17-718	2	0	0
Ho 17-720	6	0	0
Ho 17-722	3	0	0
Ho 17-724	4	0	0
HoCP 17-728	4	0	0
HoCP 17-730	4	0	0
Ho 17-733	6	0	0
Ho 17-737	2	0	0
Ho 17-738	3	0	0
Ho 17-741	2	0	0
Ho 17-742	4	0	0
Ho 17-744	6	0	0
Ho 17-745	2	0	0
Ho 17-746	3	0	0
Ho 17-747	4	0	0
Ho 17-749	6	0	0
HoCP 17-750	2	0	0
Ho 17-752	4	0	0
Ho 17-753	3	0	0
Ho 17-754	4	0	0
Ho 17-755	6	0	0

Ho 17-757	6	0	0
Ho 17-759	3	0	0
Ho 17-760	5	0	0
HoCP 17-761	6	0	0
Ho 17-762	6	0	0
Ho 17-763	4	0	0
HoCP 17-765	3	0	0
Ho 17-774	6	0	0
Ho 17-776	4	0	0
Ho 17-777	2	0	0
Ho 17-9101	6	0	0
Ho 17-9102	5	0	0
Ho 17-9103	1	0	0
Ho 17-9104	5	0	0
Ho 17-9105	6	0	0
Ho 17-9106	6	0	0
Ho 17-9107	4	0	0
Ho 17-9108	2	0	0
Ho 17-9109	4	0	0
Ho 17-9110	6	0	0
Ho 17-9111	4	0	0
Ho 17-9112	6	0	0
Ho 17-9113	3	0	0
Ho 17-9115	5	0	0
Ho 17-9116	4	0	0
Ho 17-9117	5	0	0
Ho 17-9118	6	0	0
Ho 17-9119	5	0	0
Ho 17-9120	5	0	0
Ho 17-9121	5	0	0
Ho 17-9123	6	0	0
Ho 17-9124	6	0	0
Ho 17-9125	5	0	0
Ho 17-9126	6	0	0
Ho 17-9127	5	0	0
Ho 17-9128	6	0	0
Ho 17-9129	6	0	0
Ho 17-9130	6	0	0
Ho 17-9131	6	0	0
Ho 17-9132	3	0	0
Ho 17-9133	6	0	0

Ho 17-9134	6	0	0
Ho 17-9136	6	0	0
Ho 17-9137	6	0	0
Ho 17-9138	6	0	0
Ho 17-9139	6	0	0
Ho 17-9140	5	0	0
Ho 17-9141	6	0	0
Ho 17-9142	4	0	0
Ho 17-9144	5	0	0
Ho 17-9145	5	0	0
Ho 17-9147	5	0	0
Ho 17-9148	6	0	0
Ho 17-9151	6	0	0
Ho 17-9152	5	0	0
Ho 17-9153	4	0	0
Ho 17-9154	5	0	0
Ho 17-9156	4	0	0
Ho 17-9158	3	0	0
Ho 17-9162	1	0	0
Ho 17-9163	5	0	0
Ho 17-9164	6	0	0
Ho 17-9165	6	0	0
Ho 17-9166	1	0	0
Ho 17-719	0	0	-
Ho 17-721	0	0	-
Ho 17-735	0	0	-
Ho 17-736	0	0	-
HoCP 17-751	0	0	-
LSU experiment one			
N 27	1	1	100
LCP 81-030	6	0	0
LCP 81-010	0	0	-
CP 83-644	4	2	50
HoCP 85-845	4	0	0
LCP 85-384	1	0	0
LCP 86-454	5	1	20
HoCP 91-552	2	0	0
HoCP 92-624	6	0	0
HoCP 92-618	4	0	0
L 94-428	3	0	0

L 94-433	6	0	0
L 94-426	0	0	-
Ho 95-988	6	0	0
HoCP 95-951	6	0	0
HoCP 96-561	6	0	0
HoCP 97-609	6	0	0
L 97-128	5	0	0
L 98-209	5	4	80
L 98-207	6	0	0
L 99-226	6	1	17
L 99-233	6	0	0
HoCP 00-950	2	0	0
US 01-040	5	1	20
L 01-283	5	0	0
L 01-299	3	0	0
L 01-315	5	0	0
HoCP 01-517	1	0	0
HoCP 01-523	5	0	0
HoCP 02-618	6	0	0
L 03-371	0	0	-
HoCP 04-838	3	0	0
HoCP 04-847	6	0	0
L 05-306	4	0	0
L 05-448	6	0	0
L 05-457	1	0	0
HoCP 05-902	5	0	0
Ho 06-563	5	2	40
L 06-001	5	1	20
L 06-038	6	0	0
L 06-040	4	0	0
Ho 06-530	4	0	0
Ho 06-537	6	0	0
Ho 07-613	1	1	100
Ho 07-617	2	1	50
L 07-057	1	0	0
Ho 08-717	3	3	100
Ho 08-730	4	4	100
L 08-090	4	0	0
Ho 08-711	6	0	0
Ho 09-832	6	2	33
L 09-099	6	0	0

L 09-112	4	0	0
L 09-123	1	0	0
L 09-131	6	0	0
HoCP 09-814	5	0	0
Ho 09-827	4	0	0
Ho 09-840	5	0	0
HoCP 09-846	5	0	0
Ho 09-9401	6	0	0
Ho 09-9402	4	0	0
L 10-146	1	1	100
L 11-183	1	1	100
L 11-147	5	0	0
L 11-187	6	0	0
Ho 11-532	6	0	0
Ho 11-573	6	0	0
Ho 11-9406	6	0	0
Ho 11-9405	0	0	-
L 12-218	1	1	100
L 12-201	6	0	0
L 12-202	4	0	0
L 12-227	2	0	0
Ho 12-615	6	0	0
Ho 12-9410	6	0	0
L 13-234	2	1	50
L 13-242	2	1	50
HoCP 13-726	6	3	50
HoCP 13-723	5	2	40
L 13-243	6	0	0
L 13-251	6	0	0
L 13-253	2	0	0
Ho 13-720	4	0	0
HoCP 13-738	6	0	0
Ho 13-705	1	0	0
Ho 13-755	6	0	0
L 14-275	4	3	75
L 14-266	3	1	33
L 14-265	5	1	20
L 14-264	5	0	0
L 14-267	5	0	0
L 14-269	2	0	0
L 14-270	5	0	0

L 14-273	6	0	0
L 14-276	6	0	0
L 14-282	6	0	0
L 15-337	1	1	100
L 15-311	5	2	40
L 15-305	6	2	33
L 15-298	6	0	0
L 15-300	6	0	0
L 15-301	4	0	0
L 15-304	1	0	0
L 15-317	2	0	0
L 15-319	2	0	0
L 15-303	0	0	-
L 15-312	0	0	-
L 15-320	0	0	-
LSU experiment two			
L 08-88	3	4	75
HoCP 96-540	0	5	0
Ho 08-730	0	5	0
Ho 09-832	1	5	20
L 11-183	0	5	0
L 12-201	0	6	0
L 13-251	0	6	0
HoCP 13-723	1	3	33
L 14-267	0	6	0
L 14-282	0	6	0
L 15-305	0	4	0
Check Clones			
USDA experiment one			
HoCP 96-540 #1	0	6	0
HoCP 96-540 #2	0	5	0
HoCP 96-540 #3	0	6	0
L 08-88 #1	6	6	100
L 08-88 #2	5	5	100
L 08-88 #3	6	6	100
HoCP 09-804 #1	2	5	40
HoCP 09-804 #2	2	6	33
HoCP 09-804 #3	0	6	0
Sorghum 'Rio' #1	6	6	100

Sorghum 'Rio' #2	6	6	100
Sorghum 'Rio' #3	5	5	100
HoCP96-540 UI	0	6	0
L08-88 UI	0	6	0
HoCP09-804 UI	0	6	0
Sorghum 'Rio' UI	0	6	0
USDA experiment two			
HoCP 96-540 #1	0	5	0
HoCP 96-540 #2	0	5	0
HoCP 96-540 #3	0	5	0
L 08-88 #1	4	6	67
L 08-88 #2	5	5	100
L 08-88 #3	5	5	100
HoCP 09-804 #1	1	4	25
HoCP 09-804 #2	0	3	0
HoCP 09-804 #3	1	6	17
Sorghum 'Rio' #1	6	6	100
Sorghum 'Rio' #2	6	6	100
Sorghum 'Rio' #3	6	6	100
HoCP 96-540 UI	0	3	0
L 08-88 UI	0	5	0
HoCP 09-804 UI	0	5	0
Sorghum 'Rio' UI	0	6	0
LSU experiment one			
HoCP 96-540 #1	6	0	0
HoCP 96-540 #2	5	0	0
HoCP 96-540 #3	5	0	0
HoCP 96-540 #4	4	0	0
HoCP 96-540 #5	4	0	0
L 08-88 #1	3	3	100
L 08-88 #2	2	2	100
L 08-88 #3	5	5	100
L 08-88 #4	6	6	100
L 08-88 #5	5	4	80
HoCP 09-804 #1	-	-	-
HoCP 09-804 #2	6	0	0
HoCP 09-804 #3	6	0	0
HoCP 09-804 #4	5	0	0
HoCP 09-804 #5	4	0	0

Sorghum 'Rio' #1	0	0	-
Sorghum 'Rio' #2	0	0	-
Sorghum 'Rio' #3	0	0	-
Sorghum 'Rio' #4	-	-	-
Sorghum 'Rio' #5	-	-	-
HoCP 96-540 UI	6	0	0
L 08-88 UI	1	0	0
HoCP 09-804 UI	5	0	0
Sorghum 'Rio' UI	0	0	-
LSU experiment two			
HoCP 96-540 #1	4	0	0
HoCP 96-540 #2	4	0	0
HoCP 96-540 #3	5	0	0
L 08-88 #1	5	2	40
L 08-88 #2	3	0	0
L 08-88 #3	4	3	75
HoCP 09-804 #1	5	0	0
HoCP 09-804 #2	5	0	0
HoCP 09-804 #3	5	0	0
Sorghum ‘Rio’ #1	6	6	100
Sorghum ‘Rio’ #2	6	6	100
Sorghum ‘Rio’ #3	6	6	100

[a] UI = uninoculated
[b] - = unable to evaluate

VITA

Jancee Rice is from the town of Arnaudville, Louisiana. She attended Beau Chêne High School in Arnaudville and graduated in 2012. Afterwards, she began her undergraduate studies at the University of Louisiana at Lafayette. While there, she participated in undergraduate research under the supervision of Dr. Caryl Chlan investigating the antimicrobial activity of chitinases using *Agrobacterium*-mediated plant transformations. She graduated with a B.S. in Biology in the fall of 2015. The following spring, she began her graduate studies at Louisiana State University under the supervision of Dr. Jeff Hoy. She anticipates graduating with her master's degree in Plant Pathology in December 2018.