

## ABSTRACT

**BRYAN, ADRIENNE DAWN.** Impact of Sweet Potato Feathery Mottle Virus and Micropropagation on Yield, Root Quality and Virus Incidence in Commercial Sweetpotato Production Systems. (Under the direction of Dr. J.R. Schultheis and Dr. G.C. Yencho)

Reduction in yield and root quality (decline) in sweetpotato has been attributed to the accumulation of viruses and mutations. Three studies were initiated to assess the effects of mutations and viruses in sweetpotato. In the first study, to document the effects of decline over time, two micropropagated, virus-indexed, greenhouse produced “G1” ‘Beauregard’ mericlones (clones obtained from meristem-tip culture) B94-14 and B94-34, were compared with B94-14 and B94-34 clones propagated over five years via adventitious field propagation (G2, G3, G4, G5) and non-micropropagated NCSU ‘Beauregard’ seed in field trials during 1997-2001. The trials were located in primary sweetpotato producing regions in NC each year. Yield and root quality measurements were recorded at harvest. G1 plants consistently produced higher total yield, total marketable yield (TMY), No.1 root yield (the most valued grade), and percent No.1 yield (relative to total yield) than G2-G5 plants. G1 plants produced roots with higher shape uniformity and better overall appearance than G2-G5 plants. G2-G5 roots tended to be longer than G1 roots. Linear regression analysis used to model G1-G5 yield and root quality measurements over time indicated that total yield, TMY, No.1 yield, percent No.1 yield, shape uniformity, and overall appearance decreased gradually and length/diameter (L/D) ratios increased gradually with increased field generations of adventitious propagation.

The second study was conducted to determine the effects of *Sweet potato feathery mottle virus* (SPFMV) on yield and root quality of sweetpotato. To do this, virus-indexed

mericlones (VI-), which tested free of known viruses, were compared with virus-infected clones (VI+), in two separate tests with three mericlones each of 'Beauregard' and 'Hernandez' in a two-year study. Tests were arranged in a split plot design with the initial presence or absence (+/-) of SPFMV as the whole plot factor and mericlone as the subplot factor. Yield and root quality measurements indicated that the presence of SPFMV prior to planting (VI+) reduced TMY, yield of No.1s, and percent No.1s and decreased overall appearance for 'Beauregard' mericlones. SPFMV-infected plants also produced roots with higher L/D ratios than VI- plants for mericlones of both cultivars.

The third study was initiated to assess the reinfection rate of SPFMV in the previously described experiments. Weekly observations of the number of SPFMV symptomatic plants were recorded for each plant per plot beginning 4 weeks after planting (WAP) in 2000 and 1 WAP in 2001. In addition, aphid traps were placed in the trials 1 WAP and weekly aphid counts per trap were recorded. The monitoring indicated that 100 % of the virus-indexed, micropropagated mericlones became infected by the end of the growing season as early as 5 WAP or as late as 10 WAP. In both years, the percentage of plants displaying symptoms the first week of monitoring was higher for VI+, G2-G5, and NCSU 'Beauregard' plants than VI- and G1 plants. The reinfection rate of micropropagated, virus-indexed mericlones (VI- and G1) in all trials was higher in 2000 than 2001, which may correlate with the higher number of aphids recorded per trap in 2000 than 2001.

In order to verify the presence of SPFMV in the field trials, one symptomatic vine was collected per plot at three sampling dates in 2000 and 2001, and grafted onto the indicator

plant *Ipomoea setosa* Ker and tested for SPFMV and other viruses using an enzyme linked immunosorbant assay (NCM-ELISA). All field samples induced virus symptoms on *I. setosa* after grafting. Samples tested using NCM-ELISA confirmed only the presence of SPFMV. The results from these studies indicate that adventitious propagation methods used in commercial sweetpotato production allows SPFMV to accumulate and that this contributes to cultivar decline.

**IMPACT OF SWEET POTATO FEATHERY MOTTLE VIRUS  
AND MICROPROPAGATION ON  
YIELD, ROOT QUALITY, AND VIRUS INCIDENCE  
IN COMMERCIAL SWEETPOTATO PRODUCTION SYSTEMS**

by

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This thesis is dedicated to my parents,  
and to the memory of my grandmother

## **BIOGRAPHY**

Adrienne Dawn Bryan was born on February 15, 1977 and raised in Sampson County, North Carolina outside the small town of Newton Grove. She is the oldest of three children with a younger brother and sister, 6 and 16 years her junior, respectively. Her family operated a corn mill beside the Coharie Branch of the Cape Fear River and she spent many summers there working or fishing in the pond near the mill. Adrienne began playing the piano at age 9 and occasionally plays for her church in Maple Grove. She graduated from Hobbton High School in 1995 and received her B.A. in Biology from Mount Olive College, NC in 1999.

After her junior year in college, Adrienne took a summer job with the Sweetpotato Breeding and Genetics Program. In the winter of 2000, she began a Masters degree with the program working with Dr. G. Craig Yencho, Dr. Jonathan R. Schultheis, and Dr. Zvezdana Pesic-VanEsbroeck on a sweetpotato micropropagation project.

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## CHAPTER 1

### Literature Review

#### Sweetpotato

Sweetpotato [*Ipomoea batatas* (L.) Lam.] ranks ninth amongst the most important food crops in the world (FAO 2001). The sweetpotato is a member of the family Convolvulaceae. It is a perennial dicot with its origins thought to be in northwestern South America. Sweetpotato is a hexaploid with 90 somatic chromosomes ( $2n=6x=90$ ). Most sweetpotato varieties are self-incompatible and have high levels of heterozygosity. Morphological and horticultural characteristics in sweetpotato vary considerably with a wide range of root yielding abilities, size and shapes of roots, flesh and skin color of roots, and size and shape of leaves and branches among sweetpotato germplasm. Sweetpotato is propagated both sexually and asexually. In all parts of the world, asexual methods are preferred by growers for root production while true seed is used by breeders to develop new varieties.

Because sweetpotato can be vegetatively propagated, numerous plants, theoretically of the same genotype, can be produced. In tropical climates, sweetpotato can be grown year round, thus, stems of vines are cut from a current crop and planted to produce the next. In the United States and other temperate climates in the northern hemisphere, the growing season of sweetpotato is restricted to summer and early fall, and the next season's crop is produced by using adventitious sprouts of "seed" roots saved from the previous crop. The "seed" roots are usually stored after harvest in commercial or farm warehouses first by curing the roots at 85 F and 90 to 95% relative humidity for 4 to 7 days, and then storing them for the remainder of time at 55 to 60 F and 85 to 90% relative humidity. In early spring, "seed" roots are

planted in beds covered with 5-10 cm of soil to produce adventitious sprouts which are cut and transplanted to the field. Typically a 3 to 4 month growing season is required for root development (Schultheis, 1990). Since roots harvested for sale are cured and stored in the same manner as “seed” roots and because greater than 500 adventitious sprouts can be produced from 1 bushel of “seed” roots, many growers prefer this method of plant production.

### **Mutations and Decline**

In the United States, early breeding methods involved selection of sweetpotatoes introduced from other countries in which somatic mutations had occurred because it was difficult to induce flowering in sweetpotato. Mutation induced color changes in the root epidermis and flesh (Groth, 1911; Rosa, 1926; Harter, 1926; Thompson, 1929; Miller, 1930; Miller, 1935). Selection of mutant sweetpotatoes with desirable characteristics resulted in new varieties of sweetpotato with different shape, color, and yielding ability (Edmond and Ammerman, 1971). For instance, four new cultivars were derived from adventitious propagules with mutations for color and shape from the original ‘Porto Rico’ sweetpotato (Miller, 1935). Most of the mutations were color changes, but there were also changes in shape and yield. Miller proposed that quantitative mutations for shape and yield were also occurring and produced the mutant Unit I Porto Rico which had higher yields and quality than the original ‘Porto Rico’ (Edmond and Ammerman, 1971).

The high incidence of mutations in sweetpotato, particularly off-types with less desirable characteristics, led to the development of programs to maintain the characteristics of the

variety developed by breeders. By elimination of off-types and increase of high quality seed, improved seedstock was made available to growers beginning in 1929 in North Carolina (Middleton and McLaughlin, 1978). By 1945, a foundation and certification program for sweetpotato in North Carolina was developed. Superior hill selections (roots of single plant) were made every two to three years to maintain high yield and root quality attributes. The selected roots of hills were increased through adventitious propagation methods for production of planting material or “seed” roots for use by growers. Visual selection of roots or hills most characteristic of a cultivar is still the most widely used method of preserving the genetic integrity of cultivars in US sweetpotato foundation seed programs (Dangler, 1994).

Despite efforts to maintain sweetpotato cultivars in the United States, a decrease in yield and root quality has been observed over time. This decline in yield and root quality in sweetpotato has been referred to as cultivar running-out (Miller et al., 1959). The sweetpotato cultivar ‘Centennial’ decreased in yield by 45% over a 35 year period despite vigorous selection for true-to-type roots (Villordon and La Bonte, 1995). Selection of true-to-type roots, used in foundation programs, for “seed” increase eliminates roots with mutated color, shape, and yield within the initial population used for propagation, however, mutants are still observed in roots of subsequent adventitious propagules (Dangler, 1994). Mutations are also responsible for genetic variability resulting in a range of shapes and yields among clones of a given cultivar (Edmond and Ammerman, 1971; Villordon and La Bonte, 1995). An analysis of “seed” roots used in seed foundation programs in the United States indicated that yield variability among clonal sources of ‘Jewel’ ranged from 27-46% (Villordon and La Bonte, 1995). Further studies with ‘Jewel’ indicated that adventitious propagules were

genetically more variable than nodal (stem) cuttings (Villordon and La Bonte, 1996).

Adventitious propagules of ‘Jewel’ were also found to produce roots with more chimeras and more variable yields than nodal cuttings from plants produced in tissue culture (Templeton-Summers and Collins, 1986). Nodal cuttings of sweetpotato thus have lower mutation rates than adventitious sprouts of saved roots (Templeton-Somers and Collins, 1986; Villordon and La Bonte, 1995; Villordon and La Bonte, 1996).

If deleterious mutations are not detected by visual selection, they may accumulate altering favorable genetic components for yield and root quality. Continuous adventitious propagation, which enables mutations to accumulate, leads to sweetpotato cultivar decline over time (Villordon and La Bonte, 1995). The use of planting material derived from preexisting meristematic tissue, specifically nodal cuttings, has been proposed to reduce clonal variability in sweetpotato foundation seed programs (Villordon and La Bonte, 1995; Villordon and La Bonte, 1996).

### **Virus and Decline**

In addition to mutations, viral infections also contribute to cultivar decline in sweetpotato (Gooding, 1964; Huett, 1982). Accumulation of viruses and diseases occur through the adventitious, root-to-sprout propagation method used in commercial production. Saved “seed” roots of sweetpotato plants that become infected with virus during the growing season will produce virus-infected adventitious sprouts (Clark and Moyer, 1988). If virus infection decreases yield and root quality in sweetpotato, then the continuous use of virus-infected adventitious sprouts may lead to cultivar decline. Consequently, the use of sweetpotato

planting material that is free of known viruses is recommended as a method of controlling, preventing, or slowing virus accumulation in subsequent plantings (Clark and Moyer, 1988). Healthy plants can be obtained through meristem-tip culture and these plants are tested for freedom of known viruses by grafting onto *Ipomoea setosa* Ker (virus-indexing) (Clark and Moyer, 1988). However, most viruses that infect sweetpotato, however, have insect vectors, and virus reinfection occurs rapidly during the growing season if sources of local inoculum, including virus-infected sweetpotato plants and *Ipomoea spp.*, are present (Clark and Moyer, 1988).

### **Important Viruses of Sweetpotato**

#### ***Sweet potato feathery mottle virus***

*Sweet potato feathery mottle virus* (SPFMV) infects sweetpotatoes worldwide and is the most common virus found in the United States. Because many different strains exist and symptoms induced by these strains differ, many names have been used to describe the virus. These names include internal cork virus, sweetpotato leafspot virus, sweetpotato ringspot virus, sweetpotato virus A, and russet crack virus (Moyer and Salazar, 1989). The most distinctive symptom of the virus, irrespective of strain, present is the chlorotic feathering of the leaf midrib and, in some genotypes, the expression of chlorotic spots with purple rings (Alconero, 1972; Moyer and Salazar, 1989).

SPFMV, which is aphid and graft-transmissible in sweetpotatoes, is often found in mixed-infection with other potyviruses. This characteristic caused much confusion in most of the

earlier work, since much of what was found relied heavily on symptoms, host range, and transmission (Moyer and Salazar, 1989). The disease now known to be caused by SPFMV was first described by Doolittle and Harter in 1945. Their work reported a sweetpotato virus that was different in symptoms and methods of transmission from a previously investigated virus known as mosaic. They found the virus to be only graft-transmissible as attempts of aphid and mechanical inoculations of the virus were unsuccessful. Symptoms of the virus included a feathery yellowing along the veins of leaves, which was distinctly different from symptoms of the mosaic virus. Doolittle and Harter (1945) proposed that the virus with such described symptoms and methods of transmission be known as the “feathery mottle of the sweet potato.” In 1954, Webb and Larson working with SPFMV using the same source of sweetpotato germplasm as Doolittle and Harter (1945) found the virus to be transmitted mechanically and through aphids. Webb (1954a) also described hosts of the feathery mottle virus of the sweetpotato with mechanical inoculations limited to the four genera of the family Convolvulaceae, and found the morning-glory to be a good indicator of the virus (Webb, 1954b).

Stubbs and McLean described two isolates of SPFMV, one mild strain and one severe strain in 1958. Their work found both to be non-persistently transmitted by aphids *Myzus persicae*, *Aphis apii*, and *Aphis gossypii*. However, Hildebrand found SPFMV not to be transmitted by aphids but by the whifefly *Traileurodes abutilonea*, adding uncertainty about the specific vector of SPFMV (Hildebrand, 1959a, 1959b). In 1960, Hildebrand attempted to clarify this confusion by suggesting that SPFMV is a virus complex of three distinct viruses: yellow dwarf (transmitted by whitefly) and internal cork and leafspot (both transmitted by aphids).

Hildebrand suggested that previous work describing SPFMV could have been a mixture of more than one of these viruses.

### **Internal cork virus and ringspot virus**

Internal cork was first described in 1946 by Nusbaum in which internal necrotic spots on the roots of the cultivar 'Porto Rico' were found in conjunction with foliar symptoms of SPFMV (vein-clearing, mottling, ringspotting, and bronzing). Martins' work in 1950 suggested that there may be more than one virus involved with internal cork since some 'Porto Rico' plants with foliar symptoms did not show root symptoms. However, Aycock and Hughes (1952), showed all 'Porto Rico' cultivars with foliar symptoms also developed necrotic root symptoms after inoculation, while two varieties did not. Hildebrand, Anderson, and Ball's (1956) survey of internal cork clarified misconceptions about the presence of foliar symptoms and lack of root symptoms as the result of storage periods of root samples. They emphasized that symptoms increase the longer the roots are in storage. Hildebrand and Smith (1958) found that the aphids *Myzus persicae* and *Macrosiphum solanifolii* were successful in transmitting the internal cork virus and associated the internal cork virus with chlorotic foliar symptoms. Hildebrand (1958) also found that the original source of sweetpotato that showed symptoms of SPFMV was also infected with internal cork. In 1959, Hildebrand concluded that internal cork disease of the sweetpotato was a complex of two separate viruses: ringspot virus and internal cork virus (Hildebrand, 1959c, 1961).

## **Russet crack**

The russet crack disease of sweetpotato was described by Daines and Martin in 1964 as causing leaf symptoms of dark gray to yellowish spots and distinctive darkened lesions consisting of fine cracks in the epidermis and cortex of the root. They hypothesized the disease to be caused by a virus. Hildebrand (1968) found russet crack to be naturally transmitted by aphids in field conditions and demonstrated that the whitefly was not an insect vector of the virus that caused russet crack.

The relationship between the previously described viruses of sweetpotato did not become clear until after methods of detection, purification, and characterization were refined. In North Carolina the strains of SPFMV that occur include the common strain (SPFMV-C) and the mild and severe russet crack strains (SPFMV-MRC and SPFMV-SRC, respectively) (Moyer et al., 1980; Cali and Moyer, 1981). The C strain and the RC strain are serologically distinct and are genetically different based on their coat protein sequences (Abad and Moyer, 1990; Abad et al., 1992). The RC strain causes cracking symptoms in sweetpotato roots and root necrosis in the 'Jersey' cultivar (Clark and Moyer, 1988). Russet crack has also been noted on roots of some Japanese cultivars (Usugi et al., 1994).

SPFMV has been detected nearly everywhere sweetpotatoes are grown and is the most thoroughly characterized sweetpotato virus (Moyer and Salazar, 1989). SPFMV is a member of the genus *Potyvirus* (Family *Potyviridae*) (Pringle, 1999) and is characterized by 850 nm virions containing a single, plus-sense strand of RNA with a molecular weight of approximately  $3.7 \times 10^6$ . SPFMV forms pinwheel inclusion bodies in infected cells (Clark

and Moyer, 1988). The host range of SPFMV is narrow being confined to members of the Convolvulaceae including a wide range of *Ipomoea* spp. Most strains of SPFMV do not infect *Ipomoea aquatica*. Many strains cause local lesions on *Chenopodium amaranticolor* and *C. quinoa* and are able to infect *Nicotiana benthamiana* (Clark and Moyer, 1988). A study in Louisiana found the wild *Ipomoea* spp. *I. trichocarpa*, *I. wrightii*, and *I. hederacea* to be hosts of SPFMV, with *I. trichocarpa* being a potential reservoir for SPFMV given its perennial growth (Clark et al., 1986).

### **Detection of SPFMV**

Symptoms of SPFMV on many cultivars can be mild, transient, or may not appear at all on sweetpotato foliage (Clark and Moyer, 1988; Brunt et al., 1996). Many East African cultivars do not exhibit foliar symptoms of SPFMV, but do induce SPFMV symptoms on *I. setosa* after grafting (Gibson et al., 1998). Plant age, light intensity, temperature, and leaf pigment have been shown to have an effect on SPFMV symptom expression in sweetpotato (Stubbs and McLean, 1958; Alcerono, 1972; Mukiibi, 1977; Arrendell and Collins, 1986). In addition, older, symptomatic leaves of sweetpotato may drop and new growth may be symptomless (Alcerono, 1972). Because of the variability in SPFMV expression in sweetpotato, other more reliable, consistent methods are needed to detect SPFMV.

Indicator plants reliably detect viruses infecting sweetpotato. Stubbs and McLean first introduced *Ipomoea setosa* Ker as a valuable indexing host in 1958 in aphid transmission studies with SPFMV. Since then, *I. setosa* has been used for verifying the russet crack disease as caused by a strain of SPFMV (Daines and Martin, 1964; Campbell et al, 1974).

Because *I. setosa* is susceptible to most known viruses of sweetpotato (Brunt et al, 1996), it has been used in virus-indexing systems following meristem-tip culture to verify that known viruses were eliminated (Alconero et al., 1975).

In addition to grafting onto indicator plants, serology, electron microscopy, probe hybridization, and a reverse-transcription polymerase chain reaction have been used to detect SPFMV. An enzyme-linked immunosorbant assay (ELISA) was developed by Cadena-Hinojosa and Campbell (1981) that detected four strains of SPFMV. Esbenshade and Moyer (1982) also developed an ELISA assay and determined that 5-8 wk following sprouting from roots was a sufficient time in which to detect SPFMV in sweetpotato leaves. Other methods for SPFMV detection include an in-vitro transcribed RNA probe (Abad and Moyer, 1992) and several methods including reverse transcription-polymerase chain reaction (RT-PCR) (Colinet and Kummert, 1993; Colinet et al., 1993; Nishiguchi et al, 1995; Colinet et al, 1998).

The advantage of serological assays such as ELISA for virus detection includes specificity, rapid detection, and sensitivity to small amounts of viral antigen in the plant material. To date, twenty viruses are known to infect sweetpotato (Mwanga, 2001). The majority of these have not been sequenced and have not yet been completely characterized (NCBI, 2001). The antisera for SPFMV, *Sweet potato mild mottle virus* (SPMMV), *Sweet potato latent virus* (SPLV), *Sweet potato chlorotic fleck virus* (SPCFV), *Sweet potato mild speckling virus* (SPMSV), C-6 virus, *Sweet potato chlorotic stunt virus* (SPCSV), and *Sweet potato caulimovirus* (SPCaLV) have been produced and are available in diagnostic kits with

necessary reagents for nitrocellulose membrane enzyme-linked immunosorbant assays (NCM-ELISA) through the International Potato Center (CIP), Lima, Peru (Carey et al., 1999). These kits can be used to detect the eight viruses in the same extracted sample allowing a large number of samples to be screened.

### **Reinfection rate of SPFMV in sweetpotato**

Pozzer et al. (1994) examined reinfection rates of SPFMV in healthy sweetpotatoes planted either in a plot where sweetpotato had previously been planted, or in a plot that had never been planted with sweetpotato and isolated by sugarcane. SPFMV was found to reinfect 80% of sweetpotatoes in the first plot and only 20% in the isolated field. Milgram et al., (1996) found reinfection rates of SPFMV in healthy plants was greater in fields located near virus-infected fields. In the same study, no yield differences were detected in comparing SPFMV-infected plants to healthy controls in Israel. However, the study attributed the insignificance in yield differences to low aphid populations in the area.

Since the studies by Stubbs and McLean (1958), where the aphid vectors were reported as the vector, others have found that SPFMV is experimentally transmitted by *Myzus persicae* (Sulzer) and *Aphis gossypii* (Glover) (Kennedy and Moyer, 1982; Schaefer and Terry, 1976; Ususgi et al., 1994). Most aphids are considered to be vectors and to transmit SPFMV in a non-persistent manner. Studies correlating aphid activity with the spread and re-infection of SPFMV are important to determine effects of SPFMV on sweetpotato production. In parts of Africa, the incidence and severity of *Sweet potato virus disease* (SPVD), a disease induced by the presence of SPFMV and SPCSV in the same plant, has

been found to correlate with the prevalence of whiteflies (Artua et al., 1998). To date, no studies have been conducted to examine the incidence of SPFMV with aphid populations in commercial fields in the United States.

### **Effects of SPFMV on yield of sweetpotato**

Studies of the effects of SPFMV on sweetpotato production are also scarce in the United States. Elsewhere, SPFMV has been found to reduce yield in sweetpotato including Venezuela (Olivero and Oropeza, 1985) and Brazil (Pozzer et al., 1995). The presence of SPFMV is a great concern in parts of Africa because of its role in SPVD, the main contributor to economic loss in sweetpotato in Africa (Karyeija et al., 1998). Tests analyzing yields in SPVD-infected sweetpotatoes against apparently healthy or supposedly “virus-free” sweetpotatoes have all concluded that virus-infected plants have significantly lower yields compared to healthy plants (Mukiibi, 1977; Hahn, 1979; Ngeve, 1990; Ngeve and Bouwkamp, 1991). Other viruses that occur in combination with SPFMV and reduce yield include the *Sweet potato sunken vein virus* in Israel (Milgram et al., 1996), and the *Sweet potato latent virus* in Taiwan (Liao et al., 1983).

### **Micropropagation**

Elimination of viruses in sweetpotato using meristem-tip culture either with or without heat therapy has been conducted since 1957 (Alconero, 1971; Alconero et al., 1975; Elliott, 1969; Hildebrand, 1957; Hildebrand, 1964; Hildebrande and Brierly, 1960; Holmes, 1956; Frison and NG, 1981; Liao and Chung, 1979; Liao et al., 1982; Mori, 1971; Nielson, 1960). In the 1960s, California began a meristem-tip culture program to produce clean seed to eliminate

russet crack in foundation seed (Dangler, 1994). Other countries have also implemented programs using this procedure to improve sweetpotato crop production. China, the world's largest producer of sweetpotato, produced 466,000 ha of "virus-free" sweetpotato in 1998. The program in China has helped restore sweetpotato yield and has helped protect plants from other pathogens (Feng, 2000).

The process of micropropagation, along with virus-indexing of meristemed clones (mericlones) provides material free of known viruses that can be rapidly multiplied for planting stock via greenhouse increase of nodal cuttings. In addition, the use of nodal cuttings from micropropagated plants reduces the amount of variation between propagules and retains genetic integrity (Villordon and La Bonte, 1996). Nodal cuttings of micropropagated planting stock can be used as an alternative to adventitious propagules; therefore, its use may help slow the effects of cultivar decline.

North Carolina ranks first in the nation in sweetpotato production accounting for approximately 40% of the crop sold in the United States (USDA NASS 2001). The success of production of sweetpotato in NC relies heavily on the implementation of the NC Crop Improvement Association's (NCCIA) Sweetpotato Certified Seed Program. The Micropropagation Unit at North Carolina State University was established to improve certified seed through the production and distribution of micropropagated plants to produce Foundation Seed for use by North Carolina growers. The benefits of micropropagated plants include variety availability and stability, true-to-type planting material, virus-indexed planting material, and high quality plants in size and that produced roots of characteristic,

vigor, color, and high yield. Because micropropagated plants are high quality and virus-indexed, it is thought that their use in commercial production systems would slow the accumulation of mutations and viruses. In US commercial sweetpotato production, one cultivar typically dominates the industry. The current predominant cultivar in commercial sweetpotato production in the US is 'Beauregard' (Schultheis et al., 1999) which is susceptible to SPFMV (Schultheis, 1994). Russet crack root symptoms have been reported on as much as 23% of a grower's sweetpotato crop in NC (Schultheis et al., 1994).

Several studies have been conducted to compare the effects of yield and root quality of nodal cuttings from micropropagated plants with non-micropropagated, SPFMV-infected plants from various sources. These studies indicated that the use of micropropagated plants, in addition to reducing russet crack symptoms, provided planting material that yielded as good, or better than non-micropropagated, SPFMV-infected plants that had been in field production for several years (Averre et al., 1993; Jester et al., 1994; Schultheis, 1994; Schultheis et al., 1994). However, the effects of virus and mutation accumulation, and their corresponding effect on yield and quality over several generations of propagation have not been studied.

Micropropagated sweetpotato mericlones of cultivars serve as the initial source of planting material in several state foundation programs (Dangler, 1994); however, the use of micropropagated material on a yearly basis may not be economically feasible for growers compared to adventitious propagation methods. If adventitious propagules derived from "seed" roots of micropropagated plants also retained high yields and reduced russet crack symptoms, then growers could benefit from micropropagation without drastic change in

production of planting stock. In addition, growers would have the option to renew their planting stock with nodal cuttings of micropropagated plants kept by state foundation programs. Therefore, it would benefit growers to know how quickly decline occurs and its effect on root quality and yield. Determining the reinfection rate of SPFMV in sweetpotato and its effects on yield and root quality would also be beneficial to certified seed producers and growers who produce their crop through adventitious propagation methods.

Therefore, the objectives of the research presented in this thesis were to:

- (1) document the changes observed in sweetpotato yield and root quality over time using saved “seed” from micropropagated plants over successive years;
- (2) determine the effects of SPFMV on yield and root quality of sweetpotato;
- (3) determine the reinfection rate of SPFMV in micropropagated, virus-indexed mericlones and adventitiously propagated “seed”;
- (4) compare reinfection rates with weekly aphid counts in the field trials; and
- (5) verify the presence of SPFMV and other potential viruses in field trials by grafting field samples onto the indicator *I. setosa* and testing for viruses using NCM-ELISA.

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## Chapter 2

### **Impact of Adventitious Propagation and *Sweet Potato Feathery Mottle Virus* on Yield and Root Quality in Sweetpotato**

**Abstract.** Reduction in yield and root quality (decline) in sweetpotato has been attributed to the accumulation of viruses and mutations. To document the effects of decline on yield and root quality over time, two micropropagated, virus-indexed, greenhouse produced “G1” ‘Beauregard’ mericlones B94-14 and B94-34, were compared with B94-14 and B94-34 clones propagated over five years of adventitious field propagations (G2, G3, G4, G5) and non-micropropagated NCSU ‘Beauregard’ seed in field trials in 1997-2001. Each year, at least three trials were located in primary sweetpotato producing regions in NC with 5 replications, 25 plants per plot. In 2000 and 2001, two trials were monitored weekly for the presence of *Sweet potato feathery mottle virus* (SPFMV) and three vines per plot for each monitored trial were sampled and virus-indexed using the indicator plant *Ipomoea setosa* and tested for selected viruses using a nitrocellulose enzyme linked immunosorbant assay (NCM-ELISA). Only SPFMV was detected to be present in field samples using NCM-ELISA. Monitoring indicated that all G1 plants became infected with SPFMV by the end of the growing season and that G2-G5 plants were most likely infected in the previous growing season. Evaluations at harvest indicated that G1 plants consistently produced higher yields of roots in total, total marketable yield (TMY), and No.1 root yield (the most marketable grade) and percent No.1 yield (relative to total yield) than G2-G5 plants. G1 plants produced roots with better shape uniformity and overall appearance than roots produced from G2-G5 plants. G2-G5 roots tended to be longer than G1 roots. Rank mean yield and root quality measurements of each location were consistent with means averaged over locations per year

and suggested a decrease in yield and root quality with successive seasons of adventitious propagation. Linear regression analysis used to model yield and root quality measurements of G1-G5 over time indicated that total yield, TMY, No.1 yield, percent No.1 yield, shape uniformity, and overall appearance decreased gradually and length/diameter ratios increased gradually with increased field generations of adventitious propagation. In addition, B94-34 was observed to have a higher rate of decline in No.1 yield than B94-14. The results from this study indicate that both SPFMV and mutations due to adventitious propagation contribute to decline in sweetpotato and that micropropagated, G1 plants can be used to circumvent the effects of decline.

## **Introduction**

Sweetpotato [*Ipomoea batatas* (L.) Lam.] ranks ninth among the most important food crops in the world (FAO 2001). North Carolina ranks first in the nation in sweetpotato production accounting for approximately 40% of the crop sold in the United States (USDA NASS 2001). Sweetpotato can be asexually propagated via roots or stems. In the US, growers use asexual propagation methods to produce sweetpotato by saving a portion of the crop from each year as “seed” roots. In early spring, the “seed” roots are planted in beds, and adventitious sprouts from bedded roots are pulled or cut and transplanted to the field to produce the sweetpotato crop for the following season. After several years of adventitious propagation via storage roots, however, a decrease in yield and root quality is often observed. The decline of root quality and yields in sweetpotato, referred to as cultivar running-out (Miller et al., 1959), has been attributed to the accumulation of mutations and viruses (Villordon and LaBonte, 1995).

Mutations induce color changes in the root epidermis and flesh (Groth, 1911; Rosa, 1926; Harter, 1926; Thompson, 1929; Miller, 1930; Miller, 1935). Mutations are also responsible for genetic variability resulting in a range of shapes and yields among clones of a given cultivar (Edmond and Ammerman, 1971; Villordon and La Bonte, 1995). Visual selection of roots or hills (all roots of an individual plant) most characteristic of a cultivar is the most widely used method of preserving the genetic integrity of cultivars in US sweetpotato foundation seed programs (Dangler, 1994). Though this method eliminates roots with mutated color, shape, and yield within the initial population used for propagation, mutants are still observed in roots of subsequent adventitious propagules (Dangler, 1994). If deleterious mutations are not detected by visual selection, they may accumulate and alter

favorable genetic components for yield and root quality. Continuous adventitious propagation, which enables mutations to accumulate, leads to sweetpotato cultivar decline over time (Villordon and La Bonte, 1995). Nodal cuttings of sweetpotato have lower mutation rates than adventitious sprouts of saved roots (Templeton-Somers and Collins, 1986; Villordon and La Bonte, 1995; Villordon and La Bonte, 1996). Thus, the use of planting material derived from preexisting meristematic tissue, specifically nodal cuttings, has been proposed to reduce clonal variability in sweetpotato foundation seed programs (Villordon and La Bonte, 1995; Villordon and La Bonte, 1996).

In addition to mutations, viral infections also contribute to cultivar decline in sweetpotato (Gooding, 1964; Huett, 1982). Accumulation of viruses and diseases occur through the adventitious, root-to-sprout propagation method used in commercial production. Saved “seed” roots of sweetpotato plants that become infected with virus during the growing season will produce virus-infected adventitious sprouts (Clark and Moyer, 1988). If virus infection decreases yield and root quality in sweetpotato, then the continuous use of virus-infected adventitious sprouts may lead to cultivar decline. Consequently, the use of sweetpotato planting material that is free of known viruses is recommended as a method of controlling, preventing, or slowing virus accumulation in subsequent plantings (Clark and Moyer, 1988). Healthy plants can be obtained through meristem-tip culture and these plants are tested for freedom of known viruses by grafting onto *Ipomoea setosa* Ker (virus-indexing) (Clark and Moyer, 1988). However, most viruses that infect sweetpotato have insect vectors and virus reinfection occurs rapidly during the growing season if sources of local inoculum, including virus-infected sweetpotato plants and *Ipomoea spp.*, are present (Clark and Moyer, 1988).

Thus, the level of virus infection is virtually 100% in 1-3 years, depending on virus incidence and insect vectors present.

The process of micropropagation, along with virus-indexing of meristemed clones (mericlones) provides material free of known viruses that can be rapidly multiplied for planting stock via greenhouse increase of nodal cuttings of mericlones. In addition, the use of nodal cuttings from micropropagated plants reduces the amount of variation between propagules and retains genetic integrity (Villordon and La Bonte 1996). Nodal cuttings of micropropagated planting stock can be used as an alternative to adventitious propagules; therefore, its use may help slow the effects of cultivar decline.

The process of micropropagation was incorporated into sweetpotato foundation programs when russet crack, a root symptom caused by a strain of *Sweet potato feathery mottle virus* (SPFMV), was detected in commercial sweetpotato crops in a number of locations and cultivars (Dangler, 1994). SPFMV is the most common virus of sweetpotato in the United States (Clark and Moyer, 1988). This virus is non-persistently transmitted by aphids (*Myzus persicae*, *Aphis gossypii*) and it also occurs worldwide. Foliar symptoms include veinal chlorosis, feathering, and chlorotic spots with purple borders. Root symptoms, in addition to russet crack, include internal cork, shape deformities, and surface discoloration depending on the cultivar and virus strain present (Moyer and Salazar, 1989).

In North Carolina, several studies have been conducted to compare the effects of yield and root quality of nodal cuttings from micropropagated plants with non-micropropagated,

SPFMV-infected plants from various sources. These studies indicated that the use of micropropagated plants, in addition to reducing russet crack symptoms, provided planting material that yielded as good as or better than non-micropropagated, SPFMV-infected plants that had been in field production for several years (Averre et al., 1993; Jester et al., 1994; Schultheis, 1994; Schultheis et al., 1994). However, the effects of virus and mutation accumulation and their corresponding effect on yield and quality over several generations of propagation were not studied.

Micropropagated sweetpotato cultivars serve as the initial source of planting material in several state foundation programs (Dangler, 1994); however, the use of micropropagated material on a yearly basis may not be economically feasible for growers compared to adventitious propagation methods. If adventitious propagules derived from “seed” roots of micropropagated plants also retained high yields and reduced russet crack symptoms, then growers could benefit from micropropagation without drastic change in production and quality of planting stock. In addition, growers would have the option to renew their planting stock with nodal cuttings of micropropagated plants kept by state foundation programs. Therefore, it could benefit growers to know what effects the number of years that adventitiously derived “seed” obtained from micropropagated material have on root quality and yield as they are affected by genetic mutation and viruses.

The primary objectives of this experiment were: 1) to plant virus-indexed, true-to-type micropropagated seed in the field and document the changes observed in sweetpotato yield and quality over time using saved “seed” from micropropagated plants over successive years;

and 2) construct predictive models with respect to yield and various root quality attributes over “seed” generations. Another objective was to determine the rate at which micropropagated plants are reinfected with viruses during the growing season.

## **Materials and Methods**

***Plant material.*** Micropropagated clones B94-14 and B94-34 of the sweetpotato cultivar ‘Beauregard’, released by LSU in 1987 (Rolston et al, 1987), were used throughout the study. B94-14 and B94-34 were selected in 1994 as superior hill selections made from the ‘Beauregard’ clone B73 which was received by the North Carolina Crop Improvement Association in 1993 from Dr. Christopher Clark, Plant Pathology Department, LSU. A non-micropropagated ‘Beauregard’, maintained by the Sweetpotato Breeding and Genetics Program at North Carolina State University (NCSU) of “seed” derived from the original ‘Beauregard’ was included in the study for comparison. Adventitious propagules of ‘Beauregard’ were also obtained from local growers and included in all trials in 2000 and 2001.

***Study design.*** Micropropagated, virus-indexed, greenhouse-produced “G1” (G represents generation while the number indicates the number of years in field production) ‘Beauregard’ mericlones B94-14 and B94-34, were compared with B94-14 and B94-34 clones propagated over successive field propagations (second generation “G2”, third generation “G3”, fourth generation “G4”, and fifth generation “G5”), and non-micropropagated NCSU ‘Beauregard’ stock in a 5-year study. The series of tests were initiated in 1997 to evaluate G1 plants of B94-14 and B94-34. Each year following, adventitious propagules derived from the 1997

B94-14 G1 and B94-34 G1 roots were compared to new G1 nodal cuttings of B94-14 and B94-34. In addition, successive and cumulative “seed” generations of B94-14 and B94-34 were also included and the following comparisons were made each year: G1 and G2 in 1998; G1, G2, and G3 in 1999; G1, G2, G3, and G4 in 2000; and G1, G2, G3, G4, and G5 in 2001. Field trials in each year also included non-micropropagated NCSU Breeder stock of ‘Beauregard’ for comparison. Trials conducted in 1999, 2000, and 2001 will be the focus of this study.

***Plant increase.*** In 1994, the B94-14 and B94-34 clones were placed into the Micropropagation Program at the NCSU Micropropagation Unit (MPU). Adventitious sprouts produced from roots of both clones were used for meristem-tip culture and this procedure along with virus-indexing was conducted according to the Food and Agriculture Organization of the United Nations and International Board for Plant Genetic Resources guidelines (Moyer et al., 1989). Virus-tested and vegetative planting stock was maintained in insect proof cages to avoid virus reinfection. For our studies, G1 transplants of both mericlones were obtained from nodal cuttings of micropropagated mother plants (stock grown directly from tissue culture, virus-indexed, and tested for genetic integrity of the mericlones). Mother plants of each mericlone were established in 15 cm pots filled with sand and kept in screen cages (52 mesh, 460  $\mu\text{m}^2$ ) in greenhouses with a 16 hr photoperiod and a temperature range of 25°C-28°C at the MPU. Each mother plant was treated with 1 g/pot of the insecticide Marathon®, 1% Granular Greenhouse and Nursery Insecticide (imidacloprid, Olympic Horticultural Products, Mainland, PA) every 6-8 weeks, 1 g/pot Miracle Gro All Purpose (15-30-15) water soluble fertilizer (The Scotts Co., Marysville, OH) once weekly,

and 1 g/pot of 14N-4.2P-11.6K slow release fertilizer every two to three months as needed. Plants were watered once daily. The same mother plants for each mericlone were used in all years. Cuttings of two nodes were taken from micropropagated mother plants and established in 200 cm<sup>3</sup> cells of a 25 cell tray, model IP200, IPL® Rigi-Pots™ (Stuewe & Sons, Inc., Corvallis, OR), containing moist Metro-Mix (The Scotts Co., Marysville, OH). Each cut was made with a sterile single edge razor blade. Trays of cuttings were watered daily. Once cuttings formed roots and new shoots, they were fertilized with 1 g 15-30-15 water soluble fertilizer and plant multiplication was continued through repeated cuttings of two nodes and placing them in cells of trays. Cuttings of each mericlone were grown in screen cages (52 mesh, 460 μm<sup>2</sup>) to prevent the introduction of aphids and potential virus infection.

Successive root to sprout propagules (G2, G3, G4, and G5) were obtained from cuttings of adventitious sprouts produced from bedded “seed” saved from the harvested roots of the preceding generation used in tests from the previous year, as described earlier. Saved “seed” of each generation included a random selection of US No.1 and canner grade roots (diameter of 5 cm ≤ 9 cm and length of 7.5 cm ≤ 23 cm and diameter of 2.5 cm ≤ 5 cm, respectively) from each plot. “Seed” roots of NCSU ‘Beauregard’ were selected from US No.1 and canner grade roots in field test conducted by the NCSU Sweetpotato Breeding and Genetics Program at the Cunningham Research Station, Kinston, NC. Off-types of NCSU ‘Beauregard’ “seed” roots were eliminated leaving those that were true-to-type. “Seed” roots of each generation and NCSU ‘Beauregard’ were cured, stored, and bedded in the following spring after harvest to provide adventitious propagules for the trials conducted the following growing season. The process of curing, storing, and bedding was conducted as recommended by the

Vegetable Crop Guidelines for the Southeastern US (Sanders, 2001). Stems of G1 plants and adventitious sprouts of G2-G5 and NCSU ‘Beauregard’ stock of uniform length (20 cm) were cut one day prior to planting. ‘Beauregard’ plants ( 20 cm in length) were obtained from North Carolina growers prior to planting. A different grower source of ‘Beauregard’ was used for each trial in all years.

***Trial design.*** The trials were located in the primary sweetpotato growing areas of North Carolina, and included (1) the Cunningham Research Station, Kinston, NC; (2) the Horticultural Crops Research Station, Clinton, NC; (3) on-farm, Columbus County, NC; (4) on-farm, Nash County, NC; and (5) on-farm, Wilson County, NC. For each year, at least three locations were used for tests, including one research station and two on-farm locations. Each test was arranged in a randomized complete block design with five replications. Plots were 6.1 m in length and rows were formed on 1.1 m centers. Each plot included 25 plants spaced 23 cm apart. Border plants of the sweetpotato cultivar ‘O’Henry’ were included at the end of plots at the same spacing.

***Planting procedures.*** Trials conducted in 1999, 2000, and 2001 and their corresponding location and planting and harvest dates are listed in Table 1. The soil series and texture for each location was a Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandudults). In 1999, B94-14 (G1, G2, and G3), B94-34 (G1, G2, and G3) and NCSU ‘Beauregard’ stock were tested in five field trials. Two separate trials were conducted at Kinston, an early and a later planting. In 2000, B94-14 (G1, G2, G3, and G4), B94-34 (G1, G2, G3, and G4) and NCSU ‘Beauregard’ stock were tested in three field trials. In 2001, B94-14 (G1, G2, G3, G4,

and G5), B94-34 (G1, G2, G3, G4, and G5), and NCSU 'Beauregard' stock were compared in four field trials. On-farm trials were planted and grown according to recommended cultural and pest management practices until harvest (Sanders, 2001). Fertilization and cultivation practices varied due to grower preference and equipment with each on-farm trial. With on-farm trials, at least three cultivations with incorporation of fertilizer applications were conducted at approximately 1, 2, and 4 weeks after planting (WAP). Generally, on-farm applications included 30 kg·ha<sup>-1</sup> of P, 93 kg·ha<sup>-1</sup> of K, and 1.1 kg·ha<sup>-1</sup> of B at the first cultivation, 112 kg·ha<sup>-1</sup> of K at the second cultivation, and 57 kg·ha<sup>-1</sup> of N at the third cultivation. In addition to the fertilization regime, weed and insect control were implemented in the on-farm trials. The Wilson County trial in 1999 included weed removal by hand 3 WAP and a foliar spray of the insecticide Dyfonate 10G Insecticide (fonofos, Syngenta, Greensboro, NC) at 22 kg·ha<sup>-1</sup> 4 WAP. The Columbus County trial in 1999 included weed removal by hand at 5 WAP and a foliar spray of Sevin® Brand Carbaryl Insecticide (carbaryl, United Horticultural Supply, Fremont, NE) at 2 kg·ha<sup>-1</sup> at 7 WAP. In the Nash County trial in 2000, 3 L·ha<sup>-1</sup> of Command®, 3 ME herbicide (clomazone, FMC Corp., Philadelphia, PA) was incorporated 5 days after planting and two foliar applications of the insecticide Thiodan 3 EC (endosulfan, FMC Corp., Philadelphia, PA) was applied at 3 L·ha<sup>-1</sup> at 5 and 9 WAP, respectively. In the Columbus County trial of 2000, hand removal of weeds was conducted approximately 5 and 8 WAP and a foliar insecticide spray of Sevin® at 2 kg·ha<sup>-1</sup> at 5 WAP. The Wilson County trial in 2001 included an application of Poast® 1.53 EC (sethoxydim, BASF Corp., Research Triangle Park, NC) at 1.5 L·ha<sup>-1</sup> 2 WAP. The Columbus County trial in 2001 included two foliar applications of the insecticide Sevin® at 2 kg·ha<sup>-1</sup> at 5 and 8 WAP. Research station trials were planted by hand. The fertility regime

for the Horticultural Crops Research Station trials in Clinton included three cultivations at approximately 1, 2, and 4 WAP. Fertilizer application of 18 kg·ha<sup>-1</sup> of N and 45 kg·ha<sup>-1</sup> of K was incorporated into the first cultivation, 25 kg·ha<sup>-1</sup> of P and 116 kg·ha<sup>-1</sup> of K into the second, and 57 kg·ha<sup>-1</sup> of N into the third cultivation. The fertility regime for the Cunningham Research Station trials in Kinston included two cultivations at approximately 1 and 4 WAP. Fertilizer application of 30 kg·ha<sup>-1</sup> of P, 140 kg·ha<sup>-1</sup> of K, and 1.1 kg·ha<sup>-1</sup> of B was incorporated into the first cultivation and 52 kg·ha<sup>-1</sup> of N into the second cultivation.

At harvest, sweetpotatoes were graded according to U.S. Dept. of Agriculture (U.S. Dept. of Agriculture, 1981) standards, which classify harvested root products into U.S. No.1 roots (diameter of 5 cm ≤ 9 cm and length of 7.5 cm ≤ 23 cm), canner roots (diameter of 2.5 cm ≤ 5 cm), jumbo roots (diameter > 9 cm, length > 23 cm), and cull roots (malformed or distorted roots). Yield measurements were recorded for each grade, including total marketable yield (TMY), which includes all grades except culls. The percentage of No.1s (percent No.1s) was calculated relative to the total yield for each plot. Evaluations of root quality of No.1 roots, including overall appearance, and shape uniformity were measured for each treatment and recorded on a 0 to 5 scale to the nearest half unit where 5 represents the best overall appearance and most uniform shape. Evaluations of negative epidermal characteristics were measured for each plot in 2000 and in 2001 including lenticels (small pores (2mm) for gas exchange) and pimples (brown to black protrusions (1mm diameter) on root epidermis). In trials in 2001, ratings for eyes (vegetative buds) on roots were also recorded. The presence of lenticels, pimples, and eyes were recorded on a 0 to 5 scale to the nearest half unit where 5 represents minimal or no presence of lenticels, pimples, and eyes, 3 represents roots where

lenticels, pimples, or eyes covered half the area of the root, and 0 represents roots completely covered with one of the respective skin characteristics. For each plot, No.1 roots were given visual ratings for length diameter ratios (L/D). Ratings were recorded on a 1 to 5 scale to the nearest half unit where each rating indicated the ratio of length to width for majority of No.1 roots in each plot. In trials in 2000 and 2001, ten No.1 roots were randomly selected from each treatment plot for the evaluation of flesh color and presence of chimeras. Selected roots were sliced approximately 1/3 the distance from the proximal end of roots and a score ranging from 0 to 5 for intensity of orange flesh were made. Scores of 0 indicate no carotene (white) and scores of 5 indicate very high carotene (dark orange), similar to the scale developed by Hernandez et al. (1965). True-to-type 'Beauregard' flesh color rating, based on the scale developed by the NCSU Sweetpotato Breeding program, is scored as 3, which indicates medium carotene (medium orange) (Craig Yencho, personal communication). In addition to color rating, the numbers of chimeras were recorded for each sliced root per plot.

***SPFMV monitoring.*** Tests in the 2000 Kinston and Nash County locations and 2001 Kinston and Wilson County locations were monitored for presence of SPFMV. In 2000, weekly observations began approximately 4 WAP. However, due to the large percentage of symptomatic plants recorded at 4 WAP in 2000, monitoring was initiated 1 WAP in 2001. Weekly observations of the number of SPFMV symptomatic plants were recorded for each plot in each year. Prior to harvest, symptomatic vines (approximately 30 cm in length) of three randomly selected plants were chosen per plot, cut, and grafted onto the indicator plant, *Ipomoea setosa*, to test for the presence of viruses. Indicator plants showing severe virus symptoms were tested for SPFMV, *Sweet potato mild mottle virus (SPMMV)*, *Sweet potato*

*latent virus (SPLV), Sweet potato chlorotic fleck virus (SPCFV), Sweet potato mild speckling virus (SPMSV), C-6 virus, Sweet potato chlorotic stunt virus (SPCSV), and Sweet potato caulimovirus (SPCaLV)* using a nitrocellulose membrane enzyme-linked immunosorbant assay (NCM-ELISA) kit provided by the International Potato Center (CIP) in Peru. The presence of each virus was indicated when the membrane showed some purple color, as recommended by the kit.

***Russet crack.*** In 2001, a high incidence of russet crack, the root symptom induced by the SPFMV-Russet crack strain, was observed on harvested roots in two locations. Thus, all harvested roots from three replications in Columbus County and five replications in Wilson County were evaluated for the presence of russet crack symptoms. Negligible symptoms of russet crack on roots were found in trials in 1999 and 2000 so incidence of symptoms was not quantified.

***Statistical analysis.*** To compare effects of generations of each clone on yield and root quality per year, a split plot analysis with location as the main plot factor and clone and generation as subplot factors were used. A residual analysis was conducted to determine normality of data and homogeneity of variances (SAS, 1998). For 1999, yields of graded roots and measurements of root quality for each generation were analyzed using the SAS's General Linear Models (GLM) procedure (SAS Institute, 1998) to adjust for data lost in two plots in Clinton and one plot in Kinston due to flooding caused by Hurricane Floyd. The Least Squares Means (LSMEANS) procedure was used to compare generations of each clone at alpha levels 0.05 and 0.1. For 2000 and 2001, yields of graded roots and measurements of

root quality for each generation were analyzed using the SAS Analysis of Variance (ANOVA) procedure (SAS Institute, 1998). Fisher's Protected Least Significant Difference (LSD) was used to compare generations of each clone at alpha levels 0.05 and 0.1. For comparisons of the generations to NCSU 'Beauregard' stock, a separate analysis was conducted for each year (GLM for 1999 and ANOVA for 2000 and 2001), where each clone and generation (i.e., B94-14 G1, B94-34 G2) and NCSU 'Beauregard' were analyzed as separate "treatments." The analysis, including NCSU 'Beauregard,' used comparisons of LSMEANS for 1999 data and Fisher's Protected LSD for 2000 and 2001 data for comparison at alpha levels 0.05 and 0.1. For comparisons of the generations to the grower 'Beauregard' source, a separate ANOVA was conducted for each location in each year. Means of G1 B94-14 and G1 B94-34 yield and root quality measurements were compared with those of grower 'Beauregard' sources separately for each trial in 2000 and 2001 using Fisher's Protected LSD test.

In order to construct predictive models for yield and root quality measurements over "seed" generations, a linear regression analysis was conducted using the SAS Regression (REG) procedure (SAS, 1998). G1 yield and root quality measurements of B94-14 and B94-34 and the yield and root quality measurements of roots produced from the subsequent adventitious propagules (G2-G5) derived from the original G1 plant material were used in the analysis. Thus, a total of three sets of generational lines were analyzed: (1) G1- G5 (1997-2001); (2) G1-G4 (1998-2001); and (3) G1-G3 (1999-2001). In order to account for year effects, the ratio of yield and root quality measurements of the generations, G2-G5, to the yield and root quality measurements of the G1 grown the same year was used in the regression. Because

the G2 and G3 of 1999, G3 and G4 of 2000, and G4 and G5 of 2001 were derived from the G1 material initially used in the 1997 and 1998, data from these years are included in the analysis. These data have been previously reported in the 1997 and 1998 North Carolina Sweetpotato Progress Reports. An ANOVA test was conducted to determine equality of slopes for the ratios of each clone and generational line for the yield and root quality measurements that were significant when comparing generations within each year.

## **Results**

**1999.** Differences ( $p=0.1$ ) were detected among both main effects, and first and second order interactions for yield and root quality measurements when comparing generations of clones B94-14 and B94-34 (Table 2). The effects of generation were significant for yield of No.1s, canners, culls, percent No.1s, uniformity, and L/D ratio (Table 2). The rank of mean yield and root quality measurements for generations for total yield, TMY, No.1s, culls, percent No.1s, shape uniformity, L/D, and overall appearance were generally consistent across locations (data not shown). Thus, mean yield for each clone and respective generation averaged across locations are reported in Table 3. No.1 yield of B94-34 G1 was higher than B94-34 G3. Yield of culls B94-34 G1 was lower than B94-34 G2 while yield of culls of B94-14 G1 was lower than B94-14 G2 and G3. Uniformity of G1 roots of both clones was higher than G2 or G3 roots. Percent No.1 yields of G1, G2, and G3 decreased as the number of generations in the field increased for B94-34 while percent No.1 yields of B94-14 decreased in G3 compared with G1. L/D ratios of G1 roots were lower than both G2 and G3 roots in both B94-14 and B94-34.

For the analysis comparing generations of clones to NCSU 'Beauregard', differences ( $p \leq 0.10$ ) were detected in total yield, TMY, No.1s, culls, percent No.1s, uniformity, and L/D ratio. G1 root yields were higher for both clones in total yield, TMY, and yield of No.1s than NCSU 'Beauregard' (Table 3). B94-34 G1 produced higher percent No.1 roots and fewer cull roots than NCSU 'Beauregard.' Root quality measurements of shape uniformity in B94-14 and B94-34 G1 were higher than NCSU 'Beauregard.' L/D ratios of B94-14 and B94-34 G1 were lower than NCSU 'Beauregard.'

**2000.** Significant differences ( $p=0.1$ ) were detected among the main effects and first order interactions for both yield and root quality measurements for generations (Table 2). The effects of generation were significant for total yield, TMY, No.1s, culls, percent No.1s, shape uniformity, L/D ratio, and overall appearance. As observed in 1999, the rank of most traits at each location were generally consistent for mean yield and root quality measurements averaged over locations (data not shown), therefore, mean yield and root quality for each clone and respective generation were averaged over locations (Table 3). In 2000, B94-14 G1 plants produced higher total yield and TMY than B94-14 G3 plants while B94-34 G1 plants produced higher total yield than B94-34 G4 plants. No.1 root yields of G1 plants were higher than No.1 root yields of G4 plants for both mericlones. G1 plants also yielded more No.1 roots than G3 plants derived from B94-14 and G2 plants obtained from B94-34. Cull yields of G1 plants of both clones were lower than all other generations (G2-G4). The percent No.1 yield produced by B94-34 G1 plants was higher than that produced by G2 and G4 plants, while no statistical differences were detected with B94-14. Shape uniformity and

overall appearance ratings for G1 in roots of both clones were higher than all other generations and L/D ratios for G1 roots of both clones were lower than all other generations.

For the analysis comparing clones and generations to NCSU 'Beauregard', significant differences were found between NCSU 'Beauregard' and all yield and root quality measurements of B94-34 G1 and all root quality measurements for B94-14 G1. B94-34 G1 plants yielded higher than NCSU 'Beauregard' in total yield, TMY, No.1 yield, and percent No.1s. B94-14 G1 plants produced higher yields in No.1s and percent No.1s than NCSU 'Beauregard'. Both B94-14 G1 and B94-34 G1 had lower cull yields and L/D ratios, and better shape uniformity and overall appearance ratings than NCSU 'Beauregard.' L/D ratios of B94-14 G2 were lower than NCSU 'Beauregard.' B94-14 and B94-34 G2-G4 were not significantly different from NCSU 'Beauregard' in any other measurements recorded (Table 3).

**2001.** As observed in 1999 and 2000, significant differences ( $p=0.1$ ) were detected among main effects and first order interactions for both yield and root quality measurements for generations (Table 2). Effects of generation were significant for total yield, TMY, yield of No.1s, percent No.1s, flesh color, pimples, shape uniformity, L/D ratio, and overall appearance (Table 2). As in 1999 and 2000, mean yield and root quality measurements for each clone and respective generation at each location (data not shown) were generally consistent with mean yield and root quality measurements averaged over locations (Table 3). In 2001, G1 plants produced higher total, TMY, yield of No.1s, and percent No.1s for both clones compared with all other generations (Table 3). B94-14 G5 plants produced lower

No.1 yields and percent No.1s than B94-14 G1, G2, and G3. G1 roots had higher shape uniformity ratings and lower L/D ratios when compared with all other generations for both clones. The overall appearance rating of B94-34 G1 roots was higher than all other generations with a gradual decline in appearance from G1 to G5.

When clones and generations were compared to the NCSU 'Beauregard, significant differences were found between G1 roots of both clones for all yield measurements except cull yields and all root quality measurements. Total yield, TMY, yield of No.1s, and percent No.1s and root quality measurements of uniformity and appearance ratings for G1 B94-14 and B94-34 were higher than NCSU 'Beauregard'. L/D ratios of G1 roots of both clones were lower than NCSU 'Beauregard.' Overall ratings of B94-34 G5 roots were lower and yield of culls of B94-34 G3 and B94-34 G4 were higher than NCSU 'Beauregard.'

Significant differences between generations were not detected for the number of chimeric regions observed in storage roots, and for the presence of negative epidermal characteristics such as lenticels and eyes (data not shown). However, pimples and flesh color were significant in one case in 2001. Means of epidermal characteristics, chimeras, and flesh color are not reported. The mean yield and root quality measurements for the grower 'Beauregard' sources for each trial in 2000 and 2001 are listed in Appendix 1.1.

Yield of graded roots harvested in the Wilson County trial in 2001 of generations G1-G5 of B94-34 and B94-14 are illustrated in Appendix Figures 1.1 and 1.2, respectively. No. 1 root

yield of B94-34 of generations G1-G4 harvested in Nash County in 2000 are illustrated in Appendix Figure 1.3.

**Regression analysis.** The ANOVA to test equality of slopes indicated that the loss of No.1 yields differed between clones for G1-G4 (1998-2001) and G1-G3 (1999-2001). Therefore, linear regression analysis was conducted separately for clones in No.1 yield. For all other significant yield and root quality measurements, linear regression equations were determined by combining data from both clones. The combined linear regression analyses over clones indicated that total yield, TMY, and percent No.1s decline as generation number (G2-G5) increases (Fig. 1). The percent decrease in yield (slope of regression equation) is at least 5% for total yield, 4% for TMY, and 7% for percent No.1s. The linear regression analysis indicates that the rate of decrease of yield of No.1s is higher in B94-34 than B94-14 with the rate of yield decrease for B94-34 estimated to be between 12 and 18% and ranging between 8 and 12% for B94-14 (Fig. 2). For the second and third generational lines analyzed (G1-G4 and G1-G3, respectively), R-square values were at least 0.9 for total yield, TMY, yield of No.1s, and percent No.1s, indicating that at least 90% of the variation observed was due to increases in the number of generations. Linear regression analysis also indicates that L/D ratio increases with generation, and overall appearance and shape uniformity decrease with clonal generation (Fig. 3).

**SPFMV monitoring.** Both years of SPFMV monitoring indicated that all plant sources became infected with SPFMV during the growing season (Fig. 4). In 2000, less than 2% of G1 plants and at least 30% of G2-G5 and NCSU 'Beauregard' plants displayed foliar

symptoms of SPFMV 4 WAP. By 5 WAP, the percentage of SPFMV symptomatic plants increased by roughly 40% in all clones, and the majority of G2-G5 plants were displaying symptoms of SPFMV. By 10 WAP, all sweetpotato plants, including G1 plants, were displaying symptoms of SPFMV. In 2001, approximately 10% of G1 plants and at least 90% of G2-G5 plants and NCSU 'Beauregard' displayed symptoms of SPFMV 1 WAP. All G2-G5 plants and NCSU 'Beauregard' displayed SPFMV symptoms by 5 WAP while all G1 plants displayed SPFMV symptoms by 8 WAP.

All plants sampled for grafting on the indicator plant *I. setosa* indicated the presence of SPFMV. When using only selected *I. setosa* grafts with severe symptoms, NCM-ELISA confirmed the presence of SPFMV. Tests using NCM-ELISA for the presence of SPCSV, SPMMV, SPLV, SPCFV, SPMSV, C-6 virus, SPCSV, and SPCaLV yielded negative results suggesting that these viruses were not present in the samples.

**Russet crack.** In the Columbus County and Wilson County trials of 2001, russet crack symptoms were present on No.1, canner, and cull roots of B94-14 and B94-34 G2-G5 and NCSU 'Beauregard' (Appendix 1.2). No russet crack symptoms were found to be present on G1 roots of any grade of both clones at either location. In the Columbus County location, 3.9%, 1.8%, 4.7%, 0.9%, and 0.3 % of russet crack symptoms were present on roots of G2, G3, G4, G5, and NCSU 'Beauregard', respectively. A higher percentage of russet crack symptoms was present on roots at the Wilson County location with 13.2%, 10.1%, 15.5%, 10.8%, and 2.9% present on No.1 and canner roots of G2, G3, G4, G5, and NCSU 'Beauregard', respectively.

## **Discussion**

Sweetpotato is a notoriously variable crop that is sensitive to a wide range of environmental variations (Carpena et al., 1982; Collins et al., 1987; Bacusmo et al., 1988; Kannua and Floyd, 1988; Ngeve and Bouwkamp, 1993). The significant generation by location and clone by location interactions reported in the present study can be partially attributed to differences in environmental factors in each year (not reported) and differences in cultural practices used at on-farm and research station trials. However, the results from the present study indicate that micropropagated, nodal cuttings of G1 plants consistently yield higher and produce more uniformly shaped roots of higher quality with a lower L/D ratio than adventitious sprouts produced from subsequent generations of saved “seed” from G1 plants and adventitious sprouts produced from the non-micropropagated NCSU ‘Beauregard.’ A gradual decrease in total yield, TMY, yield of No.1s, and percent No.1s and an increase in yields of culls with increased “seed” generation were also consistent in all years for both clones. Similarly, a decrease in overall appearance and shape uniformity and an increase in L/D ratio with increased “seed” generation were also consistent through years. Trends, which are generally consistent for both yield and root quality measurements, are supported by the linear regression analysis in which a decrease in total yield, TMY, yield of No.1s, percent No.1s, shape uniformity, and overall appearance and an increase in L/D ratio were observed for the three generational lines.

The present study indicates that clonal degradation occurs gradually from one propagation cycle to the next and that this is consistent in three separate populations with at least three generations of adventitious propagation cycles. In addition, the rate of decrease may differ

between clones of the same cultivar, such as observed in this study where the decrease in No.1 yield of the B94-34 clone was greater than the B94-14 clone.

The high yield and root quality achieved through the use of G1 plants are most likely due to the uniformity obtained through the nodal cuttings of high yielding, high quality micropropagated clones and the absence of SPFMV in those cuttings prior to planting. Both B94-14 and B94-34 were superior hill selections with high yield and root quality characteristic of the cultivar 'Beauregard.' The adventitious sprouts of these clones were micropropagated and the nodal cuttings of the mericlones produced high yields and high quality roots; however, an increase in the number of subsequent adventitious propagations led to decreases in yield and root quality. The decreases in yield and root quality are most likely due to the adventitious propagation method of root to shoot whereby deleterious mutations and SPFMV were allowed to accumulate.

Previous reports have indicated that adventitious propagules of sweetpotato cultivars are genetically more variable than those produced from preexisting meristematic regions and this variability alters favorable genetic combinations, resulting in deleterious mutations that may lead to decline (Villordon and LaBonte, 1996). Somaclonal variation is used to describe genetic variation in plants originating from adventitious meristems and is not observed in plants from non-adventitious meristems (Hussey, 1983). Though this description usually refers to differences observed in adventitious meristems derived from tissue culture procedures, the adventitious propagation cycle of sweetpotato from root to shoot may produce somaclonal variation in propagules (Villordon and La Bonte, 1995). Somaclonal

variation between adventitious propagules of sweetpotato may be due to an accumulation of spontaneous somatic mutations. Since adventitious buds arise from cells from one histogenic layer, most likely L2 or L3, somatic mutations occurring within that layer will be present in all three histogenic layers of the new adventitious propagule (Hartman et al., 1997).

Phenotypic variation due to somaclonal variation has been determined as the percentage of plants that display departure from one or more defined characteristics (Lee and Phillips, 1987; Lourens and Martin, 1987). This study suggests that readily detectable differences in sweetpotato yield and root quality can be observed in field plantings of G2 to G5 “seed” derived from adventitious propagules compared to the micropropagated G1 planting materials produced via meristematic propagation. Nodal stem cuttings of micropropagated G1 plants are derived directly from meristematic tissue and are not subject to somaclonal variation. This suggests that somaclonal variation may have at least partly contributed to differences observed between nodal cuttings of G1 plants and adventitious propagules, and that these mutations reduced yield and root quality.

We attempted to assess the rate of clonal degradation of the two clones in this study with respect to the original G1 planting generation using linear regression analysis. In this analysis, yield and root quality measurements are expressed as the ratio of each generation (G2-G5) to the G1 yield and root quality measurements of the same year. Since G1 propagules were stem cuttings taken from the same G1 plant of each clone for each year and trial, the rate of decline, or slope of the regression equation, represents the percentage of deviation in yield and root quality from the original G1 plant from which the adventitious

propagules were derived. For most yield and root quality measurements, the departure (slope) was at least 5% per generation.

Our studies suggest that clonal degradation occurs fairly slowly in sweetpotato. This is exemplified by the fact that we were unable to consistently detect significant differences between generations in most yield and root quality measurements. However, this was not unexpected as it has been previously shown that decreased plant vigor and yield result from accumulation of deleterious mutations over time (Muller's Ratchet) (Muller, 1964). Generally, no differences were observed between most mean yield and root quality measurements until after four generations of adventitious propagation (i.e., G5). In comparing B94-14 and B94-34, with NCSU 'Beauregard,' statistical differences in percent No.1s and overall appearance were not observed until after five propagation cycles (G5), and not in generations G2 through G4. Thus, the net accumulation of deleterious mutations between the generations (G2-G5) appears to gradually impact yield and root quality. The gradual decreases in yield and root quality are depicted in Fig. 1-3. We are unsure as to why the NCSU 'Beauregard', which has been adventitiously propagated for more than 5 years, performed comparatively well in these tests. We speculate that the true-to-type root selection methods used for the propagation of NCSU 'Beauregard' may account for an even more gradual decline. True-to-type root selection may reduce the amount of deleterious mutations and thus slow decline by sustaining the accumulation of favorable mutations which may offset the effects of deleterious mutations (Haigh, 1978) or deleterious mutations may be eliminated through random drift or intraorganismal selection (Klekowski and Kazarinova-

Fukshansky, 1984; Michod, 1995; Otto and Orive, 1995; and Pineda-Krch and Fagerstrom, 1999).

Adventitious propagation over several generations has the potential to produce more genetically different variants (somaclones) and this variability presents itself in the range of yield and root quality measurements recorded. The degree of somaclonal variation in plants has been assessed by determining the value of standard deviations (SD) for a quantitative trait (De Klerk et al., 1990). However, when genotype by location interactions are significant, such as in this study, stability variances, such as proposed by Shukla (1972) which correspond to each genotype may be more useful. Shukla's stability variance ( $\sigma^2$ ) has been reported to provide reliable parameters in estimating yield stability in sweetpotato (Ngeve and Bouwkamp, 1993). Low  $\sigma^2$  indicates a stable cultivar while high  $\sigma^2$  indicates a less stable cultivar. The  $\sigma^2$  for all yield and root quality measurements for 1999, 2000, and 2001 are listed in Appendix 1.3. In this study, the  $\sigma^2$ , averaging over locations for 2001, for No.1 root yield of B94-14 was 3.7, 7.3, 1.2, 11.2, and 2.5 and B94-34 was 34.8, 5.0, 1.9, 0.3, and 0.4 for G1, G2, G3, G4, and G5 of each mericlone respectively. The  $\sigma^2$ , averaging over locations in 2001, for No.1 root yield of NCSU 'Beauregard' was 0.5. The variability of G1 plants of B94-14 and particularly B94-34 may correspond to transplant shock and replacement of these plants within 2 WAP. Greenhouse produced transplants, such as G1, often experience difficulties in acclimating to field environments; thus G1 yield of No.1s, though consistently high, may be somewhat variable. Low  $\sigma^2$  of NCSU 'Beauregard' may indicate elimination of off-types renders adventitious propagules with higher yield stability than G2-G5 propagules in which off-type roots were not discarded. In addition, the relatively

low  $\sigma^2$  for G2-G5 of each mericlone indicates that 'Beauregard' is a stable cultivar which corresponds to the gradual decline modeled by the linear regression analysis. Interestingly,  $\sigma^2$  values for G2-G5 of B94-34 are lower than those for B94-14. As noted in the linear regression analysis, B94-34 was observed to decline at a higher rate in No.1 root yield than B94-14. The lower  $\sigma^2$  values may indicate that deleterious mutations for yield in B94-34 may have accumulated or were fixed more readily than those for B94-14. Consequently, the stability of lower yielding adventitious propagules may have led to a higher rate of decline in No.1 yield of B94-34 than for B94-14.

Methods to detect genetic variation between sweetpotato clones have been achieved using genetic markers, such as randomly amplified polymorphic DNA (RAPD) markers (Villordon and La Bonte, 1995). Though these methods were not included in this study, preliminary studies suggest that B94-14 and B94-34 can be distinguished from each other and the B73 clones from which they were derived with amplified restriction fragment polymorphism (AFLP) markers (Craig Yencho, personal communication).

In addition to somatic mutations, the presence of SPFMV may have also contributed to the decline in yield and root quality. Symptomless sweetpotato plants and virus-indexed sweetpotato plants have been reported to yield more than virus-infected sweetpotato (Mukiibi, 1977; Hahn, 1979; Liao et al., 1983; Olivero and Oropeza, 1985; Ngeve, 1990; Ngeve and Bouwkamp, 1991; Pozzer et al., 1995; Milgram et al., 1996). In this study, SPFMV was confirmed to be present in all field generations (G2-G5) after planting in the field. The G2 to G5 and NCSU 'Beauregard' materials all displayed SPFMV symptoms very

early in the growing season indicating that the planting material likely became infected with SPFMV in the previous growing season or field beds used in adventitious propagation. G1 plants became infected with SPFMV by the end of the growing season, indicating that infected sweetpotatoes in field trials or surrounding areas were present. Since G1 plants of both clones produced higher yields of more uniformly shaped roots of high quality with a lower L/D ratio, than all other generations, the presence of SPFMV in transplants may have greater effect on yield and root qualities rather than in-season infection. Kano and Nagata (1999) found SPFMV-infected plants produced roots with less weight and lower root diameters than roots produced from healthy plants. In our test, the presence of SPFMV may have caused a decrease in diameter length of roots in G2-G5 roots compared with G1 roots, causing L/D ratios to increase. The decrease in root yield of SPFMV-infected plants of G2-G5 could also contribute to the observed decreases in total yield, TMY, yield of No.1s, and percent No.1s. Russet crack symptoms on roots, due to the presence of SPFMV-RC, were observed on both B94-14 and B94-34 roots and detracted from appearance and root quality. Russet crack symptoms were present on approximately 10% or less of the total roots in each treatment in the Wilson County and Columbus County locations in 2001. In some cases russet crack symptoms were severe and resulted in detraction from appearance. In addition, skin discolorations associated with SPFMV-RC were also present and diminished appearance.

In N.C., Louisiana, California (Dangler, 1994), and China (Feng et al., 2000), the use of micropropagation has improved both yield and root quality in sweetpotato. In North Carolina, micropropagated material has been incorporated into the certified seed program for

growers. Certified seed producers purchase virus-indexed, micropropagated greenhouse produced plants from the NCSU Micropropagation Unit. The plants are cuttings derived from true-to-type mother plants and certified seed. Producers multiply these plants to sell to commercial growers or use G1 plants to produce G1 "seed" roots. The following spring, the G1 roots can be used to produce G2 plants. According to a survey of 3 of the 10 certified seed producers in North Carolina, each cutting of virus-indexed micropropagated G1 plants is sold for approximately \$0.20 per cutting while G2 plants are sold for approximately \$0.0325 per cutting. Low quality seed, such as five generations from micropropagated material (G5), is sold for \$0.02 per cutting. Our observed yield data suggests that while growers have the potential to produce higher No.1 yields with G1 plants, a greater monetary return can be achieved using G2 plants because of the high price of G1 plants. According to the 2001 budget analysis by Estes, the operating and fixed costs for commercial sweetpotato production are \$3967 and \$619 per hectare, respectively. The low price of US No.1s in 2001 was \$12.00 per 18 kg box (NCDA&CS and LDA&F, 2001). Factoring in the costs of plants as quoted by certified seed producers and using our observed data for No.1 root yields of B94-14 for 2001, growers have a potential net return of \$2203·ha<sup>-1</sup> for G1 plants, \$5030·ha<sup>-1</sup> for G2 plants, and \$4394·ha<sup>-1</sup> for G5 plants, above all specified costs. Thus, growers can produce G2 roots with a higher net return (\$636 per hectare) over the net return for producing G5 roots. Potential fluctuations based on environmental factors and market conditions influencing yield and prices of No.1 sweetpotato roots may increase or decrease these estimates. In addition, the price of plants typically drops as the growing season progresses. However, the estimates for potential net return do not take into account the price that may be

obtained with higher quality roots produced by planting material that has been in production for limited adventitious generations.

Most growers in the US prefer the bedding system because of the large amount of transplants (adventitious sprouts) produced in a short period of time. “Seed” roots need to be cured and stored over winter and this procedure is already used for the commercial sweetpotato crop by most growers. Because roughly 10% of the crop is saved as “seed” roots for production of sprouts, the time and energy spent in saving “seed” may be less than the cost of micropropagated transplants for larger growers. Thus, “seed” with less mutation and virus accumulation may be more useful to growers who choose to save their own “seed.”

Providing growers with “seed” that will consistently produce high yields and root quality is of the utmost importance. Considerable time and effort is needed to choose true-to-type sweetpotato “seed” and also avoid virus-infection in “seed” production. Knowing that mutation and virus accumulation contributes to decline, particularly in the first few “seed” generations, may help in utilizing resources to prevent the effects of decline while incorporating production methods used by growers.

## **Conclusions**

Adventitious propagules from roots that are infected with SPFMV do not retain the high yield and root quality attributes initially observed in the micropropagated, greenhouse produced nodal cuttings from which they were derived. These studies demonstrate that adventitious propagules of saved “seed” roots of micropropagated G1 plants, which are only randomly selected and not selected as true-to-type for high yield and quality, yield less and

have lower root quality measurements than nodal cuttings of G1, micropropagated plants. Mutations and virus that are allowed to accumulate through adventitious propagation methods appear to lead to decline in sweetpotato. The use of micropropagation in sweetpotato foundation seed program can be used to minimize the effects of decline. The use of limited “seed” generations for sweetpotato production may be an option for growers who choose to save their own “seed” when micropropagated plants are used as the initial planting material such as used by the NC certified seed program. “Seed” roots from G1 plants must be certified as true-to-type and produced in fields with limited virus infection. This method assumes true-to-type roots have minimal net deleterious mutations and that symptomless sweetpotato plants also produce “clean” roots with minimal or no virus. However, in order to develop and maintain a sweetpotato cultivar with high yield and root quality, these assumptions need to be validated. Further studies are needed to elucidate the role of each mutation and virus accumulation as they may have simultaneous effects which can be confounding. The stability of sweetpotato over generations of adventitious propagation needs to be considered in order to preserve the desirable characteristics initially developed by the breeder. Both virus and mutations may cause instability in sweetpotato yield and root quality. Determining how great an impact each mutation and virus contributes to decline may help breeders in cultivar selection such as developing cultivars more stable over adventitious propagations or by developing a tolerant or virus resistant cultivar. Continuous propagation of adventitious propagules kept free of virus-infection may accurately reflect decline due to accumulation of mutations over subsequent generations, but extreme isolation measures should be considered as sweetpotato may be reinfected with SPFMV by the end of the growing season, as observed in this study. Another approach would be to clarify the role

of SPFMV in decline by comparing micropropagated virus-indexed nodal cuttings of sweetpotato to those infected with SPFMV. Since nodal cuttings are not subject to somaclonal variation, differences observed would be due to virus-infection prior to planting, rather than accumulated mutations or in-season infection. By determining the effect of each mutation and virus accumulation, appropriate measures can be taken to prevent decline.

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**Table 1.** Description of location, planting and harvest date, and the number of days in the growing season for field trials conducted in each year.

Year	Site <sup>z</sup>	Planting Date	Harvest Date	No. of days <sup>y</sup>
1999	Clinton	23-Jun	29-Oct	98
	Columbus County	15-Jun	6-Oct	113
	Kinston (early)	20-May	24-Aug	96
	Kinston (late)	9-Jun	7-Oct	120
	Wilson County	22-Jun	14-Oct	114
2000	Columbus County	23-May	14-Sept	112
	Kinston	15-Jun	28-Sept	105
	Nash County	8-Jun	21-Sept	105
2001	Clinton	23-May	4-Sept	114
	Columbus County	12-Jun	4-Oct	114
	Kinston	6-Jun	20-Sept	106
	Wilson County	8-Jun	25-Sept	109

<sup>z</sup> Trials were conducted at the following sites: Cunningham Research Station, Kinston, NC; Horticultural Crops Research Station, Clinton, NC; and sweetpotato farms in Wilson County, Nash County, and Columbus County.

<sup>y</sup> Number of days indicates the length of the growing season for each trial.

**Table 2.** Statistical differences detected for yield and root quality measurements for each source of variation.<sup>z</sup>

Source of variation	Y i e l d <sup>y</sup>							R o o t Q u a l i t y <sup>x</sup>							
	Total	TMY	No.1s	Can	Jum	Culls	Percent No.1s	Flesh Color	Chim	Eyes	Lent	Pimples	Shape Uniformity	L/D Ratio	Overall Appearance
<b>1999</b>															
Site	***	***	****	***	****	***	***	***					ns	***	**
Rep(Site)	****	****	***	*	ns	**	**	*					ns	***	ns
Clone	ns	*	*	ns	ns	ns	*	ns					ns	**	ns
Generation	ns	ns	*	*	ns	*	*	ns					*	**	ns
Clone*Generation	ns	ns	ns	ns	ns	ns	ns	ns					ns	*	ns
Site*Clone	ns	ns	ns	ns	ns	ns	ns	ns					*	ns	***
Site*Generation	***	***	***	ns	***	ns	***	ns					**	**	***
Site*Clone* Generation	**	**	*	ns	*	ns	ns	ns					ns	ns	**
<b>2000</b>															
Site	ns	ns	***	****	***	ns	****	***	ns		***	****	*	****	ns
Rep(Site)	***	***	**	ns	ns	****	**	****	ns		****	****	ns	ns	*
Clone	ns	ns	ns	ns	*	*	ns	ns	ns		ns	ns	ns	*	ns
Generation	*	*	*	ns	ns	**	*	ns	ns		ns	ns	**	***	***
Clone*Generation	ns	ns	ns	ns	**	ns	ns	ns	ns		ns	ns	ns	ns	ns
Site*Clone	ns	ns	ns	**	ns	ns	ns	ns	ns		**	ns	**	ns	ns
Site*Generation	ns	ns	***	****	**	ns	**	ns	ns		*	ns	**	**	*
Site*Clone* Generation	ns	ns	ns	*	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns
<b>2001</b>															
Site	****	****	****	***	****	****	****	***	ns	****	****	****	***	***	****
Rep(Site)	***	**	**	*	ns	**	***	***	ns	****	****	***	***	ns	****
Clone	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
Generation	***	***	***	ns	ns	ns	***	*	ns	ns	ns	*	***	***	****
Clone*Generation	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
Site*Clone	ns	ns	**	ns	*	ns	ns	***	ns	ns	*	ns	ns	**	ns
Site*Generation	ns	ns	***	****	ns	*	***	ns	ns	ns	***	***	*	***	ns
Site*Clone* Generation	*	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns

(Continuation Table 2)

<sup>z</sup> A split-plot design analysis was conducted for each year where sites are treated as the whole plot factor and clones and generations treated as subplot factors using the general linear model procedure for 1999 and analysis of variance for 2000 and 2001 (SAS, 1998).

<sup>y</sup> Yield measurements were recorded and analyzed for the following: total = yield of all grades; TMY (total marketable yield)=No.1s + canners + jumbos; No.1s=roots with diameter of  $5 \text{ cm} \leq 9 \text{ cm}$  and length of  $7.5 \text{ cm} \leq 23 \text{ cm}$ ; can=canner roots with diameter of  $2.5 \text{ cm} \leq 5 \text{ cm}$ , jum=roots with diameter  $> 9 \text{ cm}$ , length  $> 23 \text{ cm}$ ; and culls=malformed or distorted roots. Percent No.1s means were analyzed as the percentage of No.1s relative to total yield.

<sup>x</sup> Root quality measurements were recorded and analyzed for the following: flesh color for 10 randomly selected roots, sliced 1/3 from proximal end and rated on a 0 to 5 scale where 0=no carotene (white) and 5=high carotene (dark orange); chim=number of chimeras recorded for each sliced root; overall appearance and shape uniformity (0 to 5 scale) where 5 represents the best overall appearance and most uniform; negative epidermal characteristics of lent=lenticels (small pores (~2 mm) for gas exchange), pimples (brown to black protrusions (~1 mm diameter) on root epidermis), and eyes (vegetative buds) (0 to 5 scale) where 5 represents minimal presence of lenticels, pimples, and eyes, 3 represents roots where lenticels, pimples, or eyes covered half the area of the root, and 0 represents roots completely covered with each skin characteristics; and visual ratings for length diameter ratios (L/D) (1 to 5 scale) where each rating indicated the ratio of length to width for majority of No.1 roots in each plot. Measurements of chimeras, eyes, lenticels, and pimples were not recorded in 1999 and measurements of eyes were not recorded in 2000.

<sup>ns</sup>, \*, \*\*, \*\*\*, and \*\*\*\* are non-significant or significant at  $p \leq 0.1, 0.05, 0.01, \text{ or } 0.001$  respectively

**Table 3.** Yield and root quality measurements for each clone and respective generation in 1999, 2000, and 2001.<sup>z</sup>

Year	Source <sup>w</sup>	Y i e l d <sup>y</sup>							R o o t Q u a l i t y <sup>x</sup>		
		Total	TMY	No.1s		Culls		Percent No.1s	Shape Uniformity	L/D Ratio	Overall Appearance
1999	B94-14 G1	26.2 A <sup>v</sup> a **	25.4 A a **	16.2 A a **	0.7 A a *	62 A a *	3.0 A a *	2.5 A a **	2.9 A a		
	B94-14 G2	23.1 A a	21.9 A a	14.2 A a	1.3 A b	59 A ab	2.8 A b	3.0 B b	2.7 A a		
	B94-14 G3	24.9 A a *	23.3 A a	14.3 A a	1.6 AB b	55 A b	2.9 A ab	3.0 B b	2.6 A a		
	B94-34 G1	25.4 A a *	24.3 A a *	15.5 A a *	1.1 A a	58 A a	3.0 A a **	2.8 A a *	2.9 A a		
	B94-34 G2	23.4 A a	21.9 A a	13.6 A ab	1.6 A b	57 A b	2.8 A b	3.0 A b	2.7 A a		
	B94-34 G3	21.9 A a	20.5 A a	11.7 A b	1.4 A ab	49 A c *	2.7 B b	3.3 B c	2.5 A a		
	NCSU 'Beauregard'	21.4	20.0	12.6	1.4	55	2.6	3.1	2.5		
2000	B94-14 G1	34.8 A a	33.8 A a	23.3 A a *	1.0 A a **	65 A a **	3.4 A a **	2.4 A a **	3.5 A a **		
	B94-14 G2	33.1 A ab	30.9 AB ab	20.5 A ab	2.3 B b	61 A a	2.9 A ab	3.0 B b **	2.6 B b		
	B94-14 G3	30.9 A b	28.8 B b	18.7 A b	2.0 B b	59 A a	2.9 A b	3.1 B bc	2.3 B b		
	B94-14 G4	32.7 A ab	30.7 AB ab	19.9 A b	2.0 B b	60 A a	2.9 A b	3.3 B c	2.3 B b		
	B94-34 G1	38.5 A a **	37.6 A a *	25.9 A a **	0.8 A a **	67 A a **	3.4 A a **	2.6 A a **	3.6 A a **		
	B94-34 G2	33.7 A ab	31.1 AB b	19.4 A b	2.6 B b	57 B b	2.6 B c	3.2 B b	2.1 C c		
	B94-34 G3	33.2 A ab	30.5 AB b	20.3 A ab	2.7 B b	60 AB ab	2.9 B b	3.1 B b	2.4 B b		
	B94-34 G4	31.4 A b	28.9 B b	18.2 A b	2.5 B b	57 B b	2.7 B bc	3.3 B b	2.3 BC bc		
NCSU 'Beauregard'	32.9	30.9	18.7	2.3	55	2.8	3.4	2.2			
2001	B94-14 G1	35.3 A a **	33.4 A a **	21.6 A a **	1.9 A a	61 A a **	3.3 A a **	2.7 A a **	3.4 A a **		
	B94-14 G2	30.9 B b	29.2 B b	16.1 BC b	1.7 A a	52 BC b	2.8 B b	3.2 B b	2.5 B c		
	B94-14 G3	29.8 B b	27.9 B b	16.2 AB b	1.9 A a	54 AB b	2.9 AB b	3.2 B b	2.7 B b		
	B94-14 G4	31.9 B ab	29.6 B b	16.3 BC bc	2.3 A a	46 BC bc	2.8 B b	3.3 B b	2.6 B bc		
	B94-14 G5	31.1 B b	29.0 B b	14.5 C c	2.2 A a	49 C c *	2.9 AB b	3.4 B b	2.5 B c		
	B94-34 G1	37.1 A a **	35.5 A a **	23.7 A a **	1.6 A a	65 A a **	3.6 A a **	2.7 A a **	3.4 A a **		
	B94-34 G2	31.0 B b	28.8 B b	16.1 B b	2.2 AB ab	52 B b	3.0 B b	3.2 B b	2.8 B b		
	B94-34 G3	31.7 B b	28.9 B b	15.9 B b	2.8 B c **	52 B b	2.8 B b	3.2 B b	2.6 BC bc		
	B94-34 G4	30.4 B b	27.9 B b	15.8 B b	2.5 B bc *	50 B b	2.9 B b	3.1 B b	2.5 BC c		
	B94-34 G5	30.0 B b	27.4 B b	14.5 B b	2.2 AB ab	48 B b	2.9 B b	3.3 B b	2.3 C c *		
NCSU 'Beauregard'	31.2	29.8	16.6	1.5	53	2.9	3.3	2.6			

(Continuation Table 3)

<sup>z</sup> Yield and root quality values are the means of five replications, 25 plants per plot averaged over sites in each year.

<sup>y</sup> Yield measurements are expressed in  $\text{kg}\cdot\text{ha}^{-1}$  for the following: total = yield of all grades; TMY (total marketable yield)=No.1s + canners + jumbos; No.1s=roots with diameter of  $5\text{ cm} \leq 9\text{ cm}$  and length of  $7.5\text{ cm} \leq 23\text{ cm}$ ; canner=roots with diameter of  $2.5\text{ cm} \leq 5\text{ cm}$ , jumbo=roots with diameter  $> 9\text{ cm}$ , length  $> 23\text{ cm}$ ; and culls=malformed or distorted roots. Percent No.1s means are expressed as the percentage of No.1s relative to total yield.

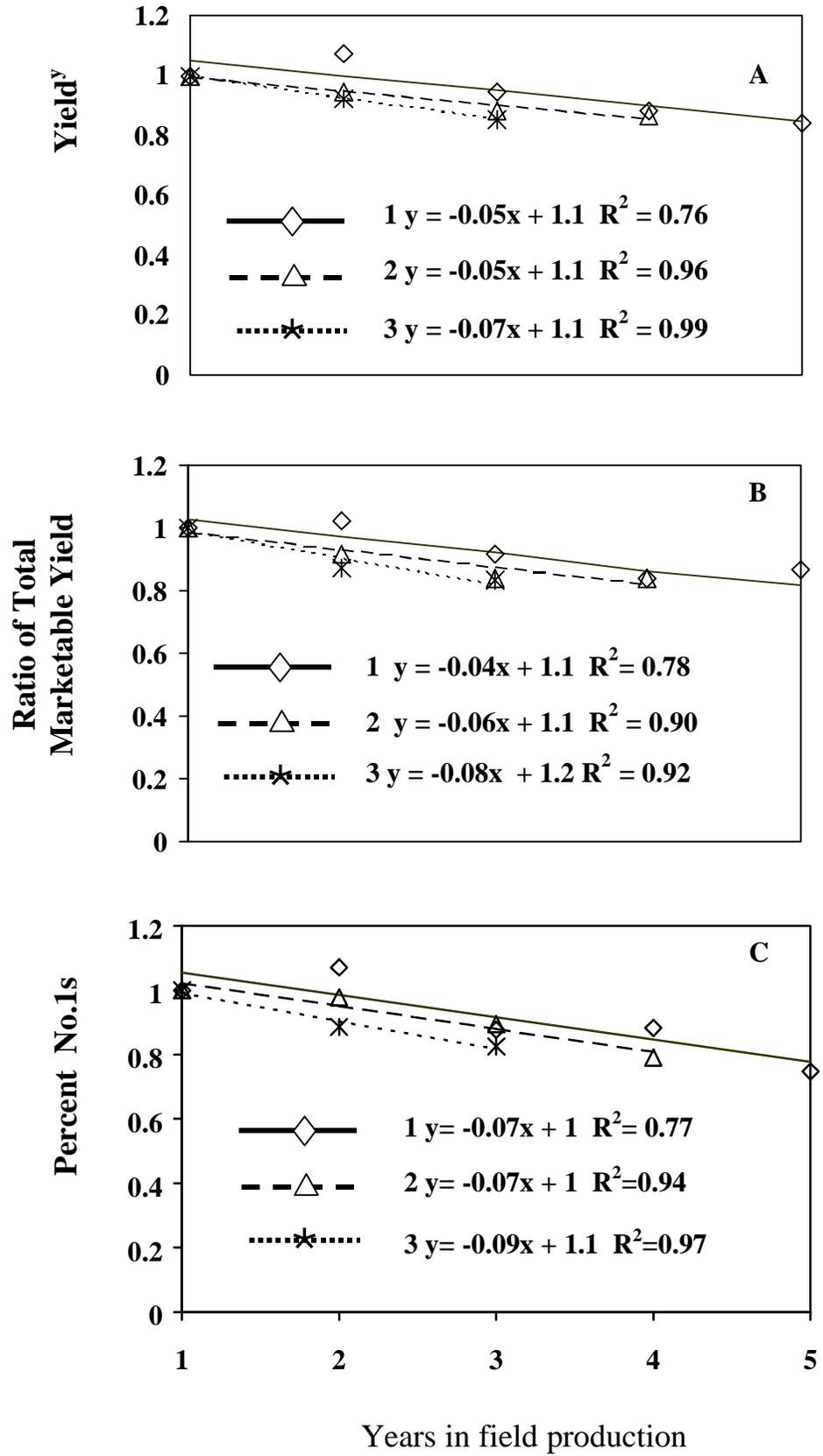
<sup>x</sup> Root quality measurements are expressed as the mean rating value for the following: overall appearance and shape uniformity (0 to 5 scale) where 5 represents the best overall appearance and most uniform and visual ratings for length/diameter ratios (L/D) (1 to 5 scale) where each rating indicated the ratio of length to width for majority of No.1 roots in each plot.

<sup>w</sup> Source indicates each mericlone, B94-14 and B94-34, of the cultivar 'Beauregard' and respective generation, G1, G2, G3, G4, and G5, (G represents generation while the number indicates the number of years in field production) or non-micropropagated NCSU 'Beauregard.'

<sup>v</sup> Mean separation within columns by Fishers Protected Least Significant Difference (LSD) at  $p \leq 0.05$  (uppercase letters) and  $p \leq 0.1$  (lowercase letters) comparing generations of each mericlone in each year. Columns with the same upper or lower case letter are not significantly different.

\* , \*\* Mean separation within columns by Fishers Protected LSD at  $p \leq 0.05$  and  $p \leq 0.1$ , respectively, comparing each clone and respective generation to NCSU 'Beauregard' in each year.

Fig. 1 Bryan



**Figure 1.** Predictive models for decline in yield of ‘Beauregard’ mericlones B94-14 and B94-34 over adventitious propagation cycles using three generational lines.<sup>z</sup>

<sup>z</sup> A linear regression analysis was conducted using the SAS Regression (REG) procedure (SAS, 1998). Yield measurements were averaged over mericlones and locations each year. Yield measurements of micropropagated, virus-tested, greenhouse produced, nodal cuttings “G1” (G represents generation while the number indicates the number of years in field production) of B94-14 and B94-34. The yield measurements of roots produced from the subsequent adventitious propagules (G2-G5) derived from the original G1 plant material were used in the analysis.

<sup>y</sup> The ratio is expressed as the mean yield measurements of the generations, G2-G5, to the yield measurements of the G1 grown the same year.

(A) Total yield=yield of all grades (No.1s + canners + jumbos + culls; No.1s=roots with diameter of  $5\text{ cm} \leq 9\text{ cm}$  and length of  $7.5\text{ cm} \leq 23\text{ cm}$ ; canner=roots with diameter of  $2.5\text{ cm} \leq 5\text{ cm}$ , jumbo=roots with diameter  $> 9\text{ cm}$ , length  $> 23\text{ cm}$ ; and culls=malformed or distorted roots

(B) Total marketable yield (TMY)= No.1s + canners + jumbos

(C) Percent No.1 yield=percentage of No.1 root yield relative to the total yield

(1) Generational line 1= yield measurements recorded for G1-G5 (1997-2001).

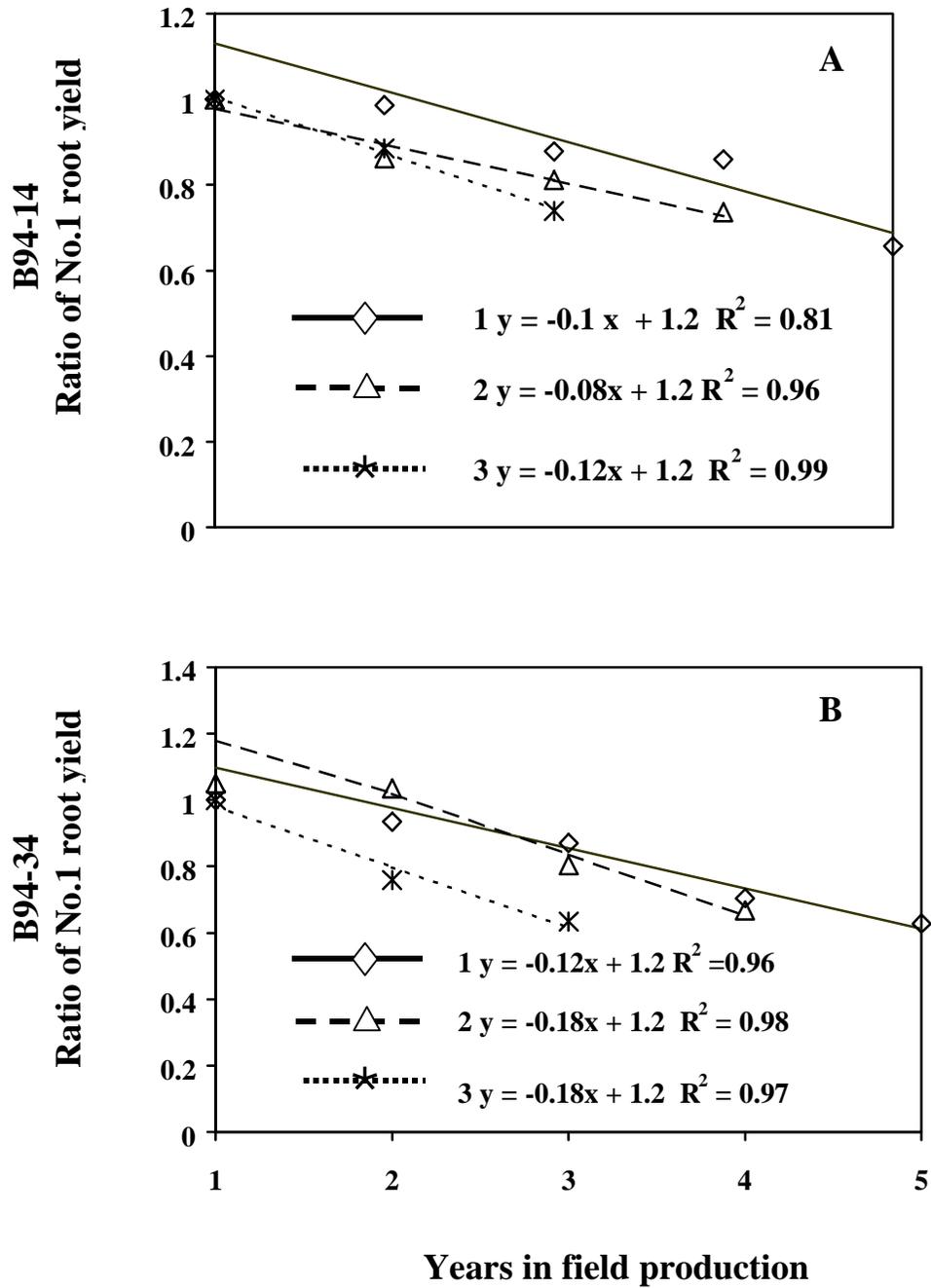
(2) Generational line 2=yield measurements recorded for G1-G4 (1998-2001).

(3) Generational line 3=yield measurements recorded for G1-G3 (1999-2001).

y= predicted yield expressed as the percentage of the yield of each subsequent generation, G2-G5, relative to the G1 yield of the same year

x=number of years in field production

Fig. 2 Bryan



**Figure 2.** Predictive models for decline in No.1 root yield of mericlones B94-14 and B94-34 over adventitious propagation cycles using three generational lines.<sup>z</sup>

<sup>z</sup> A linear regression analysis was conducted using the SAS Regression (REG) procedure (SAS, 1998). No. 1 root yields were averaged for each mericlone over locations. No.1 root yields of micropropagated, virus-tested, greenhouse produced, nodal cuttings “G1” (G represents generation while the number indicates the number of years in field production) of each B94-14 and B94-34 and the yield measurements of roots produced from the subsequent adventitious propagules (G2-G5) derived from the original G1 plant material of each mericlone were used in the analysis.

<sup>y</sup> The ratio is expressed as the mean root quality measurements of the generations, G2-G5, to the root quality measurements of the G1 grown the same year.

(A) Decline of No.1 root yield for B94-14

(B) Decline of No. 1 root yield for B94-34

(1) Generational line 1= No.1 root yield recorded for G1-G5 (1997-2001).

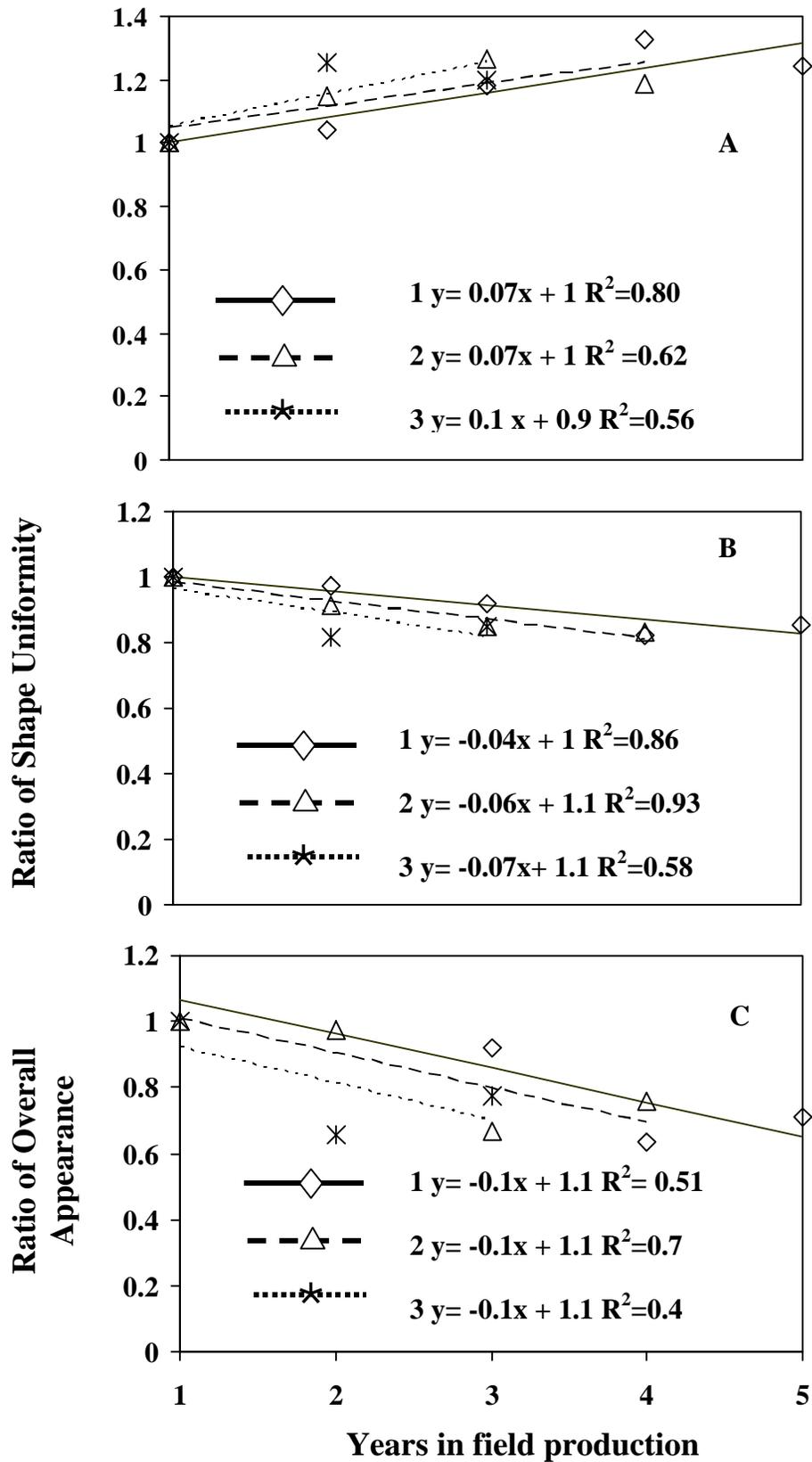
(2) Generational line 2=No.1 root yield recorded for G1-G4 (1998-2001).

(3) Generational line 3=No.1 root yield recorded for G1-G3 (1999-2001).

y=predicted No.1 root yield expressed as the percentage of the No.1 root yield of each subsequent generation, G2-G5, relative to the G1 No.1 root yield of the same year

x=number of years in field production

Fig. 3 Bryan



**Figure 3.** Predictive models for decline in root quality of ‘Beauregard’ mericlones B94-14 and B94-34 over adventitious propagation cycles using three generational lines.<sup>z</sup>

<sup>z</sup> A linear regression analysis was conducted using the SAS Regression (REG) procedure (SAS, 1998). Root quality measurements were averaged over mericlones and locations each year. Root quality measurements of micropropagated, virus-tested, greenhouse produced, nodal cuttings “G1” (G represents generation while the number indicates the number of years in field production) and the root quality measurements of roots produced from the subsequent adventitious propagules (G2-G5) derived from the original G1 plant material were used in the analysis.

<sup>y</sup> The ratio is expressed as the mean root quality measurements of the generations, G2-G5, to the root quality measurements of the G1 grown the same year.

(A) Length/diameter ratios (L/D) measured on a 1 to 5 scale where each rating indicated the ratio of length to width for majority of No.1 roots in each plot.

(B) Shape uniformity measured on a 0 to 5 scale where 5 is the most uniform.

(C) Overall appearance measured on 0 to 5 scale where 5 is the best overall appearance.

(1) Generational line 1= root quality measurements recorded for G1-G5 (1997-2001).

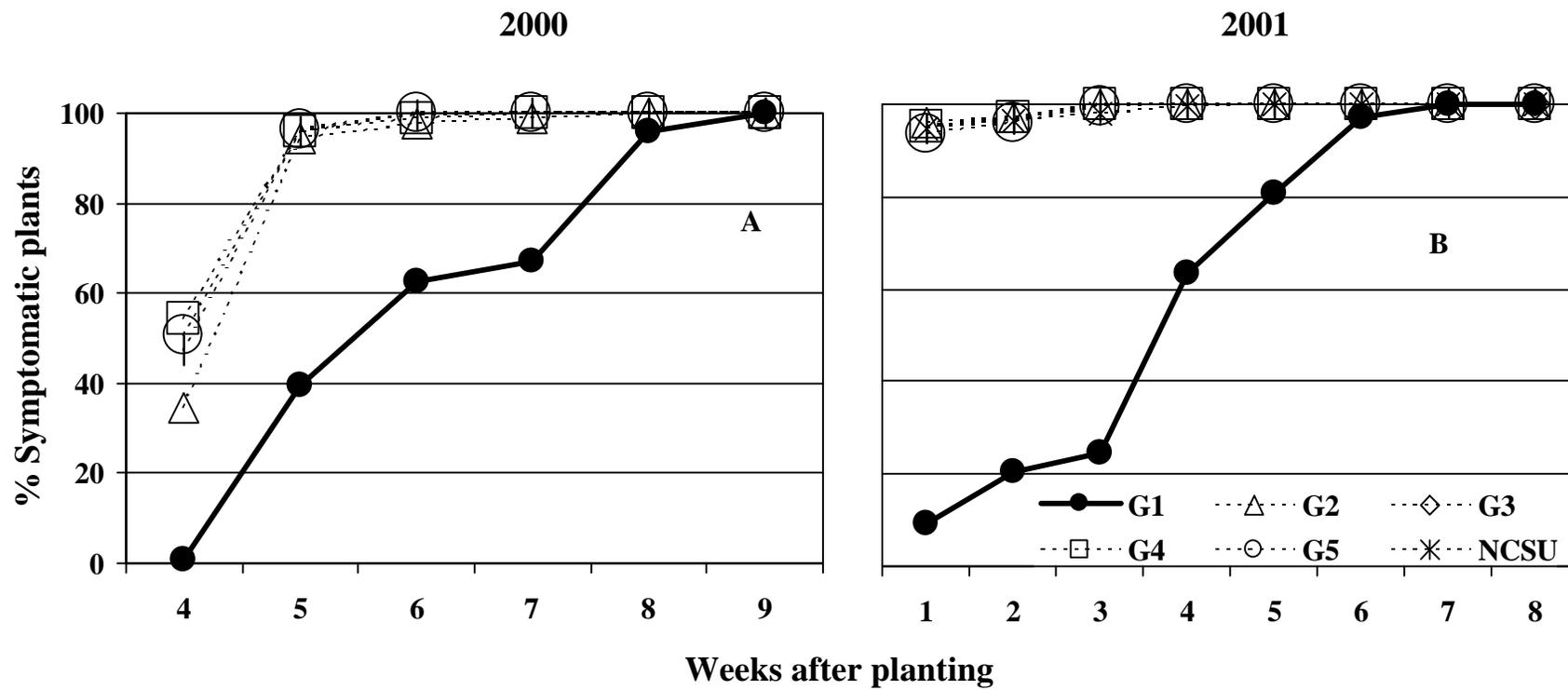
(2) Generational line 2=root quality measurements recorded for G1-G4 (1998-2001).

(3) Generational line 3=root quality measurements recorded for G1-G3 (1999-2001).

y=predicted root quality expressed as the percentage of the root quality measurement of each subsequent generation, G2-G5, relative to the G1 root quality measurement of the same year

x=number of years in field production

Fig. 4 Bryan



**Figure 4.** Weekly percentages of *Sweet potato feathery mottle virus* symptomatic plants in 2000 averaged over Nash County and Kinston trials (A) and in 2001 averaged over Wilson County and Kinston trials (B).

## CHAPTER 3

### *Sweet Potato Feathery Mottle Virus Decreases Yield and Root Quality in Sweetpotato*

**Abstract.** To determine the effects of *Sweet potato feathery mottle virus* (SPFMV) on yield and root quality, virus-indexed mericlones (VI-), which tested free of known viruses, were compared with virus-infected mericlones, in two separate tests with three mericlones each of the sweetpotato cultivars 'Beauregard' or 'Hernandez' in a two year study. Tests were arranged in a split plot design with the initial presence or absence (+/-) of SPFMV as the whole plot factor and mericlone as the subplot factor with 20 plants/plot in 2000 and 25 plants/plot in 2001. In both years, tests were monitored weekly for the symptom expression of SPFMV in VI+ plants and the rate of reinfection of VI- plants. Vine samples from three plants per plot were taken for virus-indexing on *Ipomoea setosa*. Additional testing for selected viruses was done using a nitrocellulose membrane enzyme linked immunosorbant assay. SPFMV was the only virus detected in the study. Monitoring indicated that 100% of VI- plants were reinfected with SPFMV by 9 weeks after planting. Despite reinfection rates, field tests indicated that the presence of SPFMV prior to planting (VI+) reduces yield of No.1 roots, the most valued grade, by 26% and also decreases overall appearance for 'Beauregard' mericlones. In addition, the presence of SPFMV prior to planting (VI+) increases length and reduces width of sweetpotato roots of both cultivars leading to an increase in length/diameter ratios which detracts from root quality and appearance. SPFMV presence prior to planting increased severity of russet crack symptoms, caused by a strain of SPFMV, which were only observed on VI+ roots of 'Beauregard' mericlones. The results from this study demonstrate that SPFMV contributes to cultivar decline in sweetpotato.

## Introduction

Decline in yield and root quality in sweetpotato has been attributed to the accumulation of mutations and viruses (Dangler, 1994; Villordon and La Bonte, 1995; Villordon and La Bonte, 1996). In the United States and other mild temperate climates where sweetpotato is produced, growers save a portion of “seed” roots from each crop. In early spring, the “seed” roots are planted in raised beds, and adventitious sprouts from bedded roots are pulled or cut and transplanted to the field to produce the sweetpotato crop for the following season. The disadvantage of this method of propagation is that mutations and viruses acquired by sweetpotato plants during the previous growing season will be present in adventitious sprouts produced from saved “seed” from those plants.

Planting materials derived from adventitious sprouting of sweetpotato storage roots have higher phenotypic variability than planting materials derived from nodal cuttings of sweetpotato, which may contribute to decreases in yield and root quality of sweetpotato (Templeton-Somers and Collins, 1986; Villordon and La Bonte, 1995; Villordon and La Bonte, 1996). Likewise, adventitious sprouts and nodal cuttings of sweetpotato infected with virus yield less than non-symptomatic sweetpotato and sweetpotato which were virus-indexed (tested free of known viruses). The viruses that have been found to reduce yield in sweetpotato include *Sweet potato feathery mottle virus* (SPFMV) (Olivero and Oropeza, 1985; Pozzer et al., 1995), *Sweet potato virus disease* (a synergistic interaction between SPFMV and *Sweet potato chlorotic stunt virus* (SPCSV)) (Mukiibi, 1977; Hahn, 1979; Ngeve, 1990; Ngeve and Bouwkamp, 1991), a combination of SPFMV and *Sweet potato sunken vein virus* (Milgram et al, 1996), and a combination of SPFMV and *Sweet potato latent virus* (Liao et al., 1983).

SPFMV is the most common virus infecting sweetpotato in the United States and is found nearly everywhere sweetpotatoes are grown (Clark and Moyer, 1988). SPFMV is non-persistently transmitted by aphids (*Myzus persicae*, *Aphis gossypii*). Foliar symptoms include veinal chlorosis and feathering, and chlorotic spots with purple borders that appear mostly on older leaves of sweetpotato. Root symptoms may include russet crack, internal cork, shape deformities, and surface discoloration depending on the cultivar and virus strain present (Moyer and Salazar, 1989). Cultivars released in the United States over the last 20 years appear to have a high level of tolerance to SPFMV strains found in the U.S. (Moyer and Salazar, 1989). However, decline in yield and root quality of sweetpotato is still observed. The cultivar ‘Beauregard’, released in 1987 by Louisiana State University (Rolston et al., 1987), is susceptible to the russet crack strain of SPFMV (SPFMV-RC) (Schultheis, 1994). The use of micropropagation, which eliminates viruses through meristem-tip culture, has been incorporated into several state sweetpotato foundation seed programs to produce high quality, true-to-type, virus-tested stock for growers (Dangler, 1994). The use of virus-tested stock has been found to reduce russet crack symptoms and provide planting material that yield as good as or better than non-micropropagated, SPFMV-infected plants that have been in field production for several years (Averre et al., 1993; Jester et al., 1994; Schultheis, 1994; Schultheis et al., 1994). Studies conducted to compare planting materials derived from micropropagated, virus-indexed mericlones that have been increased in the greenhouse via vegetative nodal cuttings versus “seed” that has been produced from adventitious sprouts of the same mericlones for five adventitious propagation cycles indicate that a significant decline in yield and root quality occur after the first adventitious propagation cycle and continues to decline gradually (Bryan, 2002). The

significant decline in yield and root quality after the first adventitious propagation cycle may be due to virus infection and/or the increased variability due to mutation accumulation in adventitious propagules. However, the relative importance of mutations and virus infection and their corresponding effects on yield and root quality remains unresolved.

This study was designed to elucidate the role that SPFMV infection plays in sweetpotato decline. Specifically, our objectives were to determine the effects of SPFMV on the yield and root quality of micropropagated sweetpotato. Because the micropropagated planting materials used for this study were derived from nodal cuttings of the same mericlones of the cultivars 'Beauregard' and 'Hernandez', differences in yield and root quality can be attributed to virus infection rather than effects of phenotypic variability due to propagules.

### **Materials and Methods**

Field trials comparing virus-indexed, micropropagated sweetpotato plants to micropropagated sweetpotato plants infected with SPFMV were conducted in 2000 and 2001.

*Plant material.* 'Beauregard' clones B94-14, B94-34, and B-73 and 'Hernandez' clones H98-7, H98-9, and USDA were used in the study. The B94-14 and B94-34 clones were derived from superior hill selections made in 1994 from the 'Beauregard' clone B73 which was received by the North Carolina Crop Improvement Association in 1993 from Dr. Christopher Clark, Plant Pathology Department, Louisiana State Univ. (LSU). The 'Hernandez' selections H98-7 and H98-9 were derived from superior hill selections made in a grower's field (Mr. Clay Strickland, Spring Hope, NC) 1998 from 'Hernandez' which was

released by LSU in 1992 (La Bonte et al., 1992) and had been maintained by the N.C. State Univ. (NCSU) Breeding program since its release, while the USDA ‘Hernandez’ was obtained from *in vitro* stocks submitted by LSU and maintained by the USDA-ARS Plant Genetic Resources Conservation Unit, Griffin, Georgia. The ‘Beauregard’ and ‘Hernandez’ clones were placed into the Micropropagation Program at NCSU in 1994 and 1997, respectively. Meristem-tip culture and virus-indexing (testing to determine if free of known viruses) was conducted according to the Food and Agriculture Organization of the United Nations and International Board for Plant Genetic Resources guidelines. The virus-tested, vegetative planting stock has been maintained in insect proof cages to avoid virus reinfection (Moyer et al., 1989).

***Inoculation.*** The SPFMV isolates used in the study were obtained from twelve infected ‘Beauregard’ sweetpotato plants sampled from the Sandhills Research Station, Jackson Springs, NC in 1998. The isolate was kept in the original infected ‘Beauregard’ plant in greenhouses at NCSU. The infected ‘Beauregard’ sweetpotato plants were virus-indexed on *Ipomoea setosa* and tested for SPFMV, *Sweet potato mild mottle virus* (SPMMV), *Sweet potato latent virus* (SPLV), *Sweet potato chlorotic fleck virus* (SPCFV), *Sweet potato mild speckling virus* (SPMSV), C-6 virus, SPCSV, and *Sweet potato caulimovirus* (SPCaLV) using a nitrocellulose membrane enzyme-linked immunosorbant assay (NCM-ELISA) kit provided by the International Potato Center (CIP) in Lima, Peru. Both virus-indexing and NCM-ELISA confirmed that only SPFMV was present. In March of 2000, 25 two node cuttings were obtained from the mother plants from each of the ‘Beauregard’ mericlones; B94-14, B94-34, and B-73, and ‘Hernandez’ mericlones; H98-7, H98-9, and USDA. The

cuttings were established in 200 cm<sup>3</sup> cells of a 25 cell tray, model IP200 IPL® Rigi-Pots™ (Stuewe & Sons, Inc., Corvallis, OR), containing moist Metro-Mix (The Scotts Co., Marysville, OH). Plants were grown in the greenhouse, 25°C-28°C, and watered as needed. When the plants were approximately 20 cm in length, each of the 25 plants of each mericlone was graft-inoculated with a 5 cm stem scion of the SPFMV-infected 'Beauregard' plant described earlier. A side-veneer graft procedure was used (Hartman et al., 1997). The incision for the scion was made approximately 2.5 cm from the stem base of the micropropagated mericlones and foliar symptoms were recorded 4 weeks after grafting (WAG).

***Greenhouse increase.*** Two node cuttings were taken from virus-indexed, micropropagated mother plants of each mericlone and from SPFMV graft-inoculated micropropagated mericlones and established in cells of IPL® Rigi-Pots™, as described previously, and filled with moist Metro-Mix. Each cut was made with a sterile single edge razor blade. Established cuttings were watered daily. When the cuttings formed roots and new shoots, they were fertilized with Miracle Gro 15-30-15 All Purpose water soluble fertilizer (The Scotts Co., Marysville, Ohio) biweekly and plant numbers were increased through repeated two node cuttings with the cutting being rooted in a cell of IPL® Rigi-Pots™. The virus-indexed micropropagated cuttings (VI-) of each mericlone tested negative for virus and were grown in screen cages to prevent the introduction of aphids and potential virus infection. The graft-inoculated, SPFMV-infected micropropagated cuttings (VI+) of each mericlone were kept in a separate greenhouse. Cuttings used for the greenhouse plant number increase of

both VI- and VI+ plants were taken from their respective VI- and VI+ mother plants of each mericlone for both years the study was conducted.

***Trial design.*** Field trials were arranged in a split plot design with SPFMV presence or absence (+/-) as the whole plot factor and mericlones as the subplot factor with five replications. The ‘Beauregard’ and ‘Hernandez’ trials were planted and analyzed separately for both 2000 and 2001. The trials were located at the Cunningham Research Station, Kinston, NC. The soil series and texture for each trial in both years was a Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiodults). The ‘Beauregard’ trial in 2000 was planted on 8 June and harvested on 28 Sept., 112 days after planting (DAP). The ‘Hernandez’ trial in 2000 was planted on 8 June and harvested on 16 Oct., 130 DAP. In 2001, both ‘Beauregard’ and ‘Hernandez’ trials were planted 21 June and harvested 9 October, 108 DAP. In 2000, plots were 5 m in length and rows were formed on 1.1 m centers. In 2001, plots were 6.1 m in length and rows were formed on 1.1 m centers. Each plot included 20 plants in 2000 and 25 plants in 2001, spaced 23 cm apart for ‘Beauregard’ mericlones and spaced 30 cm apart for ‘Hernandez’ mericlones. Border plants of the VI-sweetpotato cultivar ‘O’Henry’ were included at the end of plots at the same spacing. In 2000, border rows were included as a single row on each side of the ‘Beauregard’ and ‘Hernandez’ trials and as two rows between whole plots (+/-) within each trial. Plants for border rows were non-micropropagated NCSU ‘Beauregard’, maintained by the Sweetpotato Breeding and Genetics Program at NCSU and derived from the original ‘Beauregard.’ The plants for border rows were spaced 23 cm apart in the row. In 2001, border rows of VI-plants of ‘Beauregard’ and ‘Hernandez’ mericlones, spaced 23 cm and 30 cm apart

correspondingly. These cultivars were included as border row plants in the same manner as the 2000 trials for each respective trial of 'Beauregard' and 'Hernandez.' Each trial was planted by hand. The fertility regime included two cultivations at approximately 1 and 4 WAP. Fertilizer application of 30 kg·ha<sup>-1</sup> of P, 140 kg·ha<sup>-1</sup> of K, and 1.1 kg·ha<sup>-1</sup> of B was incorporated into the first cultivation and 52 kg·ha<sup>-1</sup> of N into the second cultivation. At harvest, sweetpotatoes were graded according to U.S. Dept. of Agriculture (U.S. Dept. of Agriculture, 1981) standards, which classify harvested root products into U.S. No.1 roots (diameter of 5 cm ≤ 9 cm and length of 7.5 cm ≤ 23 cm), canner roots (diameter of 2.5 cm ≤ 5 cm), jumbo roots (diameter > 9 cm, length > 23 cm), and cull roots (malformed or distorted roots). Yield measurements were recorded for each grade, including total marketable yield (TMY), which includes all grades except culls. The percentage of No.1 roots (percent No.1s) was calculated relative to the total yield for each plot. The length and diameter of each No.1 root was measured for each treatment and for comparison of length/diameter (L/D) ratios. Length of roots were measured to the nearest cm from the proximal to distal end of roots and included only 2.5 cm of ends less than 1 cm in diameter. Width was measured in cm at the midpoint of the length measurement. Evaluations of root quality of No.1 roots, including overall appearance and shape uniformity were measured for each treatment and recorded on a 0 to 5 scale to the nearest half unit where 5 represents the best overall appearance and most uniform. Evaluations of epidermal characteristics were measured for each plot including lenticels (small pores (2 mm) for gas exchange), pimples (brown to black protrusions (1 mm diameter) on root epidermis), and eyes (vegetative buds). The presence of lenticels, pimples, and eyes were recorded on a 0 to 5 scale to the nearest half unit where 5 represents minimal presence of lenticels, pimples, and eyes, 3 represents roots where lenticels, pimples,

or eyes covered half the area of the root, and 0 represents roots completely covered with a given skin characteristic. Ten No.1 roots were randomly selected from treatment plots for the evaluation of flesh color. Selected roots were sliced approximately one third the distance from the proximal end of roots and a score ranging from 0 to 5 to the nearest half unit for intensity of orange flesh. Scores of 0 indicate no carotene (white) and scores of 5 indicate very high carotene (dark orange), similar to the scale developed by Hernandez et al. (1965). True-to-type 'Beauregard' and 'Hernandez' flesh color rating, based on the scale developed by the NCSU Sweetpotato Breeding program, is scored as 3 and 3.5, respectively (Craig Yencho, personal communication).

***SPFMV monitoring.*** In both years, SPFMV foliar symptoms appeared on the graft-inoculated (VI+) plants from which cuttings were made, prior to planting. However, due to new growth of sweetpotato and dropping of older, SPFMV symptomatic leaves, (VI+) plants were not symptomatic at the time of planting in both 2000 and 2001. Monitoring of SPFMV symptom expression in VI+ plants and infection of VI- plants was conducted on a weekly basis, beginning 4 weeks after planting (WAP) for the 'Beauregard' and 'Hernandez' tests in 2000 and 1 WAP for the 'Beauregard' and 'Hernandez' tests in 2001. Weekly observations of the number of SPFMV symptomatic plants were recorded for each plot. Prior to harvest, symptomatic vines (approximately 30 cm in length) of three randomly selected plants were chosen for each plot (15 plants total per treatment) and grafted onto the indicator plant, *Ipomoea setosa*, to test for the presence of viruses. Indicator plants were tested for SPFMV, SPMNV, SPLV, SPCFV, SPMSV, C-6 virus, SPCSV, and SPCaLV using a NCM-ELISA

kit provided by the International Potato Center (CIP) in Peru. The presence of each virus was recorded for membranes showing some purple color, as recommended by the kit.

***Russet crack.*** In 2001, only russet crack symptoms were detected on harvested ‘Beauregard’ mericlones. Roots of VI+ and VI- treatments of ‘Beauregard’ mericlones were analyzed for russet crack symptoms and the discoloration of root skin associated with SPFMV-RC infection of roots. Symptoms of russet crack were separated into three categories: 1) discoloration; 2) mild russet crack (annular necrotic lesions, not detracting from epidermal appearance); 3) severe russet crack (annular necrotic lesions, detracting from epidermal appearance). Russet crack symptoms were not apparent on ‘Beauregard’ mericlones in 2000 or ‘Hernandez’ mericlones in 2000 and 2001.

***Statistical analysis.*** For each year, yields of graded roots, measurements of root quality, L/D ratios of roots, the number of weekly SPFMV symptomatic plants, and the number of russet crack symptomatic roots were recorded and analyzed for each test using the SAS’s General Linear Models procedure (SAS Institute, 1998). Mean separation was conducted using Fisher’s Protected Least Significant Difference (LSD) test for yield and root quality measurements comparing VI- and VI+ plants between each mericlone and VI- and VI+ plants averaged over all mericlones for each cultivar. Mean separation for L/D ratios of VI- and VI+ plants of each mericlone was conducted using tests for differences between Least Squares Means (LSMEANS).

## Results

**2000.** VI- plants, when averaged for all 'Beauregard' mericlones produced higher yield of No.1s and percent No.1s ( $p=0.05$ ) and TMY ( $p=0.1$ ) and fewer culls ( $p=0.1$ ) and jumbos ( $p=0.05$ ) than VI+ plants (Table 1). Yield of No.1 roots and percent No.1 roots produced by micropropagated VI- plants of 'Beauregard' compared with VI+ plants were reduced by 26% and 24% respectively. Jumbo and cull yield increased by 119% and 90%, respectively, in 'Beauregard' mericlones infected with SPFMV (VI+) than when mericlones were not infected (VI-) prior to planting. 'Beauregard' VI- plants produced roots with better overall appearance ratings than VI+ plants ( $p=0.05$ ). Differences or trends in yield and root quality attributes between VI- and VI+ plants for each mericlone were generally consistent with those found between VI- and VI+ plants when averaged over all 'Beauregard' mericlones. For instance, VI- plants from B94-34 and B94-14 produced higher yields of No.1 roots than B94-34 VI+ plants ( $p=0.05$ ) and B94-14 VI+ plants ( $p=0.1$ ) while the same trend was numerically observed, but not statistically different, between VI- and VI+ plants of B73. Similarly, differences in yield of culls were generally consistent between VI- and VI+ plants for each 'Beauregard' mericlone. VI- plants of B94-14 and B73 produced fewer culls than VI+ plants, while VI- and VI+ plants of B94-34 produced a similar amount of culls. VI+ roots of B73 had lower shape uniformity ratings than VI- roots while this difference was consistent, but not detected statistically, between VI- and VI+ roots of B94-14 and B94-34 and when averaged for VI- and VI+ ratings over all mericlones of 'Beauregard.' For diameter and L/D ratio measurements, differences were found in all three mericlones with VI- roots being wider in diameter and lower in L/D ratios than VI+ roots (Table 3).

Additionally, B94-14 and B-73 VI- roots were longer than VI+ roots of each respective mericlone.

In the 'Hernandez' test, when averaged for the three mericlones, the VI- plants produced higher yields of No.1 and percent No.1 roots ( $p=0.05$ ) and fewer canner roots ( $p=0.05$ ) than VI+ plants (Table 1). The presence of SPFMV reduced yield of No.1 and percent No.1 roots of 'Hernandez' mericlones by 22% and 20%, respectively. Canner yield was reduced by 98% when SPFMV was not present in transplants (VI-). Yield and root quality attributes were generally consistent with means of VI- and VI+ plants when the same comparisons were made for each mericlone. Length, diameter, and L/D ratios were significant ( $p=0.05$ ) for all 'Hernandez' mericlones with VI- roots shorter in length, wider in diameter, and lower in L/D ratios than VI+ roots (Table 3).

Roots produced by VI- and VI+ plants of the 'Beauregard' mericlone B94-14 and 'Hernandez' mericlone USDA in a rep in 2000 are shown in Appendix Figures 1.4 and 1.5, respectively.

**2001.** When means of VI- plants for all 'Beauregard' mericlones were combined, they produced higher TMY, No.1 yield, and canner yield ( $p=0.1$ ) and percent No.1 roots ( $p=0.05$ ) and fewer jumbo yield ( $p=0.05$ ) than VI+ plants (Table 2). As in 2000, the virus infected transplants (VI+) reduced the yield of No.1 roots and percent No.1 roots. VI- plants also produced roots with better overall appearance than VI+ plants ( $p=0.05$ ). When the same comparisons were made for each mericlone, differences in yield and root quality attributes

were generally consistent, as observed for the 2000 'Beauregard' test. For root measurements, VI+ roots of mericlones B94-14 and B94-34 were longer than VI- roots of the same mericlone respectively (Table 3). The L/D ratio of B94-14 was higher in VI+ roots than in VI- roots.

When averaged for all 'Hernandez' mericlones, VI- plants produced higher jumbo yields ( $p=0.1$ ) than VI+ plants. No other differences in yield and root quality were detected between VI- and VI+ plants when averaged for all mericlones. Few differences were detected between VI- and VI+ plants within a given mericlone for many of the root yield grades or qualities measured. Length diameter ratios of all mericlones were smaller in VI- roots than VI+ roots (Table 3). All roots from VI+ plants had smaller diameters than roots obtained from VI- plants. The USDA mericlone was the only clone to have shorter roots from VI- plants versus VI+ plants.

In 2000 and 2001, no differences were found between VI- and VI+ plant sources in both 'Beauregard' and 'Hernandez' mericlones for negative epidermal characteristics (lenticels, eyes, and pimples), flesh color, and chimeras (data not presented).

***SPFMV monitoring.*** In the 2000 'Beauregard' test, the percentage of SPFMV symptomatic plants derived from VI+ mericlones 4 WAP was approximately 16%, while all VI- 'Beauregard' mericlones remained symptomless (Fig. 1A). The additional percentage of SPFMV symptomatic plants for both VI- and VI+ becoming symptomatic from 4 to 5 WAP was approximately 58%. By 6 WAP, the percentage of VI- and VI+ SPFMV symptomatic

plants reached almost 85%. The increase in SPFMV symptomatic plants for both VI- and VI+ increased at the same rate from 7 to 10 WAP.

In the 2000 'Hernandez test, SPFMV symptomatic plants were not apparent in either VI- or VI+ plants 4 WAP (Fig. 1B). SPFMV symptomatic plants were observed in both VI- and VI+ plants by 5 WAP and the percentage of SPFMV symptomatic plants increased at similar rates through 10 WAP. Slightly more VI+ plants had SPFMV symptoms at 5 WAP than VI- plants. One hundred percent of VI+ and VI- plants of 'Hernandez' became symptomatic by 10 WAP.

In the 2001 'Beauregard' test, SPFMV symptomatic plants were recorded in VI+ plants 1 WAP. By 2 WAP, the percentage of SPFMV symptomatic plants was over 50% while only 2% of VI- plants displayed foliar symptoms of SPFMV (Fig. 1C). By 5 WAP, approximately 80% of VI+ plants were SPFMV symptomatic and the percentage gradually reached 100% by 9 WAP. The increase in percentage of VI- symptomatic plants was approximately 20% per week for 5, 6, and 7 WAP and reached 100% by 9 WAP.

In the 2001 'Hernandez' test, SPFMV symptoms were not found in VI+ plants until 2 WAP while SPFMV symptoms were not found in VI- plants until 4 WAP (Fig. 1D). Forty percent of VI+ plants displayed foliar symptoms of SPFMV during the first 4 WAP, while no symptoms were observed for VI- plants for the same time period. After 4 WAP, a steady increase in plants showing virus symptoms was observed for both VI- and VI+ plants. VI+

plants showed virus symptoms on approximately 10% more plants than VI- plants during weeks 6 and 7 with over 90% of plants with SPFMV symptoms 8 WAP.

For 'Beauregard' and 'Hernandez' tests in 2000 and 2001, NCM-ELISA confirmed the presence of SPFMV, while no other viruses were found by using NCM-ELISA.

***Russet crack.*** The severe storage root symptoms induced by the SPFMV-RC strain of SPFMV was found only on VI+ 'Beauregard' mericlones. The incidence of root symptoms were low and ranged from 0.4-1.6% based on total roots evaluated. Severe russet crack symptoms were found on all grades of VI+ roots with 0.4% and 0.8% symptoms observed on No.1 roots of B94-34 and B73, respectively. Occurrence of russet crack symptoms ranged between 0.4-0.6% for canners in all mericlones and was 0.2% for jumbo roots of B73. Mild symptoms of russet crack and discoloration associated with russet crack were found sporadically on roots from both VI- and VI+ mericlones, but incidence was also very low. Greater levels of mild symptoms were measured when roots were obtained from VI+ versus VI-. Incidence of mild symptoms of russet crack and discoloration ranged from 0.4-1.4% and 2.8-6.8% for VI- and VI+ roots, respectively. No statistical differences were found between VI- and VI+ mericlones in severe and mild russet crack symptoms and discoloration associated with russet crack symptoms due to the low incidence of russet crack.

## **Discussion**

Sweetpotato cultivar decline has been attributed to the accumulation of mutations and viruses (Villordon and La Bonte, 1996). Our studies suggest that sweetpotato nodal cuttings infected

with SPFMV and used for planting result in decreased TMY, yield of No.1s, and percent No.1s for 'Beauregard.' SPFMV-infected transplants also led to a decrease in overall appearance, for both cultivars, most notably in 'Beauregard.' Transplants infected with SPFMV consistently increases L/D ratios of roots, which has a negative effect on yield and root quality of both 'Beauregard' and 'Hernandez' sweetpotato.

The decrease in root diameter and decrease in root weight (yield) with initial presence of SPFMV is consistent with other reports comparing roots produced from SPFMV infected plants to cuttings derived from virus free cuttings derived from meristem-tip culture. For example, Kano and Nagata (1999) found the pith and vascular bundles of roots produced from virus-free cuttings (derived from meristem-tip culture) were larger than in roots produced from SPFMV-infected plants.

In our monitoring, we observed that VI+ plants began to display foliar symptoms of SPFMV as early as 1 WAP in 2001 and 4 WAP in 2000. It is likely that we could have observed SPFMV symptoms 1 WAP in the 2000 trials; however, we did not start monitoring the sweetpotato plants until 4 WAP in 2000. In contrast, VI- plants did not display foliar symptoms of SPFMV until 5 WAP although all VI- plants became infected with SPFMV by the end of the growing season in both 2000 and 2001. However, we have observed that storage roots of sweetpotato are being formed in sweetpotato very early in the season - as early as 3 WAP in some cases. Wilson and Lowe (1973) have also observed that root initiation occurs at 3 WAP. They reported that at 3 WAP, secondary vascular tissues are formed in the cambium resulting in a regular cylinder of cambium being formed, producing

uniform root thickening. We hypothesize that the initial presence of SPFMV in transplants may interfere with subsequent cambial activity and reduce root thickening resulting in smaller root diameters. In the present study, both yield and diameters of VI+ roots were consistently lower than VI- roots. Lower root diameter would result in higher L/D ratio which was also reported.

Overall appearance ratings were consistently lower in SPFMV-inoculated VI+ roots of 'Beauregard' mericlones. No statistical differences were detected in shape uniformity ratings between roots of VI- and VI+ plants averaged over 'Beauregard' mericlones at  $p=0.1$ . However, when averaged for all 'Beauregard' mericlones VI- plants produced roots with consistently higher shape uniformity ratings than VI+ plants in 2000 at  $p=0.18$  and 2001 at  $p=0.15$  (data not shown). Uneven length diameter ratios could contribute to lower overall appearance ratings. Plots with roots with consistently low L/D ratios, as produced by micropropagated VI- plants, appear more uniform and of high quality. VI+ roots of 'Beauregard' mericlones, were rated average (3) or below ( $<3$ ) for overall appearance and shape uniformity ratings while VI- roots were rated average (3) or above ( $>3$ ).

Severe russet crack symptoms also detract from root epidermal appearance and may render roots as unmarketable. Severe russet crack symptoms were only found in VI+ roots of 'Beauregard' mericlones. Why russet crack appeared in only those roots initially infected with SPFMV remains unclear. Kennedy and Moyer (1982) reported that the common strain of SPFMV and SPFMV-RC co-infects sweetpotato. It is possible that SPFMV-RC symptoms are expressed only when the titer of the SPFMV-RC virus is very high. The VI+

mericlones, infected prior to planting, may have higher virus titer compared with VI-mericlones, which, when tested, were free of viruses. Thus, when the storage roots were formed from VI+ plants, there may have been an increase in the severity of root symptoms caused by SPFMV-RC. Abad and Moyer (1992) have also observed that russet crack symptoms are more severe when SPFMV and SPFMV-RC co-infect sweetpotato than when sweetpotato is infected by SPFMV alone. Although the observation that SPFMV-inoculated VI+ plants developed more severe symptoms of russet crack than VI- plants, this explanation does not agree with previous observations by Campbell et al. (1974) in which the presence of a common strain of SPFMV was reported to offer cross-protection against SPFMV-RC.

The presence of SPFMV prior to planting (VI+) resulted in greater effects on yield and root quality in 'Beauregard' than 'Hernandez' sweetpotato. The results from our studies indicate that SPFMV reduces yield and root quality of 'Beauregard' mericlones and the effects were generally consistent in 2000 and 2001. The impact of SPFMV on yield and root quality of 'Hernandez' mericlones is less clear. No.1, canner, and percent No.1 root yield were negatively impacted by SPFMV in 2000 and not in 2001 (Table 1 and 2). In contrast only yield of jumbos were reduced by SPFMV presence in transplants in 2001 and not in 2000 (Table 1 and 2).

The variability in differences observed for 'Hernandez' mericlones in 2000 and 2001 may be due to environmental factors or may indicate that 'Hernandez' is less susceptible to SPFMV than 'Beauregard.' Different climates in 2000 and 2001 may have resulted in variable yield measurements between VI- and VI+ plants of 'Hernandez' mericlones as yield is influenced

by environment (Carpena et al., 1982; Collins et al., 1987; Bacusmo et al., 1988; Kannua and Floyd, 1988; Ngeve and Bouwkamp, 1993). In 2001, heavy rains caused excess water in soils in the 'Beauregard' test and most markedly in the 'Hernandez' test, where water was standing between rows at 4 and 7 WAP. In 2000, however, rainfall was better distributed and excess water in soils was not observed. Flooding has been reported to reduce the number, size, and diameter of sweetpotato roots (Li and Kao, 1985). In 2000, L/D ratios were higher in roots produced by SPFMV-inoculated VI+ plants than VI- plants in both 'Beauregard' and 'Hernandez.' The mean length of roots produced by both VI- and VI+ plants of each cultivar were at least 3 cm longer in 2000 than in 2001 (Table 3).

'Hernandez' mericlones may be less susceptible to SPFMV than 'Beauregard' mericlones. At least 50% of initial SPFMV symptoms on VI+ plants of 'Hernandez' were recorded at least one week later in 2000 and 3 weeks later in 2001 than those were recorded for VI+ plants of 'Beauregard' (Fig. 1). In addition, no russet crack symptoms, including the discoloration associated the SPFMV-RC strain, were observed on roots of 'Hernandez.'

Our NCM-ELISA assays confirmed the presence of only SPFMV in plants sampled from both 'Beauregard' and 'Hernandez' trials in 2000 and 2001. The presence of SPMMV, SPLV, SPCFV, SPMSV, C-6 virus, SPCSV, and SPCaLV were not confirmed by NCM-ELISA. Of approximately 20 viruses that infect sweetpotato, only seven (SPFMV, SPCSV, *Sweetpotato leaf curl virus* (SPLCV), *Ipomoea leaf curl virus* (ILCV), *Cucumber mosaic virus* (CMV), and *Tobacco mosaic virus* (TMV)) have been isolated from sweetpotato in the United States (Moyer and Kennedy, 1978; Cohen et al., 1988; Brunt et al., 1996; Pio-Ribeiro,

et al., 1996; Valverde and Clark, 1997; Lotrakul et al., 1998; Sim et al., 2000; Lotrakul et al., 2001). SPFMV is the only virus that has been reported to occur in commercial sweetpotato production fields in the United States. Samples from this study are currently being tested for the presence of SPLCV and IPLCV, which were detected in breeding lines from Louisiana and Georgia, respectively (Clark et al., 1997; Valverde and Clark, 1997; Lotrakul et al., 2001). Other viruses, including those not tested and those not yet characterized, may have been present in the current study. The source of SPFMV was obtained from foundation seed fields; therefore, any potential virus combinations or strains that likely were present in NC production areas were included in the study. After running a battery of assays, we found SPFMV to be the only virus present.

Most growers in the US use adventitious propagation methods to produce transplants for the next seasons crop (Dangler, 1994). If adventitious propagules used for transplanting were derived from roots of plants that had been infected with SPFMV in the previous growing season, a decline in yield and root quality can be expected when a susceptible cultivar such as 'Beauregard' is used. Growers who prefer adventitious propagation because of rapid increase of transplants will most likely need a cultivar that is resistant or tolerant to SPFMV, which may be the case for 'Hernandez.' However, in commercial sweetpotato production in the US, one cultivar usually dominates the industry. Presently, this cultivar is 'Beauregard' (Schultheis et al., 1999). Growers who choose to use 'Beauregard' will most likely benefit from micropropagated, virus-tested plants or adventitious propagules produced from "seed" roots of plants which have avoided virus reinfection. Production of certified "seed" roots (produced from plants not infected with SPFMV) will most likely be difficult given the rapid

rate of infection of SPFMV observed in this study. Certified "seed" production will require extreme measures such as isolation in order to produce "seed" for growers.

Viruses have been noted to contribute to cultivar decline in sweetpotato. In this study, SPFMV was observed to contribute to decrease in yield and root quality in both 'Beauregard' and 'Hernandez' cultivars, but more significantly with 'Beauregard.' A previous study comparing micropropagated, virus-indexed 'Beauregard' mericlones, B94-14 and B94-34, to the same mericlones adventitiously propagated over five field generations indicated that yield and root quality decreased with increased adventitious propagation (Bryan, 2002). Though this study indicated a gradual, but significant, decrease in yield and root quality over time, the presence of SPFMV in adventitious propagules may account for the larger, more significant decrease after the first adventitious propagation. Further studies are needed to determine whether continuous use of virus-infected sweetpotato planting material, whether derived from infected adventitious propagules or nodal cuttings of infected plants, allows virus titer to increase and if this also increases the effect of SPFMV and contribute to the severity of russet crack symptoms. Nevertheless, the development and use of cultivars resistant or tolerant to SPFMV and other viruses that contribute to decrease in yield and root quality will be needed to prevent or inhibit decline in sweetpotato.

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**Table 1.** Yield and root quality measurements for ‘Beauregard’ and ‘Hernandez’ mericlones in field trials in 2000 (Cunningham Research Station, Kinston, NC).<sup>z</sup>

Mericlone	SPFMV	Y i e l d							R o o t Q u a l i t y	
		Total <sup>x</sup>	TMY	No.1s	Canners	Jumbos	Culls	Percent No.1s	Shape Uniformity <sup>w</sup>	Overall Appearance
<b>‘Beauregard’</b>										
B94-14	VI-	28.0 A a <sup>v</sup>	26.1 A a	19.2 A a	5.0 A a	1.9 A a	2.0 A a	68.4 A a	3.4 A a	3.2 A a
	VI+	29.0 A a	22.0 A a	14.6 A b	3.8 A a	3.6 A a	7.0 B b	50.1 B b	2.6 A a	2.5 A b
B94-34	VI-	32.9 A a	29.8 A a	23.3 A a	5.2 A a	1.2 A a	3.0 A a	70.9 A a	3.5 A a	3.6 A a
	VI+	27.5 B b	26.0 A a	14.0 B b	8.4 A a	3.6 A a	1.5 A a	49.5 A b	3.4 A a	2.7 A a
B73	VI-	27.8 A a	26.9 A a	20.8 A a	4.4 A a	1.7 A a	0.9 A a	74.9 A a	3.6 A a	3.6 A a
	VI+	28.8 A a	25.9 A a	18.2 A a	4.5 A a	3.2 A a	2.9 A b	62.8 A b	3.2 B b	3.1 A b
Mean	VI-	29.6 A a	27.6 A a	21.1 A a	5.6 A a	1.6 A a	2.0 A a	71.4 A a	3.5 A a	3.5 A a
	VI+	28.4 A a	24.6 A b	15.6 B b	4.9 A a	3.5 B b	3.8 A b	54.2 B b	3.1 A a	2.8 B b
<b>‘Hernandez’</b>										
H98-7	VI-	30.4 A a	29.3 A a	21.2 A a	5.3 A a	2.8 A a	1.2 A a	69.6 A a	2.9 A a	2.8 A a
	VI+	26.0 A a	24.6 A a	14.8 A b	7.2 B b	2.6 A a	1.5 A a	54.7 A b	3.4 B b	2.8 A a
H98-9	VI-	29.6 A a	27.5 A a	18.8 A a	6.4 A a	2.3 A a	2.1 A a	63.7 A a	3.2 A a	3.2 A a
	VI+	29.2 A a	28.1 A a	17.1 A a	5.2 A a	5.8 B b	1.1 A a	57.2 A a	3.2 A a	3.0 A a
USDA	VI-	28.5 A a	27.9 A a	21.2 A a	6.0 A a	0.7 A a	0.6 A a	74.5 A a	3.2 A a	3.1 A a
	VI+	29.8 A a	28.9 A a	15.7 B b	11.9 B b	1.3 A a	0.9 A a	53.2 B b	3.3 A a	3.3 A a
Mean	VI-	29.5 A a	28.2 A a	20.4 A a	5.9 A a	1.9 A a	1.3 A a	69.3 A a	3.1 A a	3.0 A a
	VI+	28.4 A a	27.2 A a	15.9 B b	8.1 B b	3.2 A a	1.2 A a	55.0 B b	3.3 A a	3.0 A a

(Continuation Table 1)

<sup>z</sup> Yield and root quality values are the means of five replications, 20 plants per plot.

<sup>y</sup> SPFMV represents micropropagated sweetpotato infected with *Sweet potato feathery mottle virus* prior to planting by graft-inoculation with a field isolate (VI+) and micropropagated sweetpotato, virus-indexed and tested free of known viruses (VI-).

<sup>x</sup> Yield means are expressed in  $\text{kg}\cdot\text{ha}^{-1}$  for the following: total = yield of all grades; TMY (total marketable yield)=No.1s + canners + jumbos; No.1s=roots with diameter of  $5\text{ cm} \leq 9\text{ cm}$  and length of  $7.5\text{ cm} \leq 23\text{ cm}$ ; canner=roots with diameter of  $2.5\text{ cm} \leq 5\text{ cm}$ , jumbo=roots with diameter  $> 9\text{ cm}$ , length  $> 23\text{ cm}$ ; and culls=malformed or distorted roots. Percent No.1s means are expressed as the percentage of No.1s relative to total yield.

<sup>w</sup> Root quality means are expressed on a 0 to 5 scale where 5 is the best overall appearance and most uniform.

<sup>v</sup> Mean separation within columns by Fisher's Protected Least Significant Difference test at  $p \leq 0.1$  (lowercase letters) and  $p \leq 0.05$  (upper case letters) for comparisons between VI- and VI+ plants of each mericlone, and the mean of VI- and VI+ plants of all mericlones for 'Beauregard' and 'Hernandez.'

**Table 2.** Yield and root quality measurements for ‘Beauregard’ and ‘Hernandez’ mericlones in field trials in 2001, Cunningham Research Station, Kinston, NC.<sup>z</sup>

Mericlone	SPFMV	Y i e l d							R o o t Q u a l i t y	
		Total <sup>x</sup>	TMY	No.1s	Canners	Jumbos	Culls	Percent No.1s	Shape Uniformity <sup>w</sup>	Overall Appearance
<b>‘Beauregard’</b>										
B94-14	VI-	32.6 A a <sup>y</sup>	32.1 A a	19.5 A a	5.7 A a	7.0 A a	0.5 A a	59.7 A a	3.0 A a	3.1 A a
	VI+	26.2 A a	24.4 A a	15.2 A b	6.3 A a	2.9 A a	1.9 B b	57.7 A a	2.9 A a	2.6 A b
B94-34	VI-	32.1 A a	30.7 A a	22.4 A a	7.8 A a	0.5 A a	1.4 A a	69.3 A a	3.5 A a	3.3 A a
	VI+	27.8 A a	25.9 A a	16.1 A b	6.1 A a	3.7 A a	1.9 A a	56.4 A a	3.1 A b	2.4 A b
B73	VI-	29.0 A a	27.6 A a	19.0 A a	6.5 A a	2.1 A a	1.4 A a	65.2 A a	3.3 A a	3.6 A a
	VI+	26.2 A a	24.1 A a	13.6 A b	4.7 A a	5.8 A a	2.1 A b	52.2 B b	2.5 A b	2.0 B b
Mean	VI-	31.2 A a	30.2 A a	20.3 A a	6.7 A a	3.2 A a	1.1 A a	64.7 A a	3.3 A a	3.3 A a
	VI+	26.8 A a	24.8 A b	15.0 A b	6.7 A b	4.1 B b	2.0 A a	55.4 B b	2.8 A a	2.3 B b
<b>‘Hernandez’</b>										
H98-7	VI-	25.3 A a	28.1 A a	14.7 A a	5.3 A a	4.8 A a	0.5 A a	58.1 A a	2.9 A a	3.0 A a
	VI+	28.8 A a	24.8 A a	20.7 A a	5.2 A a	2.2 A b	0.7 A a	69.5 A a	2.6 A a	2.9 A a
H98-9	VI-	26.2 A a	24.9 A a	17.3 A a	5.2 A a	2.3 A a	1.3 A a	66.2 A a	3.7 A a	3.1 A a
	VI+	22.2 A a	20.8 A a	14.0 A b	4.3 A a	2.5 A a	1.4 A a	62.6 A a	2.9 B b	2.8 A a
USDA	VI-	24.2 A a	24.0 A a	14.3 A a	6.1 A a	3.6 A a	0.2 A a	58.0 A a	3.3 A a	2.9 A a
	VI+	24.3 A a	23.6 A a	14.6 A a	7.9 B b	1.2 B b	0.6 A b	58.3 A a	3.0 A a	2.7 A a
Mean	VI-	25.2 A a	24.6 A a	15.4 A a	5.5 A a	3.6 A a	0.7 A a	60.8 A a	3.3 A a	3.0 A a
	VI+	25.1 A a	24.2 A a	16.4 A a	5.8 A a	2.0 A b	0.9 A a	63.4 A a	2.8 A a	2.8 A a

(Continuation Table 2)

<sup>z</sup> Yield and root quality values are the means of five replications, 25 plants per plot.

<sup>y</sup> SPFMV represents micropropagated sweetpotato infected with *Sweet potato feathery mottle virus* prior to planting by graft-inoculation with a field isolate (VI+) and micropropagated sweetpotato, virus-indexed and tested free of known viruses (VI-).

<sup>x</sup> Yield means are expressed in  $\text{kg}\cdot\text{ha}^{-1}$  for the following: total = yield of all grades; TMY (total marketable yield)=No.1s + canners + jumbos; No.1s=roots with diameter of  $5\text{ cm} \leq 9\text{ cm}$  and length of  $7.5\text{ cm} \leq 23\text{ cm}$ ; canner=roots with diameter of  $2.5\text{ cm} \leq 5\text{ cm}$ , jumbo=roots with diameter  $> 9\text{ cm}$ , length  $> 23\text{ cm}$ ; and culls=malformed or distorted roots. Percent No.1s means are expressed as the percentage of No.1s relative to total yield.

<sup>w</sup> Root quality measurements are recorded on a 0 to 5 scale where 5 is the best overall appearance and most uniform.

<sup>v</sup> Mean separation within columns by Fisher's Protected Least Significant Difference test at  $p \leq 0.1$  (lowercase letters) and  $p \leq 0.05$  (upper case letters) for comparisons between VI- and VI+ plants of each mericlone, and the mean of VI- and VI+ plants of all mericlones for 'Beauregard' and 'Hernandez.'

**Table 3.** Root measurements of length, width of diameter, and length/diameter (L/D) ratios for ‘Beauregard’ and ‘Hernandez’ mericlones in 2000 and 2001 tests.

Mericlone	SPFMV <sup>y</sup>	2 0 0 0 <sup>z</sup>			2 0 0 1		
		Length <sup>x</sup>	Width	L/D	Length	Width	L/D
B94-14	VI -	15.7	6.0	2.7	12.0	6.3	1.9
	VI+	16.8 ** <sup>w</sup>	5.4 ***	3.2 ***	13.5 *	6.3 ns	2.2 *
B94-34	VI -	16.9	5.9	2.9	12.5	5.8	2.2
	VI+	17.3 ns	5.0 ***	3.5 **	13.7 ***	5.9 ns	2.3 ns
B-73	VI -	15.5	5.7	2.8	12.3	6.0	2.1
	VI+	16.7 ***	5.3 **	3.2 ***	12.9 ns	6.3 ns	2.1 ns
H98-7	VI -	18.1	5.6	3.3	12.4	6.7	1.9
	VI+	19.4 ***	5.3 ***	3.7 ***	12.7 ns	6.3 **	2.1 **
H98-9	VI -	18.3	5.9	3.2	12.4	6.7	1.9
	VI+	19.2 **	5.6 **	3.5 ***	17.1 ns	6.4 **	2.7 ns
USDA	VI -	16.3	6.0	2.8	11.4	6.8	1.7
	VI+	18.5 ***	5.1 ***	3.7 ***	12.4 ***	6.3 ***	2.0 ***

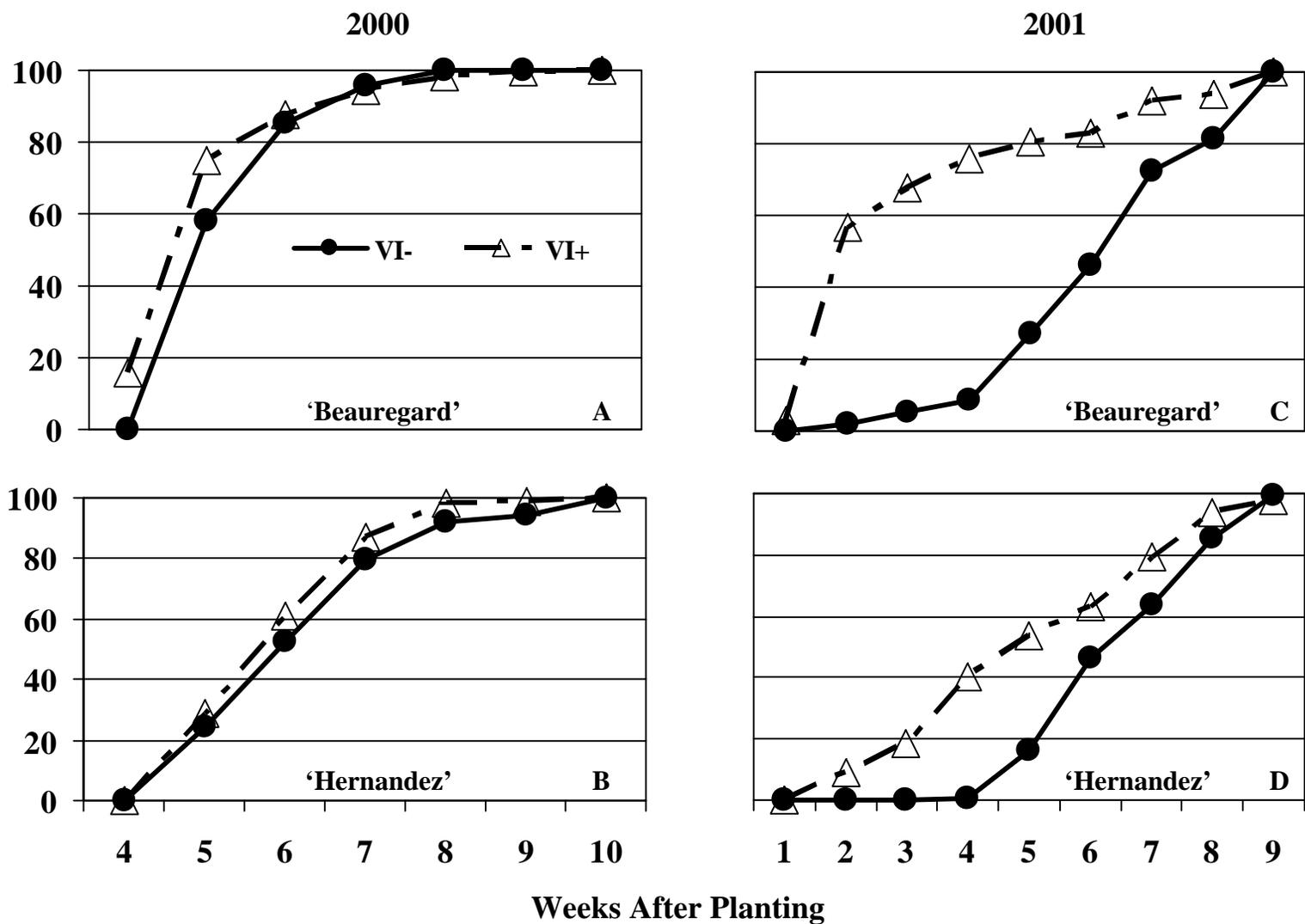
<sup>z</sup> Root measurement means for 5 replications 20 plants per plot (2000) and 25 plants per plot (2001).

<sup>y</sup> SPFMV represents micropropagated sweetpotato infected with *Sweet potato feathery mottle virus* prior to planting by graft-inoculation with a field isolate (VI+) and micropropagated sweetpotato, virus-indexed and tested free of known viruses (VI-).

<sup>x</sup> Length of roots were measured to the nearest cm from the proximal to distal end of roots and included only 2.5 cm of ends less than 1 cm in diameter. Width was measured in cm at the midpoint of the length measurement. L/D ratio is the length divided by diameter.

<sup>w</sup> Mean separation by least significant means test comparing VI- and VI+ plants of each mericlone. <sup>ns</sup>, \*, \*\*, and \*\*\* are non-significant or significant at  $p \leq 0.05$ , 0.01, or 0.001, respectively.

Fig. 1 Bryan



**Figure. 1.** Weekly percent sweetpotato feathery mottle virus (SPFMV) symptom expression in plants of ‘Beauregard’ and ‘Hernandez’ mericlones infected with SPFMV prior to planting (VI+) and mericlones, initially virus-indexed (VI-) and infected with SPFMV during the growing season for 2000 and 2001(Cunningham Research Station, Kinston, NC).

(A) and (B) Mean weekly percentages of ‘Beauregard’(A) and ‘Hernandez’ (B) mericlones of each VI- and VI+ plants displaying SPFMV symptoms during the growing season in 2000 beginning 4 weeks after planting (WAP). Percentages are means of 5 replications 20 plants per plot.

(C) and (D) Mean weekly percentages of ‘Beauregard’ (C) and ‘Hernandez’ (D) mericlones of each VI- and VI+ plants displaying SPFMV symptoms during the growing season in 2001 beginning 1 WAP. Percentages are means of 5 replications 25 plants per plot.

## CHAPTER 4

### **Reinfection Rate of *Sweet Potato Feathery Mottle Virus* in Micropropagated, Virus-Indexed Sweetpotato**

**Abstract.** The reinfection rate of *Sweet potato feathery mottle virus* (SPFMV) was monitored for two types of field trials: 1) comparing micropropagated, virus-indexed G1 plants of two 'Beauregard' mericlones to four generations of their adventitious propagules (G2-G5) and 2) comparing micropropagated, virus-indexed (VI-) plants to those graft-inoculated with SPFMV (VI+). Weekly observations of the number of SPFMV symptomatic plants were recorded for each plant per plot beginning 4 weeks after planting (WAP) in 2000 and 1 WAP 2001. Aphid traps were placed in all trials 1 WAP and weekly aphid counts per trap were recorded for 2000 and 2001. The results from monitoring indicated that 100 % of virus-indexed, micropropagated mericlones became infected by the end of the growing season, as early as 5 WAP or as late 10 WAP. In both years, the percentage of plants displaying symptoms the first week of monitoring was higher for VI+ plants, G2-G5 plants and NCSU 'Beauregard' than in those for VI- and G1 plants. The reinfection rate of micropropagated, virus-indexed mericlones (VI- and G1) in all trials were higher in 2000 than in 2001 which may correlate with higher number of aphids recorded per trap in 2000 than in 2001.

## **Introduction**

*Sweet potato feathery mottle virus* (SPFMV), non-persistently transmitted by aphids *Myzus persicae*, *Aphis gossypii*, and *Aphis apii* (Stubbs and McLean, 1958), infects sweetpotato worldwide and is the most common virus of sweetpotato in the United States (Clark and Moyer, 1988). The most distinctive symptom of the virus for all SPFMV strains present in the US is chlorotic feathering of the leaf midrib, and in some genotypes, the expression of chlorotic spots with purple rings (Moyer and Salazar, 1989). Root symptoms may include russet crack, internal cork, shape deformities, and surface discoloration depending on the cultivar and virus strain present (Moyer and Salazar, 1989). The strains of SPFMV which occur in North Carolina are the common strain (SPFMV-C) and the mild and severe russet crack strains (SPFMV-MRC and SPFMV-SRC, respectively) (Cali and Moyer, 1981).

The success of high quality sweetpotato production in NC and other areas relies heavily on the implementation of certified seed programs, which provide growers with true-to-type planting material free from known diseases. Sweetpotato is asexually propagated by roots and/or stems. In commercial production in the US, growers save “seed” roots from each crop and plant them in beds in early spring for the production of adventitious sprouts. The sprouts produced are cut and used as planting material for the next season. However, the adventitious propagation method used by growers allows for the accumulation of viruses and mutations in planting materials.

The current predominant cultivar in commercial sweetpotato production in the US is ‘Beauregard’ (Schultheis et al., 1999). This cultivar is high yielding, resistant to a number of

important diseases and attractive, but it is susceptible to SPFMV (Schultheis, 1994). Russet crack root symptoms have been reported to be as high as 23% in some fields of 'Beauregard' in NC (Schultheis et al., 1994). The use of sweetpotato planting material that is free of known viruses is recommended as a method of controlling, preventing, or slowing virus accumulation in subsequent plantings (Clark and Moyer, 1988). Thus, sweetpotato certified seed programs in the US have incorporated micropropagation including meristem-tip culture procedures to eliminate known viruses in planting stock (Dangler, 1994). The meristemed clones (mericlones) are virus-indexed (tested for freedom of known viruses) using the indicator plant *Ipomoea setosa* Ker (Clark and Moyer, 1988). Virus-indexed mericlones can be rapidly multiplied for planting stock via greenhouse increase of nodal cuttings of mericlones.

Micropropagation programs have been used to improve sweetpotato crop production in California (Dangler, 1994), China (Feng, 2000), Louisiana and NC. In the NC foundation seed program, virus-indexed, micropropagated plants are used as the initial source of planting material (Bryan, 2002). Saved "seed" roots and adventitious propagules derived from saved "seed" produced from micropropagated plants are also certified for use by growers if produced in fields with limited virus infection for a limited number of generations.

The use of micropropagated, virus-indexed plants as the initial or stock planting material has been found to reduce russet crack symptoms as well as providing planting material that yields as good as or better than non-micropropagated, SPFMV-infected plants that had been in field production for several years (Averre et al., 1993; Jester et al., 1994; Schultheis, 1994;

Schultheis et al., 1994). However, adventitious propagation from saved “seed” of micropropagated, virus-indexed ‘Beauregard’ plants over several generations leads to a gradual decline in yield and root quality and the decline is largely due to the presence of SPFMV in the propagules prior to planting (Bryan, 2002). If adventitious propagation methods are used as in commercial production systems, keeping the planting stock free from virus reinfection is of the utmost importance in order to retain high yields and root quality. Because SPFMV and most other viruses of sweetpotato are vectored by aphids or other insects, virus reinfection can occur rapidly during the growing season if sources of local inoculum, including virus-infected sweetpotato plants and *Ipomoea spp.*, are present (Clark and Moyer, 1988). To date, no detailed studies have been conducted to examine incidence of SPFMV and aphid flight activity in the United States.

The objectives for this study were to: 1) determine the reinfection rate of SPFMV in micropropagated, virus-indexed mericlones grown in field trials and saved "seed" used for production of adventitious propagules and 2) correlate reinfection rates with weekly aphid counts in the field trials.

## **Materials and Methods**

The SPFMV reinfection and aphid monitoring trials reported here were conducted as part of two larger experiments designed to determine the effect of SPFMV and mutations on sweetpotato yield and quality. Detailed descriptions of these studies are provided by Bryan (2002). In this paper, we report on aphid flight into these experimental plots and subsequent SPFMV incidence.

**Plant material.** Micropropagated ‘Beauregard’ clones B73, B94-14, B94-34 and ‘Hernandez’ clones H98-7, H98-9, and USDA were used in the study. The sweetpotato cultivar ‘Beauregard’ was released by LSU in 1987 (Rolston et al, 1987). B94-14, B94-34, and B94-75 were selected in 1994 as superior hill selections made from the ‘Beauregard’ clone B73 which was received by the North Carolina Crop Improvement Association in 1993 from Dr. Christopher Clark, Plant Pathology Department, Louisiana State University (LSU). A non-micropropagated ‘Beauregard’, maintained by the Sweetpotato Breeding and Genetics Program at North Carolina State University (NCSU) through yearly true-to-type root selections of “seed” derived from the original ‘Beauregard’ was included in the study for comparison.

The ‘Hernandez’ selections H98-7 and H98-9 were derived from superior hill selections made in a grower’s field (Mr. Clay Strickland, Spring Hope, NC) in 1998 from ‘Hernandez’ which was released by LSU in 1992 (LaBonte et al., 1992). The USDA ‘Hernandez’ was obtained from *in vitro* stocks submitted by LSU and maintained by the USDA-ARS Plant Genetic Resources Conservation Unit, Griffin, Georgia.

The ‘Beauregard’ and ‘Hernandez’ clones were placed into the Micropropagation Program at NCSU in 1994 and 1997, respectively.

**Experiment 1: Virus epidemiology in virus-indexed nodal cuttings propagated over generations.** In the larger study (Bryan 2002), micropropagated, virus-indexed, greenhouse-produced “G1” ‘Beauregard’ mericlone “seed” was compared with G2 to G5 “seed” derived

from adventitious propagules of different ages produced by successive field propagations (G represents generation while the number indicates the number of years “seed” was grown in the field), and non-micropropagated NCSU ‘Beauregard’ stock (G10+) in a 5-year study. The series of tests were initiated in 1997 to evaluate G1 plants of B94-14 and B94-34. Each year following, adventitious propagules derived from the 1997 B94-14 G1 and B94-34 G1 roots were compared to new nodal cuttings of B94-14 G1 and B94-34 G1. In addition, successive and cumulative “seed” generations of B94-14 and B94-34 were also included and the following comparisons were made each year: G1, G2, G3, and G4 in 2000; and G1, G2, G3, G4, and G5 in 2001. Additionally, field trials in each year included non-micropropagated NCSU Breeder stock of ‘Beauregard’ and grower stock of ‘Beauregard’ for comparison. In 2000 B73 G1 and B94-75 G1 plants were also included in field trials. In 2001, new nodal cuttings of B73 G1 and adventitious sprouts derived from B73 G1 roots were also included. The procedures for plant increase, planting, and cultural methods for B94-75 and B73 G1 and G2 were conducted in the same manner described for micropropagated G1 plants and adventitious propagules of B94-14 and B94-34 (Bryan, 2002).

## **Experiment 2: Virus epidemiology in virus-indexed and virus-infected mericlones**

Monitoring of field trials comparing virus-indexed, micropropagated sweetpotato ‘Beauregard’ clones B73, B94-14, and B94-34 and ‘Hernandez’ mericlones H98-7, H98-9, and USDA to micropropagated sweetpotato plants of each mericlone graft-inoculated with SPFMV prior to planting were also conducted in 2000 and 2001. The procedures for plant multiplication, planting, and cultural methods are provided by Bryan (2002).

***SPFMV monitoring.*** Experiments one and two were monitored for presence of SPFMV. In 2000, weekly observations began approximately four weeks after planting (WAP) for all trials. Due to the large percentage of symptomatic plants recorded at 4 WAP in 2000, monitoring was initiated 1 WAP in 2001 for the trials. Weekly observations of the number of SPFMV symptomatic plants were recorded for each plot in each year and subjected to an analysis of variance (ANOVA) using the SAS ANOVA procedure (SAS, 1998).

***Aphid monitoring.*** Aphid traps were included in all trials and weekly aphid counts per trap were recorded for 2000 and 2001. Traps were placed in each trial approximately 1 WAP and yellow sticky cards were changed weekly. The number of aphids per trap as observed through a stereoscope was counted and recorded for each week. Each trial comparing mericlones to their adventitious sprouts contained 5 traps placed at the four corners and center of the field trial. Each trap consisted of a pole (PVC pipe, 1.5 m in height) with 4 yellow sticky cards (9 cm X 3 cm) (IPM Tech, Portland, OR) attached at the top of the poles, with one of each facing north, south, east, and west. In 2000 and 2001, corner traps were placed approximately 9 m from the front or back edge of the trial and 3 m from the side of the trial. Center traps were placed approximately 17 m from the front of the trial and 6 m from the sides in 2000 and approximately 7 m from the sides in 2001. In 2000 and 2001, the ‘Beauregard’ and ‘Hernandez’ trials comparing VI- and VI+ plants were less than 5 m in proximity, thus 5 poles were placed among both trials. In 2000, two corner traps were placed on the northeast and southwest corners of the ‘Beauregard’ trial approximately 1.5 m from the side and 5 m from the front or back of the trial. In the 2000 ‘Hernandez’ trial, 2 corner traps were placed on the southwest and northwest corners of the trial 1.5 m from the side and

8 m from the front or back of the trial, respectively. The third trap in the 2000 ‘Hernandez’ trial was placed on the center of the east side of the trial approximately 3 m from the front and back of the trial and 1.5 m from the side.

In the 2001, two corner traps were placed approximately 1.5 m from the south side and 6m from the front or back side of the ‘Beauregard’ trial and 1.5 m from the north side and 8 m from the front or back side of the ‘Hernandez’ trial. A single center trap was placed approximately 1 m from each of the ‘Beauregard’ and ‘Hernandez’ trials.

## **Results**

### **Experiment 1**

*SPFMV and aphid monitoring.* At 4 WAP in the 2000 Kinston trial, the G1, G2, G3 and NCSU ‘Beauregard’ plants had a lower percentage of SPFMV symptomatic plants compared to the G4 plants (Fig. 1A). Approximately 20% of G4 and 8% of G2, G3, and NCSU ‘Beauregard’ were displaying symptoms of SPFMV at 4 WAP while G1 plants remained symptomless. By 5 WAP, G2-G4 and NCSU ‘Beauregard’ plants were 100% symptomatic and G1 plants were 70% symptomatic. The highest average numbers of aphids collected per trap were recorded at 4 and 5 WAP when the number averaged at 475 and 400, respectively (Fig. 1A). Preceding and succeeding these dates, numbers of aphids collected per week remained at 100 or below per trap.

In the 2000 Nash County trial, the percentage of symptomatic plants of G1 and G2 were less than those for G3, G4, and NCSU ‘Beauregard’ at 4 WAP (Fig. 1B). Approximately 60% of

G2 plants and 90% of G3, G4, and NCSU 'Beauregard' plants were symptomatic at 4 WAP while no symptoms were detected on G1 plants. At 5 WAP, 10% of G1 plants and 100% of G2-G5 and NCSU 'Beauregard' were displaying symptoms of SPFMV. The percentage of symptomatic plants of G1 gradually increased to 30% at 7 WAP to 90%, and 100% at 8 and 9 WAP, respectively. The highest average number of aphids collected per trap was 375 at 4 WAP (Fig. 1B). Following this date, the average number of aphids collected decreased to 150 aphids per trap and continued to decrease to less than 100 aphids per trap for the remainder of the weeks monitored.

For the first 4 WAP in the 2001 Kinston trial, the percentage of SPFMV symptomatic plants for G1 was lower compared to the G2-G5 and NCSU 'Beauregard' plants (Fig. 1C). One WAP, 18% of G1 plants and 95% of G2-G5 and NCSU 'Beauregard' plants were displaying symptoms of SPFMV. At 3 WAP, the percentage of SPFMV symptomatic plants of G2-G5 and NCSU 'Beauregard' had increased to 100%. At the same time, 20% of the the G1 plants were symptomatic. Within two weeks, at 5 WAP, the percentage of symptomatic plants for G1 had reached 100%. At 3 WAP, the average number of aphids collected per trap was 90 while the average number dropped to 30 aphids per trap one week later (Fig. 1C). At 6 and 7 WAP, the average number of aphids per trap increased to 100 and 110, respectively, and decreased to 50 aphids per trap or lower for the remainder of weeks monitored.

In the 2001 Wilson County trial, the percentage of symptomatic plants of G1 was lower than those for G2-G5 and NCSU 'Beauregard' until 6 WAP (Fig. 1D). By 4 WAP, percentage of

symptomatic G1 plants had increased to 40%. In contrast, roughly 100% of G2-G5 and NCSU 'Beauregard' were recorded to show SPFMV symptoms at 1 WAP while no symptomatic plants were recorded for G1 plants. By 5 WAP 60% symptomatic plants were recorded for G1 and this number increased by 35% to reach 95% one week later. By 7 WAP, 100% of G1 plants were displaying symptoms of SPFMV. Approximately 50 and 45 aphids per trap were collected at 3 and 4 WAP, respectively (Fig. 1D). The highest number of aphids per trap was 80, recorded at 8 WAP. By 10 WAP, the number decreased to 20 aphids per trap.

## **Experiment 2**

***SPFMV and aphid monitoring.*** In the 2000 'Beauregard' trial comparing VI- and VI+ plants, 18% of the VI+ plants displayed symptoms of SPFMV at 4 WAP while the VI- plants remained symptomless (Fig. 2A). By 5 WAP 60% of VI- plants were showing symptoms while 75% of VI+ plants displayed symptoms. By 8 WAP 100% of VI- and VI+ plants were symptomatic. In the 2000 'Hernandez' trial, symptomatic plants for VI- and VI+ were not detected until 5 WAP (Fig. 2B). And, 25% of VI- and 55% of VI+ plants displayed symptoms of SPFMV at this time. By 12 WAP, 100% of VI- and VI+ plants were showing symptoms of SPFMV. The highest numbers of aphids per trap were recorded at 4 and 5 WAP with 350 and 300 aphids collected each week, respectively (Fig. 2A-B). Preceding and following these weeks the numbers of aphids collected per trap were recorded to be 100 or less.

In the 2001 'Beauregard' trial comparing VI- and VI+ plants, the percentage of SPFMV symptomatic plants was lower in the VI- plants compared to the VI+ plants until 7 WAP (Fig.2C). Approximately 5% of VI- plants and 60% of VI+ plants were symptomatic at 2 WAP. The percentage of SPFMV symptomatic plants gradually increased for VI- to 30% and 75% by 5 and 7 WAP, respectively. By 9 WAP, 100% of VI- and VI+ plants were displaying symptoms of SPFMV. For the 2001 'Hernandez' trial, no symptoms were recorded on VI- plants until 5 WAP and the percentage of symptomatic plants remained lower than those recorded for VI+ until 7 WAP (Fig. 2D). The highest number of aphids per trap collected was 100, recorded at 3 and 4 WAP, and remained below this number for the remainder of the 11 weeks aphid collecting was conducted (Fig. 2C-D). At 5 WAP, the number of aphids per trap was 60 and then increased to 80 at 6 WAP. The number of aphids collected per trap decreased to below 20 by 11 WAP.

Though not quantified, the initial symptoms of SPFMV of VI+ plants, G2-G5, and NCSU 'Beauregard' plants were typically on older leaves and consisted of chlorotic spots with purple rings while the initial symptoms on VI- and G1 plants were located on leaves in the middle of the vine and typically included only veinal chlorosis. Symptomatic leaves of 'Hernandez' mericlones consisted of veinal chlorosis and chlorotic spots, while purple rings were not usually observed.

## **Discussion**

Our studies indicate that, if SPFMV inoculum is present and aphids are not controlled, virtually all of the virus-indexed, micropropagated "seed" planted in a sweetpotato field will

become infected with SPFMV by the end of the growing season. Further, the early detection of SPFMV symptomatic plants in the majority of the adventitious propagules G2-G5 of 'Beauregard' mericlones 1 WAP in 2001 suggests that the sprouts planted in our trials were derived from roots of previously infected plants. Thus, SPFMV is not only present in leaves, it is also translocated to the roots. In 2000, field trials were not monitored until 4 WAP; therefore it is likely that adventitious propagules of G2-G4, NCSU 'Beauregard', and grower 'Beauregard' could have displayed symptoms 1 WAP. As expected, in the trials comparing virus-indexed (VI-) mericlones to those that were graft-inoculated with SPFMV (VI+), the VI+ plants displayed symptoms much earlier than the VI- plants. Again, the 2000 trials were not monitored until 4 WAP so it is likely that the VI- and VI+ plants displayed symptoms of SPFMV as early as 1 WAP.

The expression of SPFMV symptoms in sweetpotato is complex. We have observed that VI+ plants in the greenhouse typically display symptoms of SPFMV in roughly three-week cycles. SPFMV symptoms have been observed on older foliage of infected sweetpotato plants for approximately 1 week followed by a period in which the plants remain symptomless for roughly three weeks, after which they recur. Plant age, light intensity, and temperature have been shown to affect symptom expression of SPFMV in sweetpotato (Stubbs and McLean, 1958; Alcerono, 1972; Mukiibi, 1977; Arrendell and Collins, 1986). In addition, older, symptomatic leaves of sweetpotato may drop and new growth may be symptomless (Alcerono, 1972). In our experiments, all the VI+ plants of 'Beauregard' and 'Hernandez' mericlones displayed symptoms in the greenhouse on the older foliage one week prior to cutting for transplanting to the field in 2000 and 2001. The cut transplants, however,

were mostly symptomless. In the field, any of the older leaves of transplants, which would have shown symptoms of SPFMV first, were covered during cultivation after transplanting thus, only new symptomless growth was observed above ground. These factors may have led to slower symptom expression of SPFMV in VI+ plant and possibly G2-G5, and NCSU 'Beauregard' plants that were most likely derived from roots of previously infected plants.

In both years, the percentage of plants displaying symptoms during the first week of monitoring was higher for G2-G5 and NCSU 'Beauregard' compared to the G1 plants (Fig. 1A-D). Also, the percentage of G1 plants displaying SPFMV symptoms in the 2000 and 2001 Kinston trials exceeded 80% two weeks prior to those in Nash County in 2000 and Wilson County in 2001 (Fig.1A-D). The higher infection rate of G1 plants at the Kinston research station location may be due to local sources of inoculum including other trials using virus-infected sweetpotato planting material. The trials located in Nash and Wilson County were located on farms that used micropropagated, virus-indexed planting stock, thus the local source of inoculum from infected sweetpotato was likely lower and may have delayed the reinfection rate of SPFMV.

Our studies also indicate that increased number of aphids into a field results in increased virus incidence when sources of SPFMV inoculum are present. The reinfection rate of SPFMV in the micropropagated, virus-indexed mericlones in all trials was higher in 2000 than in 2001, and it appears to be correlated with the number of aphids recorded per trap per week. This relation is obvious comparing the peak aphid populations and SPFMV incidence curves for each year. In 2000, the number of aphids per trap was higher compared to 2001

and virus incidence was greater. Further, in 2000 the greatest increase in the percentage of SPFMV infected plants occurred 1 week later in all trials in Kinston (Fig. 1A and 1C and Fig. 2A-B) and four weeks later in Nash County (Fig. 1B) compared with 2001. Contrasted with 2000, in 2001, aphid counts per trap were lower, with the highest number of aphids recorded to be 110 or less per trap per week for all trials. The lower aphid counts recorded in 2001 resulted in a slower rate of SPFMV reinfection compared to 2000.

In comparing the rate of SPFMV reinfection amongst the various planting materials, a slower rate of reinfection of VI- or G1 plants was observed for most trials, with the exception of the trial comparing G1-G5 mericlones and NCSU 'Beauregard' in Kinston where a 60% increase was observed in the percentage of SPFMV symptomatic plants of G1, 2 weeks after the largest peak in aphid counts. Planting date also undoubtedly affects the rate of SPFMV reinfection. For example, although the 'Beauregard' and 'Hernandez' VI-/VI+ trials were also located in Kinston, the large increase in the percentage of symptomatic plants 2 WAP for the 'Beauregard' generational trials was not observed. This trial was planted two weeks prior to the VI-/VI+ trials.

Genotype also appears to affect the expression of virus symptoms. The 'Beauregard' VI- and VI+ plants displayed symptoms earlier than those of 'Hernandez' (Fig. 2A-D). In 2001, 'Beauregard' VI- plants displayed symptoms 2 WAP while 100% of VI+ plants displayed symptoms at 5 WAP. In contrast, the VI- plants of 'Hernandez' displayed symptoms at 5 WAP while only 50% of VI+ plants of 'Hernandez' were displaying symptoms at the same time. In addition, 'Hernandez' mericlones typically only displayed SPFMV symptoms of

veinal chlorosis and chlorotic spots while 'Beauregard' displayed these symptoms as well as chlorotic spots with purple rings. Purple rings were not usually observed on 'Hernandez.' Other studies that we have conducted clearly show that yield and root quality measurements of 'Beauregard' and 'Hernandez' are negatively impacted by SPFMV, though the result vary dependent on timing of infection and plant genotype (Bryan, 2002).

In summary, these studies indicate that micropropagated virus-indexed mericlones become rapidly infected with SPFMV during the growing season. The rate of reinfection is influenced by aphid populations in field trials and possibly the amount of local inoculum. Further, the sprouts obtained from infected planting materials (roots and/or cuttings) may not show symptoms of SPFMV at the time of transplanting. This work may have significant implications for certified seed producers whose fields are inspected yearly for presence of SPFMV. This is because fields certified for production of G1 "seed" roots from initially micropropagated, virus-indexed plants (G1), must have less than a 1% infection of SPFMV. Fields of G2 plants, which are planted from adventitious sprouts of G1 roots, must have less than 5% SPFMV infection. In order to obtain such low infection rates, certified seed producers must take great care in isolating certified fields from SPFMV-infected commercial fields as well as roguing SPFMV-infected plants within the fields. If SPFMV-infected plants are not identified and rogued the first year, adventitious propagules from saved G1 "seed" used for transplanting may not show SPFMV symptoms until later in the season and thus provide a source of inoculum for aphid transmission and increase SPFMV incidence within the field. Certified seed producers would benefit from incorporating aphid monitoring programs in order to predict and thus reduce SPFMV in certified fields. In addition further

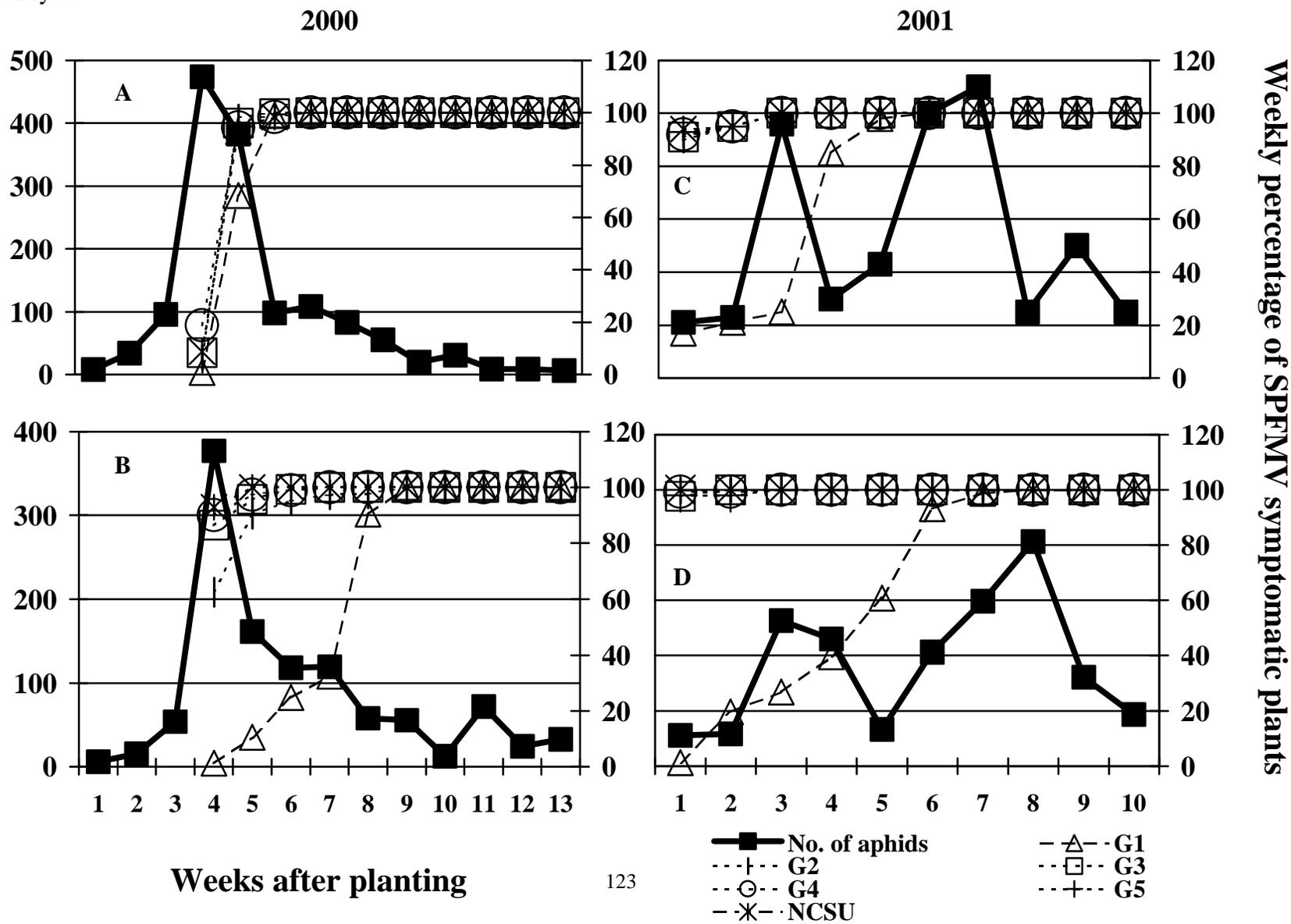
methods of detection may be needed to identify the presence of SPFMV within the field and also in saved “seed” roots of sweetpotato in order to produce clean planting stock for growers.

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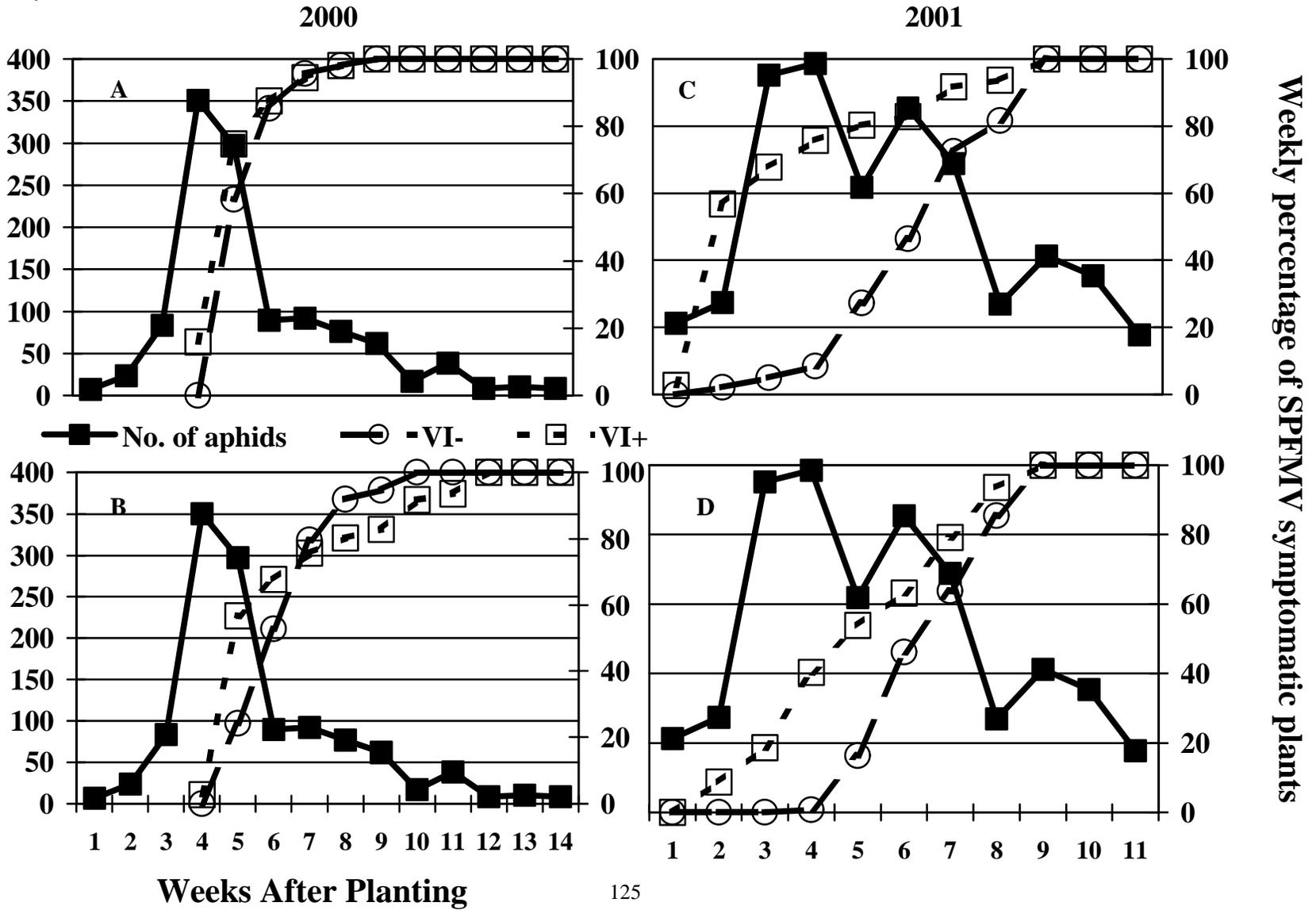
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Fig. 1 Bryan



**Figure 1.** Weekly aphid counts per trap (numbers on left of graph) and the weekly percentage of *Sweet potato feathery mottle virus* symptomatic plants (numbers on right of graph) in trials comparing micropropagated, virus-indexed, greenhouse produced G1 mericlones of 'Beauregard' to their adventitious propagules G2-G4 at the Cunningham Research Station (CRS), Kinston, NC (**A**) and commercial fields in Nash County (**B**) in 2000 and G2-G5 at the CRS in 2001 (**C**) and commercial fields in Wilson County (**D**) in 2001.

Fig. 2 Bryan



**Figure 2.** Weekly aphid counts per trap (numbers on left of graph) and the weekly percentage of *Sweet potato feathery mottle virus* symptomatic plants (numbers on right of graph) in trials at the Cunningham Research Station, Kinston, NC, comparing micropropagated, virus-indexed, greenhouse produced VI- mericlones of 'Beauregard' (**A** and **C** for 2000 and 2001, respectively) and 'Hernandez' (**B** and **D** for 2000 and 2001, respectively) to the same mericlones graft-inoculated with SPFMV prior to planting.

## CHAPTER 5

### **Virus Testing of Sweetpotato Field Samples Using the Indicator Plant *Ipomoea setosa* Ker and a Serological Assay (NCM-ELISA).**

**Abstract.** In order to verify the presence of *Sweet potato feathery mottle virus* (SPFMV) in field trials, one SPFMV symptomatic vine, approximately 30 cm in length, of a randomly selected plant was collected per plot of each trial at three sampling dates prior to harvest. In the 2000 Nash County trial, 1 root of a randomly selected micropropagated, virus-indexed G1 plant per plot initially displaying symptoms at 5, 6, 7, and 8 weeks after plantings were selected from each rep for two mericlones. Vine and adventitious sprouts of root samples were tested for viruses by grafting onto the indicator plant *Ipomoea setosa* Ker and using an enzyme linked immunosorbant assay (NCM-ELISA) to test for SPFMV, *Sweet potato mild mottle virus* (SPMMV), *Sweet potato latent virus* (SPLV), *Sweet potato chlorotic fleck virus* (SPCFV), *Sweet potato mild speckling virus* (SPMSV), C-6 virus, *Sweet potato chlorotic stunt virus* (SPCSV), and *Sweet potato caulimovirus* (SPCaLV) in selected samples. All field samples induced virus symptoms on *I. setosa* after grafting including: chlorotic vein feathering, leaf deformation, shoestringing, chlorotic spots, veinal chlorosis, cupping, crinkle, leaf curl, necrosis, rugosity, stunting, and leaf chlorosis. No differences were detected between symptoms of *I. setosa* grafted with micropropagated virus-indexed G1 plants, its adventitious propagules, G2-G5, and NCSU 'Beauregard' or the date of sampling. No differences were detected between symptoms of *I. setosa* grafted with samples of VI- and VI+ or the date of sampling. All samples tested using NCM-ELISA confirmed the presence of only SPFMV.

## Introduction

Indicator plants reliably detect viruses infecting sweetpotato. Stubbs and McLean first introduced *Ipomoea setosa* Ker as a valuable indexing host in 1958 in aphid transmission studies with *Sweet potato feathery mottle virus* (SPFMV). Since that time, *I. setosa* has been used for verifying the russet crack disease as caused by a strain of SPFMV (Daines and Martin, 1964; Campbell et al, 1974). Because *I. setosa* is susceptible to most known viruses of sweetpotato (Brunt et al, 1996), it has been used in virus-indexing systems following meristem-tip culture in order to verify that known viruses were eliminated (Alconero et al., 1975).

Other procedures have also been developed for detection of viruses in sweetpotato. An enzyme-linked immunosorbant assay (ELISA) was developed by Cadena-Hinojosa and Campbell (1981) that detected four strains of SPFMV. Esbenshade and Moyer (1982) also developed an ELISA assay and determined that 5-8 wk following sprouting from roots was a sufficient time to detect SPFMV in sweetpotato leaves. Additional methods for SPFMV detection include an in-vitro transcribed RNA probe (Abad and Moyer, 1992), and several reverse transcription-polymerase chain reaction (RT-PCR) procedures (Colinet and Kummert, 1993; Colinet et al., 1993; Nishiguchi et al, 1995; Colinet et al, 1998).

To date, twenty viruses are known to infect sweetpotato (Mwanga, 2001). The majority have not been sequenced and have not yet been completely characterized (NCBI, 2001). The antisera for SPFMV, *Sweet potato mild mottle virus* (SPMMV), *Sweet potato latent virus* (SPLV), *Sweet potato chlorotic fleck virus* (SPCFV), *Sweet potato mild speckling virus*

(SPMSV), C-6 virus, *Sweet potato chlorotic stunt virus* (SPCSV), and *Sweet potato caulimovirus* (SPCaLV) have been produced and are available in diagnostic kits with necessary reagents for nitrocellulose membrane enzyme-linked immunosorbant assays (NCM-ELISA) through the International Potato Center (CIP), Lima, Peru (Carey et al., 1999).

Previous field trials indicated that foliar symptoms of SPFMV could be detected on the sweetpotato cultivars 'Beauregard' and 'Hernandez' (Bryan, 2002). The objectives for this study were: 1) to verify the presence of SPFMV in field trials by randomly selecting plant samples and grafting them onto the indicator *I. setosa* and documenting the symptoms and 2) testing for SPFMV and additional viruses in the plant samples by using NCM-ELISA.

## **Materials and Methods**

***Study design and vine sampling.*** Plant cuttings were obtained from two experiments designed to determine the effect of SPFMV and mutations on sweetpotato yield and quality. Detailed descriptions of these studies are provided in Bryan (2002). Prior to harvest, one SPFMV symptomatic vine, approximately 30 cm in length, of a randomly selected plant was collected per plot of each trial at three sampling dates. The stem of each vine was cut below the bottom leaf displaying symptoms of SPFMV. The sampling dates for each trial are listed in Table 1.

***Virus-indexing.*** Within one day after sampling, a 5 cm section of each sampled vine was virus-indexed by grafting onto a 3-wk-old indicator plant, *Ipomoea setosa*, using a side-

vener graft procedure (Hartman et al, 1997). A 5 cm section of the same sampled vine, with at least two nodes, was planted in IPL® Rigi-Pots™ filled with moist Metro-Mix. *I. setosa* plants were grown directly from seed. Healthy *I. setosa* were also kept in the greenhouse as a control. The grafted *I. setosa* plants and the sweetpotato plants were fertilized weekly with 1g/pot or 25mg/cell of 15-30-15 and treated with 1g/ pot (*I. setosa* grafts) or 50mg/cell (sweetpotato samples) of the insecticide Marathon. Symptoms of *I. setosa* grafts were recorded 12 weeks after grafting (WAG) for samples taken in 2000 and 8 WAG for samples taken in 2001.

**Serological assay.** A nitrocellulose membrane enzyme-linked immunosorbant assay (NCM-ELISA) kit provided by the International Potato Center (CIP), Lima, Peru was used to test for the presence of SPFMV, SPMMV, SPLV, SPCFV, SPMSV, C-6 virus, SPCSV, and SPCaLV. Selected sweetpotato vine samples from all field trials and *I. setosa* grafts were tested in the 2000 and 2001 trials. Healthy *I. setosa* were also included. Methods for extraction were conducted according to the kit. An Optitran® supported nitrocellulose transfer and immobilization membrane (Scheicher & Schuell, Keene, NH) was used. Three leaves of *I. setosa* grafts of sweetpotato field samples or leaves of sweetpotato field samples were used with one symptomatic leaf chosen from the bottom, middle and top of the *I. setosa* plant or sweetpotato field sample. Samples, 1 cm diameter, were taken from each leaf (1 g total for the 3 cuttings) with a cork borer and ground in the extraction buffer provided with the kit using a mortar and pestle. Fifty microliters of each extracted sample was blotted onto the nitrocellulose membrane using a vacuum filtered Minifold® Microsample filtration manifold (Scheicher & Schuell, Keene, NH). In 2001, the leftover extracted samples were

stored in 1mL microtubes at -18°C. Each membrane consisted of 12 samples blotted on eight rows. Membranes were allowed to dry over night and then stored at 4°C. The procedures for sampling, extracting, and blotting for the healthy *I. setosa* plants were conducted in a similar manner. The membranes of blotted healthy *I. setosa* were cut with into squares containing 50 µL blots of the extracted leaves. Prior to testing, the rows of the membranes blotted with samples were cut for testing for each of the eight viruses. Each assay for testing of each virus included 6 cut membranes, for a total of 66 samples, a positive and negative control provided with the kit, and 3 healthy *I. setosa* membrane blots. The NCM-ELISA procedure was conducted according to instructions provided with the kit. The membranes were placed into solution reservoirs (Denville Scientific, Metuchen, NJ) and covered with aluminum foil for each of the steps. A new solution reservoir was used for the addition of the final substrate. The presence of each virus was indicated when the membrane showed purple color, as recommended by the kit.

In 2000, 15 randomly selected subsamples from all samples collected from field trials in 2000 were tested for viruses using the NCM-ELISA kit. For each of the 15 subsamples, symptomatic leaves of the *I. setosa* graft and leaves of the sweetpotato vine of the same sample were used. Of the 15 subsamples, SPFMV was detected in all *I. setosa* grafts of samples and only 6 of the samples using sweetpotato leaves. Thus, only *I. setosa* leaves of grafted samples were used for NCM-ELISA assays throughout the remainder of the study. In 2000, all *I. setosa* grafts of samples were grouped according to predominant virus symptoms. Selected *I. setosa* grafts of samples from each group were tested using the NCM-ELISA kit at 8 and 12 WAG. In 2001, 12 *I. setosa* grafts of samples were arbitrarily chosen (based on

virus symptoms) from each trial and sampling date at 3 WAG for virus testing using NCM-ELISA. At 8 WAG, 4 additional *I. setosa* grafts of samples were arbitrarily chosen (based on virus symptoms) from each trial and sampling date for virus testing using NCM-ELISA. In 2001, samples which tested positive for SPLV and SPMSV and one sample randomly selected from samples extracted from each trial and sampling date were retested by NCM-ELISA using stored extracted samples described previously.

**Root sampling.** In the 2000 Nash County trial, 1 root of a randomly selected G1 plant initially displaying symptoms at 5, 6, 7, and 8 WAP were selected from each rep for the mericlones B73 and B94-14. One third of each root was cut from proximal end and placed in a 10 cm clay pot filled with moist Metro-Mix. The roots were fertilized weekly with 1g/pot of 15-15-15. One month after planting, adventitious sprouts from roots were counted and the number of sprouts displaying symptoms of SPFMV was recorded. A sprout from five randomly selected roots was virus-indexed on *I. setosa* and tested for viruses using NCM-ELISA, using the procedures described previously.

**Sampling of *Ipomoea* spp.** Three wk old *I. setosa* and *I. nil* plants were placed in the 2001 Kinston trials comparing mericlones to their adventitious propagules and trials comparing virus-indexed and virus-infected mericlones of 'Beauregard' and 'Hernandez.' Two plants each of *I. setosa* and *Ipomoea nil* were placed at the ends and middle of all border rows (7 m in length) 4 and 6 WAP for each type of trial, respectively. At 15 WAP, one *I. setosa* plant and three *I. nil* plants placed in field trials were removed. In addition, three plants of native *Ipomoea hederaceae* var. *integriuscula* and one plant each of native *Ipomoea hederaceae* and

*Ipomoea lacunosa* were collected from the field trials. All *Ipomoea* species collected were tested for viruses using NCM-ELISA as described previously.

## **Results**

**Virus-indexing.** All field samples, (540 in 2000 and 600 in 2001), induced virus symptoms on *I. setosa* after grafting. The majority of symptoms recorded on *I. setosa* grafts of samples from all trials in the study included: chlorotic vein feathering, leaf deformation, small chlorotic spots, large veinal chlorosis, small veinal chlorosis, crinkling, and cupping with rugosity (Table 2). Other symptoms also recorded included: shoestringing (extreme leaf deformation), bright veinal chlorosis, large chlorotic spots, leaf curl, necrosis, rugosity, stunting, and leaf chlorosis (Table 2). Illustrations of these symptoms are provided in Clark et al. (2001) and Fig. 1. An ANOVA indicated that no differences were detected between symptoms of *I. setosa* grafted with micropropagated virus indexed G1 plants; its adventitious propagules; G2-G5, and NCSU 'Beauregard' regardless of sampling date. No differences were detected between symptoms of *I. setosa* grafted with samples obtained from VI- and VI+ plants at the various sampling dates throughout the growing season.

**Serological assay.** In 2000, all 138 of the selected sweetpotato vine samples virus-indexed onto *I. setosa* tested positive for SPFMV using NCM-ELISA. Fifty-eight of these (42%) presented a light purple reaction using the SPMSV antiserum, and one presented a light purple reaction to the SPLV antiserum. Sixty-two of the saved sweetpotato vine samples, including those presenting mild reactions to SPMSV and SPLV were re-grafted onto *I. setosa*

for testing using NCM-ELISA 4 WAG. The re-grafts of the saved sweetpotato vine samples all tested positive for SPFMV and did not react to other antiserum (no purple color).

In 2001, the selected samples from each trial and sampling date tested positive for SPFMV using NCM-ELISA. For all sweetpotato vine samples, only one sample from the 'Beauregard' test comparing virus-indexed mericlones to virus-infected mericlones resulted in a light purple color for SPLV, while three yielded a light purple reaction to the SPMSV antiserum. The four samples and a random selection of one sample per each trial and sampling date (28 total) were retested by NCM-ELISA using the saved extraction. The retesting yielded a light purple reaction to the SPLV antiserum in the same sample originally yielding a light purple reaction for SPLV indicating a possible positive response. For SPMSV, the samples that yielded a light reaction in the first test did not react with the antiserum when re-tested using saved extract. However, one of the random selections did produce a light purple reaction to SPMSV in the retest. The positive controls for SPMSV provided by the kit and included in the assays for the samples which gave a light purple reaction to the SPMSV antiserum in 2000 and 2001 were medium to dark purple. The positive controls for SPLV included in the assays for the samples which gave a light purple reaction to SPLV were light to medium purple.

For all the NCM-ELISA tests conducted in 2000 and 2001, positive and negative controls provided by the kit tested positive and negative, respectively, for the assays of all viruses tested except SPCSV. The positive control for SPCSV did not test positive (show any purple color) for 4 out of the 6 assays conducted in 2000. In 2001, the positive controls provided

with the kit for SPCSV were light purple. None of the viruses included in the NCM-ELISA assay were detected in the healthy *I. setosa* plants included in the each assay.

**Root sampling.** An average of 4 adventitious sprouts was recorded for potted roots sampled from the 2000 Nash County test. All sprouts of each potted root displayed symptoms of SPFMV, including chlorotic spots with purple rings. The *I. setosa* grafts of sprouts of five randomly selected roots tested positive for SPFMV using NCM-ELISA. No other viruses were detected using the kit.

## **Discussion**

Virus-indexing and NCM-ELISA confirmed the presence of SPFMV in all sampled vines displaying symptoms of SPFMV. A small number of field samples may have reacted with the antiserum for SPLV and SPMSV included in the kit from CIP (indicated by light purple color). The presence of SPFMV in *I. setosa*, despite the strain present, has been characterized by symptoms of vein clearing, chlorotic mottle, veinbanding, and recovery (Moyer and Kennedy, 1978; Moyer et al., 1980). The results from our virus-indexing indicate that SPFMV may induce other symptoms which have yet to be associated with SPFMV including: severe leaf deformation (shoestringing), cupping with rugosity, stunting, necrotic spots, and bright veinal chlorosis. The symptoms of the 2000 field samples yielding mild reactions to the SPMSV antiserum, included shoestringing, chlorotic spots, and veinclearing. The 2001 field samples that resulted in reactions to the SPMSV antiserum included shoestringing, chlorotic spots, veinclearing, crinkling, and cupping with rugosity. The field samples that reacted with the antiserum for SPLV included symptoms of chlorotic

spots, chlorotic vein feathering of the leaf midrib, and leaf distortion. These symptoms could correspond to SPLV, SPMSV, other potyviruses, or the combination of these or other viruses with SPFMV or perhaps more severe strains of SPFMV.

The presence of SPFMV may have also interfered with the detection of these other viruses, if they were present. SPFMV is often found in mixed-infections with other potyviruses (Moyer and Salazar, 1989). Previous studies with SPLV co-infecting sweetpotato with SPFMV have indicated that SPLV was more unevenly distributed and had a lower incidence of detection by ELISA than SPFMV (Green et al., 1988).

Both SPLV and SPMSV are in the potyviridae family (Brunt et al., 1996). SPLV and SPFMV are closely related to typical aphid-transmitted members of the potyvirus group (Hammond et al., 1992). SPLV, originally SPV-N, has been previously reported in Taiwan (Liao et al., 1979), Uganda, Kenya (Wambugu, 1991; Gibson et al., 1996), China (CIP, 1991), Japan (Usugi, 1991), Egypt, India, Peru, and the Philippines (Brunt et al., 1996). The vector for SPLV has not been identified (Brunt et al., 1996), however, some strains have been found to be transmitted by *Myzus persicae* (Usugi, 1991). SPLV does not usually induce symptoms on sweetpotato. SPMSV has previously been reported in Argentina (Di Feo and Nome, 1990), Peru, Indonesia, and the Philippines (Brunt et al., 1996; Alvarez et al., 1997). SPMSV is transmitted by *Myzus persicae* and causes mild mosaic in first true leaves on *I. setosa* (Di Feo et al., 2000).

In the United States, SPFMV is the only virus that has been reported to occur in commercial fields. Virus-like symptoms associated with whiteflies have been reported in the United States (Hildebrande, 1959; Girardeau, 1960; Hildebrand, 1961). Most recently the whitefly transmitted geminiviruses, *Sweet potato leaf curl virus* (SPLCV) and *Ipomoea leaf curl virus* (IPLCV), were detected in breeding lines in Louisiana and Georgia, respectively (Lotrakul et al., 1998; Lotrakul et al., 2000). A random selection of samples, grafted onto *I. setosa*, from the field trials in 2001 were tested for the presence of SPLCV using the polymerase chain reaction (PCR) following the methods of Lotrakul et al. (1998), however no samples tested positive (data not shown). Mild leaf curl symptoms were observed on a small percentage of *I. setosa* grafts of field samples (Table 2 and 3).

SPCSV has been reported in a sweetpotato accession (Pio-Ribeiro et al., 1996) and in Louisiana (Sim et al., 2000). *I. setosa* co-infected with SPFMV and SPCSV have been reported to induce severe stunting, general chlorosis, and necrosis of old infected leaves in Uganda (Mwanga, 2001). According to the positive controls included with the kit, SPCSV was not reliably detected with NCM-ELISA, thus other methods may be needed to screen field samples for SPCSV.

In micropropagation programs used for the increase of planting stock devoid of known viruses, the most reliable method has been grafting onto the indicator plant *I. setosa*. Though this method does take some time and greenhouse space, it is the most reliable method of determining whether the planting material is infected with a virus. Further testing using

serological assays such as NCM-ELISA are useful to rapidly verify if a virus has re-infected the planting material, especially SPFMV.

Saved "seed" roots from virus-indexed 'Beauregard' mericlones, which were infected with SPFMV during the growing season and displayed initial symptoms of the virus at 5, 6, 7, and 8 WAP, produced sprouts that were also infected with SPFMV. The observation that saved "seed" roots of SPFMV-infected plants produce infected sprouts has also been reported by Esbenshade and Moyer (1982). Our studies indicate that adventitious sprouts infected with SPFMV are produced from plants displaying SPFMV symptoms as late as 8 WAP and those symptoms on sprouts can be observed 4 weeks after planting "seed" roots of 'Beauregard.'

All field vine samples produced symptoms on *I. setosa* after grafting, however, vine samples were taken below leaves with symptoms of SPFMV. Symptoms of SPFMV on many cultivars can be mild, transient, or may not appear on foliage (Clark and Moyer, 1988; Brunt et al., 1996). Many East African cultivars do not exhibit foliar symptoms of SPFMV, but do induce SPFMV symptoms on *I. setosa* after grafting (Gibson et al., 1998). In our studies, symptoms on 'Hernandez' require more time to develop than symptoms of SPFMV on 'Beauregard' (Bryan, 2002). Additionally, SPFMV symptoms on 'Beauregard' and 'Hernandez' are not the same; both have chlorotic spots, but 'Hernandez' mericlones seldom produce purple rings. In order to verify virus presence, virus-indexing on *I. setosa* should be a significant part of detecting SPFMV or other viruses in fields as symptoms may not be reliably detected on leaves.

Other methods of detection should be used and developed in addition to virus-indexing on *I. setosa*. The NCM-ELISA kit used in this study was developed by CIP and includes all viruses in which antisera have been developed. However, a more accurate assessment of the viruses occurring in the United States and elsewhere, and methods to detect these viruses are also needed so that reliable screening can be done in order to produce clean planting stock and ensure that the planting stock remains free of these viruses.

Virus-indexing of field samples on *I. setosa* and NCM-ELISA assays should be used in addition to visual monitoring of viruses in certified seed producing sweetpotato fields. The North Carolina Crop Improvement Association monitors fields of certified seed producers for foliar symptoms of SPFMV in order to verify minimal viral infection in the field.

'Beauregard', the most widely grown cultivar in NC displays symptoms of SPFMV and its presence correlates with virus indexing and NCM-ELISA assays, as observed in this study. Symptoms of other viruses, if present, may not occur on foliage of 'Beauregard' such as SPLCV, which has been reported to not induce symptoms on 'Beauregard' (Lotrakul et al., 1998). Other cultivars grown on a smaller scale in NC have been micropropagated, virus-indexed and increased for grower use. The foliar symptoms induced by SPFMV have not yet been determined on these cultivars. In addition, newly developed cultivars also may not display SPFMV or other virus symptoms. Further studies will be needed in order to determine whether viruses other than SPFMV occur in NC. Virus indexing on *I. setosa* and NCM-ELISA can be used in order to enhance detection of SPFMV and the other seven viruses included in the kit.

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**Table 1.** Sampling dates of SPFMV symptomatic vines randomly chosen in each plot at three sampling dates in trials in 2000 and 2001.

Trial	Year	Location <sup>x</sup>	Sampling Date		
			1	2	3
Comparing field generations (G1, G2, G3, G4, and G5) <sup>z</sup>	2000	Kinston	28 Jul/24 Aug <sup>w</sup>	10 Aug/31 Aug	24 Aug/7 Sep
		Nash County	20 Jul/19 Aug	3 Aug/31 Aug	19 Aug/7 Sep
	2001	Kinston	20 Jul/6 Aug	6 Aug/21 Aug	21 Aug/11 Sep
		Wilson County	25 Jul/ 18 Aug	18 Aug/11 Sep	11 Sept/18 Sep
Comparing micropropagated, virus-indexed plants to SPFMV-infected plants <sup>y</sup>	2000	Kinston	28 Jul/24 Aug	10 Aug/31 Aug	24 Aug/7 Sep
	2001	Kinston	8 Aug <sup>v</sup>	21 Aug	18 Sep

<sup>z</sup> Trials at each location compared micropropagated, virus-indexed, greenhouse produced G1 mericlones of ‘Beauregard’ to their subsequent adventitious propagules G2, G3, and G4 in 2000 and G2-G5 in 2001. Each trial contained 25 plants per plot and 5 replications.

<sup>y</sup> Trials at each location compared micropropagated, virus-indexed, greenhouse produced VI- mericlones of ‘Beauregard’ and ‘Hernandez’ in separate trials. Each trial contained 20 plants per plot in 2000 and 25 plants per plot in 2001, with 5 replications.

<sup>x</sup> Trials in Kinston were on the Cunningham Research Station while trials in Nash and Wilson County were located in commercial grower fields.

<sup>w</sup> The first date indicates sampling for G2-G4 in 2000, G2-G5 in 2001, and VI+ plants in 2000 and the second date indicates sampling for G1 plants in 2000 and 2001 and VI- plants in 2000.

<sup>v</sup> The date indicates sampling for both VI- and VI+ plants.

**Table 2.** The percentage of various symptoms induced on *Ipomoea setosa* grafted with field samples of virus-indexed, micropropagated, greenhouse produced (G1) mericlones of ‘Beauregard’ and their successive adventitious propagules derived from saved “seed” roots of subsequent generations (G2, G3, G4, G5), non-micropropagated NCSU ‘Beauregard’, and grower ‘Beauregard’ collected in field trials located in a commercial field in Wilson County and the Cunningham Research Station, Kinston, NC in 2001<sup>z</sup>.

Symptoms	Wilson							Kinston						
	G1	G2	G3	G4	G5	Grower	NCSU	G1	G2	G3	G4	G5	Grower	NCSU
CVF	13	18	33	13	13	33	33	24	20	23	33	23	13	27
LD	20	49	40	40	30	53	60	47	31	33	53	47	33	33
SCS	22	22	23	27	17	13	13	24	47	47	40	57	40	27
LCS	7	2	3	0	0	0	0	7	7	10	7	7	0	7
LVC	24	27	23	27	10	40	27	20	7	10	20	20	13	7
BVC	9	2	10	0	0	00	7	2	9	0	7	0	0	0
SVC	42	56	60	50	50	60	40	49	58	53	53	67	53	47
SS	0	4	10	7	13	13	20	16	11	10	7	20	20	13
CUP	22	20	20	17	23	13	40	11	13	17	30	10	13	7
CRK	31	27	33	30	23	13	33	22	31	47	37	27	33	27
LC	0	0	0	10	0	0	0	0	0	3	7	3	0	0
NS	16	13	10	3	20	0	7	4	4	17	3	3	0	7
RG	2	2	3	3	7	7	0	9	7	0	3	10	0	0
ST	4	2	0	7	0	7	0	18	9	10	10	10	7	7
CHL	18	20	13	13	13	13	13	38	24	27	27	23	20	7

(Continuation Table 2)

<sup>z</sup> Numbers in each column represent the mean percentage of symptoms observed for *Ipomoea setosa* grafts of field samples averaged over the 'Beauregard' mericlones B94-14, B94-34, and B-73 for G1 and G2 and averaged over B94-14 and B94-34 for G3-G5 and three sampling dates. The means of generations (G1-G5) are not statistically different.

CVF=chlorotic vein feathering

LD=leaf deformation

SCS=small chlorotic spots

LCS=large chlorotic spots

SVC=small veinal chlorosis

LVC=large veinal chlorosis

BVC=bright veinal chlorosis

SVC=small veinal chlorosis

SS=shoestringing (severe leaf deformation)

CUP=cupping and rugosity of leaves

CRK=crinkling

LC=leaf curl

NS=necrotic spots

RU=rugosity (no cupping)

ST=stunting

CHL=chlorosis

**Table 3.** Symptoms of the indicator *Ipomoea setosa* grafted with samples of virus-indexed (VI-) and virus-infected mericlones (VI+) of the cultivars ‘Beauregard’ and ‘Hernandez’ collected from their respective field trial at the Cunningham Research Station, Kinston, NC in 2001.

Symptoms	'Beauregard'		Hernandez'	
	VI-	VI+	VI-	VI+
CVF	0.27	0.33	0.09	0.09
LD	0.31	0.42	0.22	0.27
SCS	0.33	0.36	0.27	0.22
LCS	0.31	0.16	0.16	0.31
LVC	0.18	0.22	0.00	0.18
BVC	0.0	0.0	0.0	0.0
SVC	0.42	0.49	0.22	0.36
SS	0.09	0.09	0.00	0.00
CUP	0.22	0.13	0.18	0.07
CRK	0.29	0.40	0.40	0.40
LC	0.02	0.04	0.00	0.00
NC	0.09	0.09	0.04	0.07
ST	0.11	0.07	0.07	0.04
CHL	0.33	0.31	0.40	0.18

<sup>z</sup> Numbers in each column represent the mean percentage of symptoms observed for *Ipomoea setosa* grafts of field samples averaged over the ‘Beauregard’ mericlones B94-14, B94-34, and B-73 and ‘Hernandez’ mericlones H98-7, H98-8, and USDA and three sampling dates. The means of generations (G1-G5) are not statistically different.

CVF=chlorotic vein feathering

LD=leaf deformation

SCS=small chlorotic spots

LCS=large chlorotic spots

SVC=small veinal chlorosis

LVC=large veinal chlorosis

BVC=bright veinal chlorosis

SVC=small veinal chlorosis

SS=shoestringing (severe leaf deformation)

CUP=cupping and rugosity of leaves

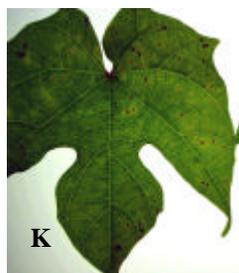
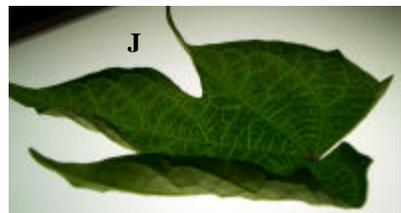
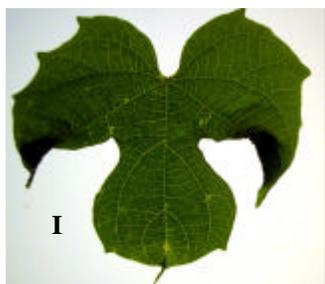
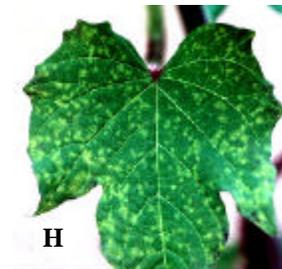
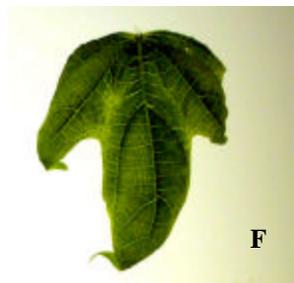
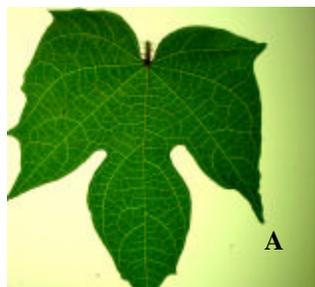
CRK=crinkling

LC=leaf curl

NS=necrotic spots

ST=stunting

CHL=chlorosis



**Fig. 1**

**Figure 1.** Symptoms observed on *Ipomoea setosa* plants grafted with field samples of sweetpotato plants.

- A)** Healthy *I. setosa*
- B)** Small veinal chlorosis
- C)** Large veinal chlorosis
- D)** Chlorotic vein feathering and leaf deformation
- E)** Shoestringing (extreme leaf deformation)
- F)** Cupping (downward curl of leaves)
- G)** Rugosity (raised leaf lamina)
- H)** Chlorotic spots
- I)** Crinkling
- J)** Leaf curl
- K)** Necrotic spots

## CHAPTER 6

### Summary

Sweetpotato is a notoriously variable crop that is sensitive to a wide range of environmental variations (Carpena et al, 1982; Collins et al, 1987; Bacusmo et al., 1988; Kannua and Floyd, 1988; Ngeve and Bouwkamp, 1993). However, our studies indicated that micropropagated, virus-indexed G1 plants consistently produced higher yields of roots in total yield, total marketable yield (TMY), No.1 root yield (the most marketable grade), and percent No.1 yield (relative to total yield) than G2-G5 plants. G1 plants produced roots with better shape uniformity and overall appearance than roots produced from G2-G5 plants. Generally, high length/diameter (L/D) ratios, which negatively impact root quality, were observed in G2-G5 roots while G1 roots were observed to have lower L/D ratios. Rank mean yield and root quality measurements of each location were consistent with means averaged over locations per year and suggested a decrease in yield and root quality with increased adventitious propagation. Linear regression analysis used to model yield and root quality measurements of G1-G5 over time indicated a gradual decrease in total yield (5-7%), TMY (4-8%), percent No.1 yield (7-9%), shape uniformity (4-7%), and overall appearance (10%) and an increase of L/D ratios (7-10%) with increased field generations of adventitious propagation. In addition, a decline was observed in No.1 yield with a higher rate noted in B94-34 (12-18%) compared to B94-14 (8-12%).

Monitoring indicated that all G1 plants became infected with *Sweet potato feathery mottle virus* (SPFMV) by the end of the growing season and that G2-G5 plants were most likely infected in the previous growing season. Monitoring also indicated that 100% of virus-

indexed (VI-) plants were reinfected with SPFMV by 9 weeks after planting. Despite re-infection rates of VI- plants, field tests indicated that the presence of SPFMV prior to planting (VI+) reduced yield of TMY, yield of No.1s, and percent No.1s and decreased overall appearance for 'Beauregard.' In addition, the presence of SPFMV prior to planting (VI+) increased storage root length and reduced width of sweetpotato storage roots leading to an increase in L/D ratios which detracted from root quality and appearance for mericlones of both cultivars studied. SPFMV presence prior to planting may also increase the severity of russet crack symptoms, caused by a strain of SPFMV, as these symptoms were only observed on VI+ roots of 'Beauregard' mericlones and G2-G5 roots.

Monitoring conducted in these studies demonstrate that increased numbers of aphids within field trials result in increased virus incidence. The higher rate of reinfection of SPFMV in VI- and G1 plants in 2000 compared to VI- and G1 plants in 2001 seems to correlate with the higher number of aphids recorded per trap in 2000.

Monitoring for foliar symptoms of virus may be difficult as they may not display virus symptoms at all times of production. As observed in this trial, VI+ plants did not show SPFMV symptoms at the time of planting in both 2000 and 2001. However, virus-indexing and NCM-ELISA confirmed the presence of SPFMV in all sampled vines displaying symptoms of SPFMV. Virus-indexing on *I. setosa* indicated that field samples from trials induce other symptoms which have yet to be associated with SPFMV including severe leaf deformation (shoestringing), cupping with rugosity, stunting, necrosis, and bright veinal

chlorosis. However, only SPFMV was confirmed to be present in all field samples using NCM-ELISA. Both methods of detection should be used to test for viruses in field samples.

Most growers in the US may still prefer the bedding system because of the large amount of transplants (adventitious sprouts) produced in a short period of time. “Seed” roots need to be cured and stored over winter, and this procedure is already used for the commercial sweetpotato crop by most growers. Because roughly 10% of the crop is saved as “seed” roots for production of sprouts, the time and energy spent in saving “seed” may be less than the cost of producing or purchasing micropropagated transplants. Thus, “seed” with less mutation and virus accumulation may be more useful to growers who choose to save their own “seed.” Providing growers with “seed” that will consistently produce high yields and root quality is of the utmost importance. Considerable time and effort is needed to choose true-to-type sweetpotato “seed” and also avoid virus-infection in “seed” production.

Knowing what each mutation and virus accumulation contributed to decline, particularly in the first few “seed” generations, may help in utilizing resources to prevent the effects of decline while incorporating production methods used by growers.

Adventitious propagules from roots that are infected with SPFMV do not retain the high yield and root quality attributes initially observed in the micropropagated, greenhouse produced nodal cuttings from which they were derived. Mutations and virus that are allowed to accumulate through adventitious propagation methods lead to decline in sweetpotato. The use of micropropagation in the sweetpotato foundation seed program can be used to minimize the effects of decline. The use of limited “seed” generations for sweetpotato

production is an especially attractive option for growers who choose to save their own “seed” when micropropagated plants are used as the initial planting material, such as used by the NC certified seed program, and “seed” is saved for a limited number of generations. However, for this system to be effective, “seed” roots from G1 plants must be certified as true-to-type and produced in fields with limited virus infection. This method assumes true-to-type roots have minimal net deleterious mutations and that symptomless sweetpotato plants also produce “clean” roots with minimal or no virus. However, in order to develop and maintain a sweetpotato cultivar with high yield and root quality, these assumptions need to be clarified. Our studies indicate that SPFMV contributes to cultivar decline. Since nodal cuttings were not subject to somaclonal variation, the yield and root quality differences we observed were due to virus-infection prior to planting, rather than accumulated mutations or in-season infection.

### **Future Considerations**

Previous reports have indicated that adventitious propagules of sweetpotato cultivars are genetically more variable than those produced from preexisting meristematic regions and this variability alters favorable genetic combinations, resulting in deleterious mutations that may lead to decline (Villordon and LaBonte, 1996). The study comparing G1 plants to its adventitious propagules G2-G5 indicates that clonal degradation occurs gradually from one propagation cycle to the next and that this is consistent in three separate populations with at least three generations of adventitious propagation cycles. Further studies are needed to elucidate the role of mutations in decline. It appears that B94-14, B94-34, and B73 can be distinguished from one another using amplified fragment length polymorphism markers

(Craig Yencho, preliminary data). However, in order to verify that mutations contributed to the decline observed in this study, an analysis should be conducted to identify any polymorphisms in genomes in the generations (G1, G2, G3, G4, G5).

The study comparing VI- and VI+ plants indicated that there was a 26% reduction in No. 1 yield when SPFMV was present prior to planting (VI+) while the yield decline observed in adventitious propagules (G2-G5) infected with SPFMV was 8-12% for B94-14 and 12-18% for B94-34 per generation propagated. Variability in yield reduction could be due to differences in the strains used to inoculate VI+ plants and those infecting G2-G5 plants in field production. In addition, higher titers of SPFMV may have been present in the VI+ plants, which were graft-inoculated with an infected sweetpotato vine collected from the field, and may have caused greater effects on yield compared to G2-G5 adventitious sprouts, which became infected by aphids during the growing season and possibly had lower concentrations of SPFMV. The titer of SPFMV in samples was not measured in this test. In future studies, determining virus concentration in inoculum may help determine whether increased titer in inoculum resulted in greater yield effects. Virus concentration could be measured in the adventitious propagules of saved “seed” roots of ‘Beauregard’ to determine if virus titer increases with increased generation of adventitious propagation as a result of reinfection by aphids with each year in field production.

The grower source of certified G2 plants (produced from G1 roots of plants with less than 1% SPFMV infection ) used in the Nash County trial in 2000 produced similar No.1 root yield ( $24.6 \text{ kg}\cdot\text{ha}^{-1}$ ) in comparison to the No.1 yield for G1 B94-14 and G1B94-34 grown in

the same location (Appendix 1.1). The G2 plants used in field trials in this study would not have met the certification requirement given reinfection of SPFMV in 100% of G1 plants. G2 plants obtained from roots of G1 plants with limited virus infection, however, may retain high yields and may experience decline even more slowly than observed in this study. Various certified seed or plants could be tested for presence of SPFMV and evaluated in field trials in order to determine if limited SPFMV reduces decline.

Determining how great of an impact each mutation and virus contributes to decline may help breeders in cultivar selection such as developing cultivars more stable over adventitious propagation cycles or by developing a tolerant or virus resistant cultivar. Until then, micropropagated, virus-indexed sweetpotato can be used in foundation seed programs in order to provide growers with high quality, high yielding seed tested for freedom of viruses.

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## **Appendices**

**Appendix 1.1** Yield and root quality measurements for various grower sources of ‘Beauregard’ evaluated in trials in 2000 and 2001.

Year	Location <sup>z</sup>	Source <sup>y</sup>	No.1 <sup>x</sup>	Canners	Jumbos	Culls	Shape <sup>w</sup>	Flesh	Lenticels	Pimples	L/D	Overall
							Uniformity	Color				Appearance
2000	Kinston	G2	27.2	6.5	2.8	0.6	3.4	3.0	3.2	5.0	2.9	3.4
	Columbus	G4+	*11.1	11.1	0.9	*5.5	*2.0	3.0	4.0	4.0	*3.1	*1.3
	Nash	G2	24.6	5.1	3.7	0.9	3.0	2.8	3.5	3.9	2.5	3.1
2001	Columbus	G2	*12.1	*7.9	0.3	*3.8	*2.7	3.0	4.4	5.0	*3.1	*2.6
	Wilson	G4+	8.7	11.5	2.5	2.6	2.6	2.9	4.0	4.9	2.9	2.2
	Kinston	G2	*14.9	*5.0	*2.5	*2.9	*2.5	3.0	4.3	4.9	2.9	*2.3
	Clinton	G3	8.5	7.6	0.0	0.6	2.4	2.9	2.8	4.0	3.1	2.2

<sup>z</sup> Trials were conducted at the following sites: Cunningham Research Station, Kinston, NC; Horticultural Crops Research Station, Clinton, NC; and sweetpotato farms in Wilson County, Nash County, and Columbus County.

<sup>y</sup> Source indicates the generation of planting material obtained from growers. G2 and G3 are certified for minimal *Sweet potato feathery mottle virus* infection. G4+ has never been micropropagated.

<sup>x</sup> Yield measurements were recorded and analyzed for the following: No.1s=roots with diameter of 5 cm ≤ 9 cm and length of 7.5 cm ≤ 23 cm; canners=roots with diameter of 2.5 cm ≤ 5 cm, jumbo=roots with diameter > 9 cm, length > 23 cm; and culls=malformed or distorted roots.

<sup>w</sup> Root quality measurements were recorded and analyzed for the following: flesh color for 10 randomly selected roots, sliced 1/3 from proximal end and rated on a 0 to 5 scale where 0=no carotene (white) and 5=high carotene (dark orange); chim=number of chimeras recorded for each sliced root; overall appearance and shape uniformity (0 to 5 scale) where 5 is the best overall appearance and most uniform; negative epidermal characteristics of lent=lenticels (small pores (~2 mm) for gas exchange) and pimples (brown to black protrusions (~1 mm diameter) on root epidermis) (0 to 5 scale) where 5 represents minimal presence of lenticels and pimples, 3 represents roots where lenticels and pimples covered half the area of the root, and 0 represents roots completely covered with each skin characteristics; and visual ratings for length diameter ratios (L/D) (1 to 5 scale) where each rating indicated the ratio of length to width for majority of No.1 roots in each plot.

\* Indicates yield and root quality measurements of each grower source in each location that is statistically different (p=0.05) than yield and root quality of B94-14 G1 and B94-34 G1 roots produced in each respective location. Means were separated using Fisher’s Protected LSD. Grower sources were not compared to G1 mericlones for Wilson County due to insufficient stands.

**Appendix 1.2.** Percentage of roots with russet crack symptoms were recorded on sweetpotato roots harvested on two on-farm locations.

		Percentage of Russet Crack									
Location	Source <sup>z</sup>	Total		No.1s		Canners		Jumbos and Culls			
Columbus County	B94-14G1	0.00	a <sup>x</sup>	0.00	a	0.00	a	0.00	a		
	B94-14G2	1.80	ab	1.10	a	2.90	a	0.00	a		
	B94-14G3	1.50	ab	1.00	a	1.90	a	2.20	a		
	B94-14G4	0.60	ab	1.10	a	0.00	a	2.50	a		
	B94-14G5	0.00	a	0.00	a	0.00	a	0.00	a		
	B94-34G1	0.00	a	0.00	a	0.00	a	0.00	a		
	B94-34G2	2.10	b	1.70	ab	2.30	a	2.50	a		
	B94-34G3	0.30	ab	0.00	a	0.70	a	0.00	a		
	B94-34G4	4.10	c	3.70	b	7.10	b	0.00	a		
	B94-34G5	0.90	ab	1.50	ab	0.60	a	0.00	a		
	NCSUBeau	0.30	ab	0.00	a	0.50	a	0.00	a		
Location	Source	Total		No.1s		Canners		Jumbos		Culls	
Wilson County	B94-14G1	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a
	B94-14G2	7.20	bc	7.10	b	8.10	b	3.60	b	8.80	ab
	B94-14G3	3.70	ab	2.80	ab	2.00	ab	0.00	a	5.40	ab
	B94-14G4	10.20	c	0.00	a	9.30	b	0.00	a	24.90	b
	B94-14G5	2.80	ab	2.70	ab	2.80	ab	0.00	a	0.00	a
	B94-34G1	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a
	B94-34G2	6.00	ac	3.20	ab	5.30	ab	0.00	a	13.90	ab
	B94-34G3	6.40	ac	0.00	a	6.80	ab	0.00	a	33.30	b
	B94-34G4	5.30	ac	5.60	b	4.30	ab	0.00	a	12.90	a
	B94-34G5	8.00	bc	6.80	b	5.20	ab	0.00	a	22.40	b
	NCSUBeau	2.90	ab	3.30	ab	2.10	ab	0.00	a	6.10	a

<sup>z</sup> Source indicates each mericlone, B94-14 and B94-34, of the cultivar ‘Beauregard’ and respective generation, G1, G2, G3, G4, and G5, (G represents generation while the number indicates the number of years in field production) or non-micropropagated NCSU ‘Beauregard.’

<sup>y</sup> Mean separation within columns by Fishers Protected Least Significant Difference (LSD) at  $p \leq 0.05$ . Columns with the same letter are not significantly different.

**Appendix 1.3** Stability variance (Shukla, 1972) of yield and root quality measurements for each planting source averaged over trials in 1999, 2000, and 2001.<sup>z</sup>

Year	Source <sup>y</sup>	Total	TMY	No1s	Culls	Percent No.1s	Shape Uniformity	L/D	Overall Appearance
1999	B94-14G1	0.5	0.4	3.0	0.1	62.3	0.1	0.1	0.1
	B94-14G2	0.3	2.3	1.4	0.0	21.0	0.0	0.0	0.0
	B94-14G3	9.5	20.4	10.9	0.5	29.7	0.1	0.0	0.3
	B94-34G1	16.4	22.9	12.2	0.2	46.4	0.1	0.0	0.1
	B94-34G2	3.7	5.3	2.2	0.1	6.2	0.0	0.0	0.2
	B94-34G3	14.4	19.0	7.5	0.1	56.4	0.1	0.0	0.1
	NCSU 'Beauregard'	-0.3	-0.4	-0.7	0.3	-0.8	0.0	0.0	0.0
2000	B94-14G1	5.6	7.2	20.7	-0.3	59.8	0.5	-0.3	0.6
	B94-14G2	1.7	2.1	4.6	0.1	17.6	0.0	0.0	0.0
	B94-14G3	3.2	1.1	5.1	0.3	29.3	0.0	0.0	0.0
	B94-14G4	-1.1	-0.7	-1.0	0.2	1.7	0.0	0.1	-0.1
	B94-34G1	29.4	28.9	21.8	-0.1	31.6	0.2	-0.2	0.4
	B94-34G2	-0.3	0.8	-1.3	0.7	1.9	-0.1	0.2	-0.2
	B94-34G3	8.8	9.8	7.9	0.5	6.2	0.0	0.1	-0.1
	B94-34G4	-1.6	-1.4	0.7	0.3	12.1	0.0	0.1	-0.1
NCSU 'Beauregard'	12.8	12.6	2.9	0.2	-4.1	-0.1	0.2	-0.1	
20001	B94-14G1	15.8	6.7	3.7	2.6	23.3	0.1	0.0	0.1
	B94-14G2	3.6	2.7	7.4	0.4	36.6	0.1	0.0	0.0
	B94-14G3	7.2	4.1	1.3	0.8	17.3	0.0	0.1	0.0
	B94-14G4	9.0	11.3	11.2	0.2	32.9	0.0	0.0	0.0
	B94-14G5	0.7	1.3	2.5	0.6	19.8	0.1	0.0	0.0
	B94-34G1	15.0	15.6	34.8	0.0	141.9	0.0	0.1	0.1
	B94-34G2	0.3	1.1	5.0	0.1	27.2	0.0	0.1	0.0
	B94-34G3	0.7	2.0	1.9	0.7	5.0	0.0	0.0	0.0
	B94-34G4	0.5	1.6	0.3	0.2	28.6	0.0	0.0	0.0
	B94-34G5	1.1	1.2	0.4	0.1	-0.1	0.1	0.1	0.0
	NCSU 'Beauregard'	4.8	6.8	0.5	0.3	1.7	0.1	0.0	0.0

(Continuation Appendix 1.3)

<sup>z</sup> Low stability variance ( $\sigma^2$ ) values indicate a more stable cultivar, while higher values are less stable.

<sup>y</sup> Source indicates each mericlone, B94-14 and B94-34, of the cultivar 'Beauregard' and respective generation, G1, G2, G3, G4, and G5, (G represents generation while the number indicates the number of years in field production) or non-micropropagated NCSU 'Beauregard.'

Shukla, G.K. 1972. Some statistical aspects of partitioning genotype-environment components of variability. *Heredity*. 29:237-245.

## **Appendix of Figures**



G1

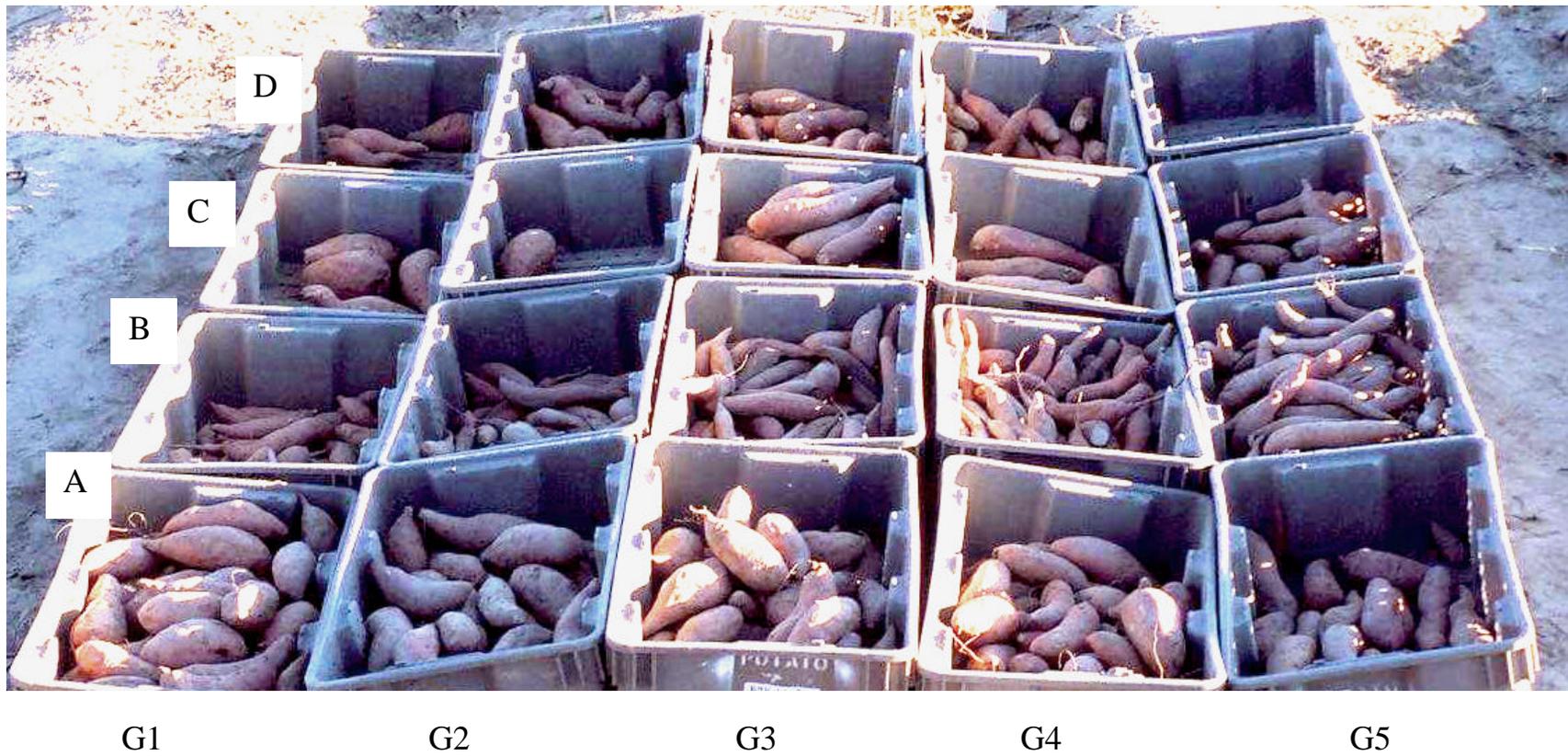
G2

G3

G4

G5

**Appendix Figure 1.1.** Graded roots produced by G1-G5 plants of B94-34 in a rep in Wilson County, 2001. (A) No.1 roots (B) Canner roots (C) Jumbo roots and (D) Cull roots.



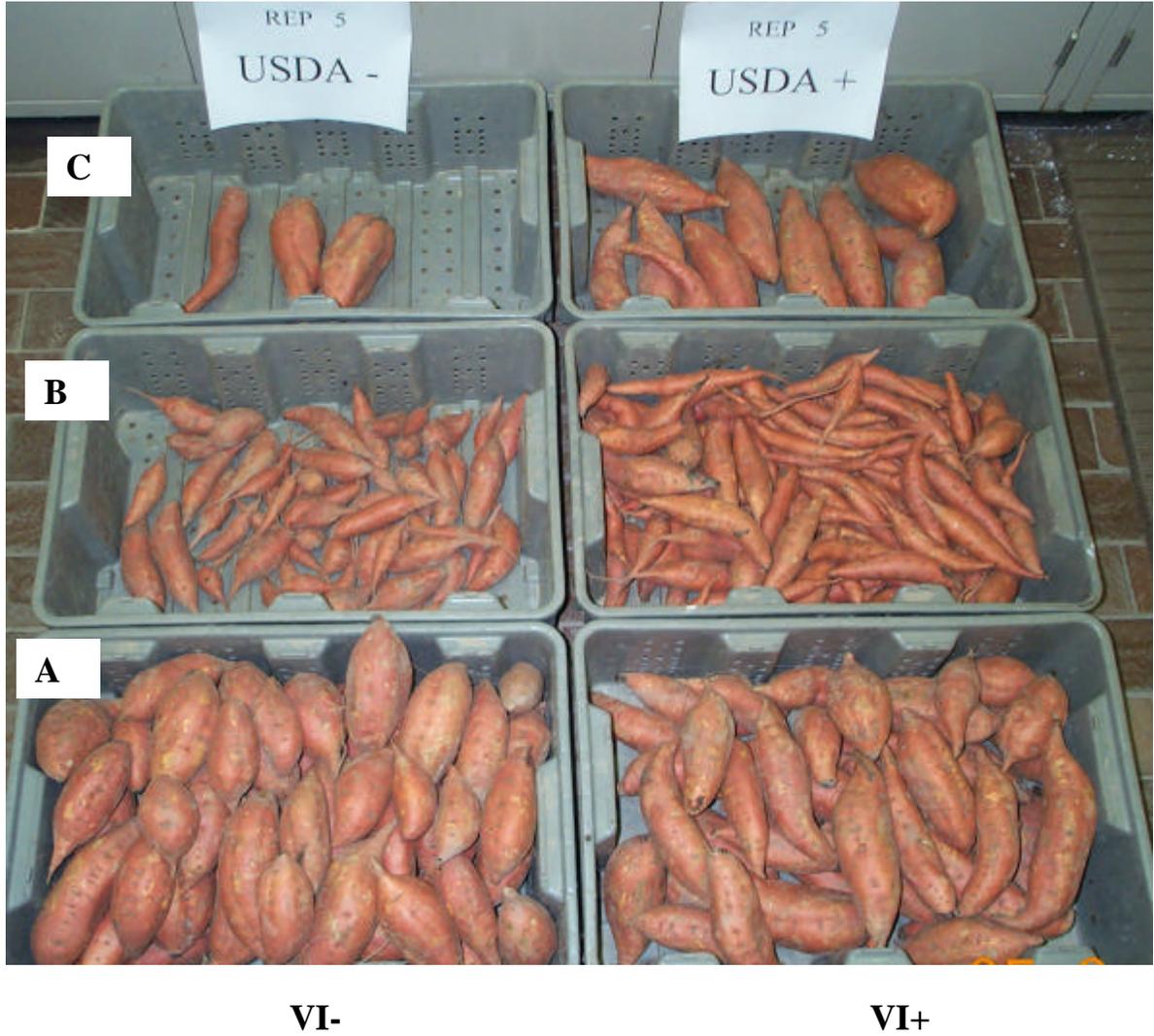
**Appendix Figure 1.2.** Graded roots produced by G1-G5 plants of B94-14 in a rep in Wilson County, 2001. (A) No.1 roots (B) Canner roots (C) Jumbo roots and (D) Cull roots.



**Appendix Figure 1.3.** No. 1 roots produced by G1-G4 plants of B94-34 in a rep in Nash County, 2000. No.1 root yield, shape uniformity, and overall appearance decreased with increased generation. Note off-type B94-34 G4 roots with white flesh.



**Appendix Figure 1.4.** Graded roots produced from VI- and VI+ plants of the ‘Beauregard’ mericlone B94-14 in a rep at the Cunningham Research Station, Kinston, NC, 2000. (A) No.1 roots (B) Canner roots and (C) Jumbo and Cull roots.



**Appendix Figure 1.5.** Graded roots produced from VI- and VI+ plants of the ‘Hernandez’ mericlone USDA in a rep at the Cunningham Research Station, Kinston, NC, 2000. (A) No.1 roots (B) Canner roots and (C) Jumbo and Cull roots.