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Studies on Development of End Rot in Sweetpotato

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STUDIES ON DEVELOPMENT OF END ROT IN SWEETPOTATO

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I dedicate this study to my family and all my professors.

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ABSTRACT

End rot development in sweetpotato is caused by several pathogens and exacerbated by unfavorable environments. A critical need exists to know what factors in the environment trigger end rot development and how to manage the crop to minimize the incidence of end rots which are the overall objectives of this study. This research was divided into three studies: 1) Effect of environmental factors on expression of end rot in sweetpotato roots. Factorial combinations consisted of flooding/non-flooding; skinned/non-skinned; cured/non-cured; recommended storage/ambient storage are environmental variables not tested previously together in a systematic way. Results showed that curing at 29ºC and 85-90% RH for five days and storage at 13ºC and 85-90% RH were the critical factors mitigating end rot incidence. 2) Understand the role of calcium deficiency on end rot incidence. Relationship was observed between calcium and ethephon. Ethephon induced proximal and distal end rot incidences and decayed areas in sweetpotato. End rot symptoms are akin to blossom end rot in tomato caused by calcium deficiency. Increased rates of calcium in hydroponic solution increased calcium content in storage roots and reduced incidence of end rot. Calcium deficiency had impact on end rot incidence. 3) Identify expressed genes in storage roots treated with ethephon and 1-MCP. Molecular mechanisms triggered by the onset of end rot are unknown and may provide insight into plant protective mechanisms to exploit in a breeding program. This study identified differentially expressed genes (DEGs) using the annealing control primers (ACPs). DEGs identified are involved in protective mechanisms, transcriptional regulation, and an expressed protein (unknown). All 5 genes expressed in sweetpotato with end rot were confirmed by semi-quantitative reverse transcription polymerase chain reaction (sqRT-PCR) analysis. Genes differentially responded to 3.9 mM ethephon and 1 ppm 1-MCP. 1-MCP induced higher expression of *TH2 and ATG*8 than ethephon treated storage roots and minimized end rot

incidence. This study furthered our knowledge of the role environment plays in inciting end rot development and how to minimize the incidence of end rots. Also, new genes were found that putatively lessen end rot and may have value as markers in breeding programs.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Sweetpotato, [*Ipomoea batatas* (L.) Lam.], is versatile and important. In Asia and Africa, vines and roots are used as animal fodder and as an important source of carbohydrates, beta-carotene and vitamin C for humans (Woolfe, 1992). Bovell-Benjamin (2007) reported that the protein content of sweetpotato leaves range from 4.0% to 27.0% and roots range from 1.0% to 9.0%. The nutrient content of a medium orange flesh sweetpotato baked with skin contains about 7% carbohydrate, 15% dietary fiber, 438% of the daily requirement for vitamin A and 37% of the daily requirement for vitamin C (USDA, 2012). The distinctive flesh colors of sweetpotatoes (white, cream, yellow, orange and purple) provide different nutritional profiles. Teow *et al*., (2007) concluded that antioxidant activities varied widely among the sweetpotato clones and the purple fleshed sweetpotato had the highest antioxidant activity. The significance of sweetpotato is underscored by the vast acreage under cultivation in the world, with 13 MT/Ha of yield and 104,453,966 tons of production (FAO, 2014). China has the most extensive acreage, accounting for 67.7% of total world production in 2014 (FAO, 2014).

The US industry is small and unlike others in the world targets fresh produce markets. Annual growth rate from 2005 to 2014 was 4.97% area harvested, 7.43% production, and 2.35% yield (FAO, 2014). The 130,300 acres in 2011 (Sweet Potato Statistical Yearbook, 2012) under cultivation generates over 500 million in gross farm and value added income in USD. Consumption is also increasing and has risen from 1,514.5 to 2,266.1 million pounds since 2005 representing an increase of 4.5 pounds to 6.9 pounds per person. California, Louisiana, Mississippi and North Carolina are the major production areas (Smith *et al*., 2009). Sweetpotatoes are costly to produce at up to $6,000/acre in California. Costs are less in other regions ($3,500-4,000/acre). This high

cost is currently countered with low prices, hence profit margins are narrow. Any loss due to disease or insect infestation can erase any profit. A particular concern is loss in storage when all the costs of production have been incurred.

Ray *et al*. (1994) found that mean weight loss of nearly 20% was observed during 45 days of storage due to fungal decay and weevil infestation. Rees *et al*. (2001) noted that damaged roots due to breakages, cuts, infestation by weevils, rotting and superficial damage had a shorter shelf life. Moreover, these roots also had increased fresh weight loss and rotting. Decline of the crop is slow in storage. Weight loss over months of storage is a hidden loss to most growers. Rots are obvious and represent several different diseases each brought about by a different set of circumstances. End rots of sweetpotato storage roots are commonly caused by *Fusarium solani* (Clark, 1980; da Silva, 2013) and *Macrophomina phaseolina* (da Silva, 2013). It has been recommended that mechanical damage, wet and cold or excessively dry soil at harvest, exposure to high or low temperatures for extended time after harvest and conditions favoring desiccation of wounded tissue may activate end rot in sweetpotato during storage (Clark *et al*., 2013a)

A USDA Specialty Crop Research Initiative (SCRI) project found that fungi can be isolated from within healthy sweetpotato sprouts in beds where transplants are produced, and in vines and storage roots during the growing season, long before symptoms develop. These endophytic fungi are living within the plant without causing disease, until something in the environment triggers them to initiate a pathogenic phase. Critical to knowing how to manage the crop to minimize the incidence of end rots is to understand how and when infection occurs, and what factors in the environment trigger development. The SCRI project has provided clear indications that these endophytic fungi enter the plants long before the storage roots are harvested and put into storage (da Silva, 2013). Growers recognize the importance of carefully handling roots at harvest and

disposing of those with obvious rots and overt skinning. Still growers have encountered significant loss to a disease/physiological complex now affecting a significant portion of the stored crop. Yet, end rot is not usually found in stored roots across the industry. The greatest incidence is reported in Mississippi. Growers elsewhere are very concerned.

1.2 Environmental factors involved in end rot incidence on sweetpotato

Many environments induce end rot development in sweetpotato from preharvset to postharvest such as flooding, mechanization, lack of curing and poor storage. Each has a unique impact on production and storage.

Sweetpotato loss due to flooding can be catastrophic. Louisiana frequently encounters hurricanes and tropical rains that damage sweetpotato fields. Roberts and Russo (1991) found that flooding at midseason reduced sweetpotato yield by 36% in 1989 and by 53% in 1990. Ray *et al*. (1997) found soil moisture at 40% resulted in postharvest loss. Loss is often (asphyxiation, rot) not easily diagnosed. Clark and da Silva (2012) reported that flooded fields led to an increase in the incidence of end rots and other diseases during sweetpotato storage.

Mechanized harvesting operations can reduce labor expense by 13-25% of the total production cost (Agron, 2009). Roughly handled sweetpotatoes are more prone to skinning damage. Wounding has been shown to increase the respiration rate and weight loss of sweetpotato (Picha, 1986). A plausible hypothesis is that water loss results in tissue stress, which reduces the ability of the tissue to defend itself against pathogen invasion (Rees *et al*., 2003).

Curing and proper storage reduce the incidence of diseases because wounded sweetpotato roots are highly susceptible (Aidoo, 1993). Roots must be cured immediately at 29 ^{o}C and a relative humidity of 85 to 90 percent for 4 to 7 days for wound healing after harvest. Arancibia *et al*. (2013) found that cured roots had lower tip rot and end rot incidence. Ray and Ravi (2005) supported that

curing promoted wound healing and as the most suitable method to reduce microbial spoilage. After curing, sweetpotatoes should be stored at 13°C with 85-90% relative humidity (Smith *et al*., 2009). Chilling injury caused by low temperature (10ºC) leads to tissue breakdown (Clark *et al*. 2013). Controlling spoilage and increasing shelf life of sweetopotato storage roots were impacted by fungicide, bio-control, irradiation, and storage method (Ray and Ravi, 2005). Environmental variables could influence the extent of end rot in stored sweetpotato.

1.3 Calcium and end rot incidence on sweetpotato

Another hypothesis may involve calcium deficiency which causes desiccation at root tips and promote end rot (Villordon personal communication, 2013). Calcium is a macronutrient and a non-mobile element in phloem primarily using passive pathway and transpiration. Main functions of calcium are as a constituent of cell walls, and an enhancer of membrane stability and cell integrity. Calcium is also important in root development, with roles in cell division and cell elongation, and as a secondary messenger; e.g., regulating developmental processes and Ca^{2+}-binding proteins (Hawkesford *et al*., 2012). Calcium content in plants range from 0.1-5% of dry weight of tissue and 0.2-0.3% in the fruit (Marschner *et al*., 1997). Calcium deficiency is characterized by necrosis on young leaf tissue. The plant fails to grow.

Blossom end rot (BER) can occur in tomato, sweet pepper, and watermelon. BER can be caused by poor absorption of calcium by plants because of water stress, or low transportation of calcium to the distal fruit tissue. Geraldson *et al*. (1956) showed that high concentrations of potassium, sodium, or ammonium decreased calcium uptake and enhanced BER in tomato. Ho and White (2005) reported that BER is promoted by stressful environments that limit the uptake and transport of calcium in tomato. Increased calcium and reduced BER is notable under high irrigation conditions (Bar-Tal and Aloni, 2013). Calcium deficiency stimulated polygalacturonase which

breakdowns pectic polysaccharides in cell walls and induced BER (Seling *et al*., 2000). However, some research indicated that BER was caused by a stress-related disorder. Saure (2001) found that BER was caused by a deterioration of the cell membrane and increased ion permeability. Transpiration causes a high flow of calcium to accumulate in the leaves at the expense of root tissue (Palta, 2010). Mantsebo et al. (2014). Showed that calcium reduced the soft rot incidence and severity of potato tuber during storage. Calcium plays an important role in plant defense against microbial pathogens.

1.4 Ethylene and end rot incidence on sweetpotato

Ethylene emission is a plant's response to environmental stress. Ethylene is produced from methionine which is converted to S-adenosylmethionine (S-Adomet) by adenosylmethionine synthetase (SAM synthase). Aminocyclopropane-1-carboxylic acid synthase (ACC synthase) catalyzes S-Adomet to ACC and 5'-methylthioadenosine. The last reaction is oxidation of ACC to ethylene (Yang and Hoffman, 1984). Ethylene binds to receptors and inactivated receptors. Plant ethylene responses therefore are stimulated. Enhanced ethylene production may signal to plants that they are under pathogen attack and induce defense reactions. Healthy plants and plant organs also are affected by exposure to ethylene and disease development may occur because ethylene exposure accelerates ripening or senescence. Villordon (2012) found that sweetpotato roots which have been bruised or cut produce 20 times more ethylene than uninjured sweetpotato roots. Sweetpotato roots responded to ethylene with increasing respiration, phenolic content, and decay (Pankomera, 2015). Buescher (1981) found that storage roots exposed to 100 ppm ethylene for 5 days at 60^{o}F was detrimental to storability of the four varieties of sweetpotatoes studied. Arancibia *et al*. (2013) showed that end rot was observed mainly in ethephon-treated sweetpotato plants while end rot was less prevalent in non-treated plants.

A common ethylene inhibitor is 1-MCP (1-methylcyclopropene) which functions by interfering with ethylene receptor sites. It is widely used for agricultural applications such as the inhibition of ripening or senescence. Villordon (2012) applied 1 ppm 1-MCP to uninjured and injured sweetpotato roots (1-1.5 inch proximal and distal end) and found that 1-MCP reduced breakdown in sweetpotato roots. Plums were treated with 0.5 $\mu l\ l^{-1}$ 1-MCP that prevented or retarded bruising after 4 weeks of cold storage (Lippert and Blanke, 2004). However, different concentrations of 1-MCP affect quality of strawberries. At low concentration of 1-MCP (5 to 15 $nL \cdot L^{-1}$) prolonged postharvest life by 35% at 20 °C and 150% at 5 °C, but at high concentration (500 $nL \cdot L^{-1}$) quality declined at both at 20 and 5 °C (Ku *et al*., 1999).

Many plants also use ethylene signaling during programmed cell death (PCD) and induce PCD only in sensitized cells or tissues (Trobacher, 2009). Villordon *et al*. (2012) found that samples submerged in ethephon and water showed signs of localized tissue death (necrosis) in 'Beauregard' sweetpotatoes. The development of lenticel proliferation was also observed on the surface of storage roots treated with ethephon, but not found in 1-MCP treated storage roots which failed to show evidence of localized tissue death even after three months of observation.

1.5 Ethylene-induced gene expression and analytical methods for gene expression

Ethylene induces gene expression. Ethylene affects transcript production of mRNA of many genes such as those involved in cellulose synthesis, ripening process and ethylene synthesis (Taiz and Zeiger, 2002). In the absence of ethylene, ethylene receptor (*ETR1*) and other ethylene receptors activate the kinase activity of the constitutive triple response (*CTR1*) gene which leads to repression of the ethylene response pathway. In the presence of ethylene, ethylene receptors found in membranes; require a copper cofactor to bind ethylene. When ethylene receptors are turned off, the response pathway proceeds as follows: ethylene binding inactivates *CTR1* resulting

in ethylene insensitive (*EIN2*) activation; *EIN2* induces *EIN3* which is a member of transcription factors; *EIN3* activates an ethylene response element binding protein (*EREBP*) to regulate gene expression; *EREBP* interacts with the GCC-box that encodes effector proteins used for ethylene responses (Chang and Shockey, 1999; Taiz and Zeiger, 2002; Guo and Ecker, 2004). Previous research found that ethylene at low concentration increased the activity of peroxidase and polyphenoloxidase and resistance to infection by *Ceratocvstis fimbriata* in sweetpotato tissue (Stahmann *et al*., 1966). This supported results of Birecka and Miller (1974) that ethylene stimulated peroxidase activity. Peroxidase can detoxify hydrogen peroxide (Dietz *et al*., 2006). Haga *et al*. (1988) studied this in rice and found that exogenous ethylene induced phenylalanine ammonia lyase (PAL) which is an enzyme in the phenylpropanoid pathway involved in structural support, plant defense, and survival (Vogt, 2010). However, ethylene highly stimulated camptothecin-induced hydrogen peroxide production and cell death. L-α-(2-aminoethoxyvinyl) glycine or silver thiosulphate inhibited ethylene synthesis or ethylene perception, respectively. This blocked camptothecin-induced hydrogen peroxide production and PCD (de Jong *et al*., 2002). Thus, the concentration of ethylene and different tissues exposed to the ethylene may affect the expression of genes differently.

Gene expression quantification. Gene expression studies enable one to understand underlying physiological responses. Researchers can identify differential mRNA expression levels and compare between treatments. Common techniques include DNA microarrays, RNA-seq, and annealing control primers (ACPs). DNA microarrays require intensive labor, are expensive, and have low sensitivity and limited specificity (Burgess, 2001; Corney and Basturea, 2014). RNA-seq is high cost and requires complex computation for data analysis (Hitzemann *et al*., 2013). However, ACP is a differential display technique that overcomes low sensitivity and high cost.

According to GeneFishing™ DEG Premix Kit's manual, ACP has three structures; core sequence at the 3'-end core, regulator, and universal sequence at the 5'-end core. The ACP system consists of a two-stage PCR amplification. The first stage PCR was set for the annealing of the core sequence at the 3'-end core, but not for the annealing of the regulator. Consequently, universal sequence at the 5'-end core is interrupted. The second stage PCR was set to amplify the product which generated from the first stage PCR. These conditions protect annealing between the 3'-end core portion of the ACP and the original template. The primers only amplify from the 3' and 5' of the first PCR product (Diagram 1) (Seegene, Rockville, MD).

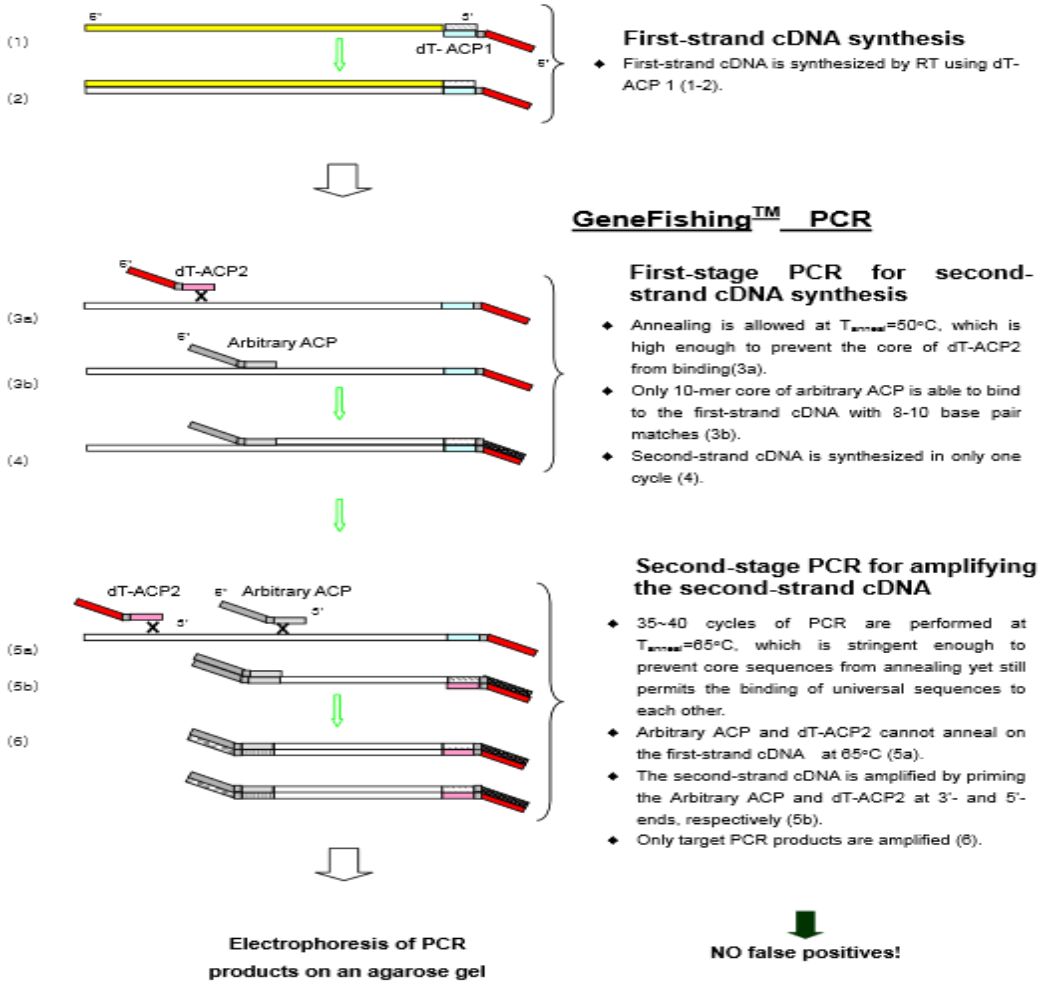

Diagram 1 Flow chart of annealing control primers system and the GeneFishing™ PCR. (https://www.funakoshi.co.jp/data/datasheet/SEE/K1022.pdf).

Thus, ACP is designed to identify differentially expressed genes in certain biological processes and disclose abundant, transient, and rarely expressed transcripts (Ramanarao *et al*., 2012). End rots appear to be diseases incited by physiology. Our understanding of variables contributing to enhanced incidence of end rot is circumstantial. The research objectives of this study were: 1) Effect of environmental factors on expression of end rot in sweetpotato roots; 2) Understand the role of calcium deficiency on end rot incidence; 3) Identify expressed genes in storage roots treated with ethephon and 1-MCP.

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CHAPTER 2. EFFECT OF ENVIRONMENTAL FACTORS ON EXPRESSION OF END ROT IN SWEETPOTATO ROOTS

2.1 Introduction

Storage rots in sweetpotato reduce the profitability of the crop. Growers recognize the importance of carefully handling roots at harvest and disposing of those with obvious rots and skinning. Still growers have encountered significant loss to a disease/physiological complex now affecting a significant portion of the stored crop. Ray *et al*. (1994) found that mean weight loss of 20% was observed during 45 days of storage due to fungal decay and weevil infestation. Rees *et al*. (2001) noted that damaged roots due to breakage, cuts, infestation by sweetpotato weevils, rotting and superficial damage had a shorter shelf life. Moreover, these roots also had increased fresh weight loss and rotting. Weight loss due to water loss over months of storage is a hidden loss to most growers. Rots in contrast are obvious and represent several different diseases each brought about by a different set of circumstances. In the past decade, end rot has emerged in some sweetpotato production regions, with the highest incidence reported in Mississippi (Aranciabia *et al*., 2013). Biotic and abiotic stress can be equally implicated. End rots of sweetpotato storage roots are commonly caused by *Fusarium solani* (Clark, 1980; da Silva, 2013) and *Macrophomina phaseolina* (da Silva, 2013). It has been suggested that mechanical damage, wet and cold or excessively dry soil at harvest, exposure to high or low temperatures for extended time after harvest and conditions favoring desiccation of wounded tissue could predispose sweetpotatoes to end rot development during storage (Clark *et al*., 2013a).

It is likely that many environments may trigger or favor end rot development in sweetpotato such as flooding, mechanized harvest, lack of curing and poor storage. Each has a unique but unknown impact on quality and storage. Louisiana frequently encounters hurricanes and tropical rains that damage sweetpotato fields and crop loss due to flooding can be catastrophic. Roberts

and Russo (1991) found that flooding at midseason reduced sweetpotato yield by 36% in 1989 and by 53% in 1990. Loss occurs in many ways (asphyxiation, rot) and is not easily diagnosed. Clark and da Silva (2012) reported that varieties differ greatly. Beauregard had more damage in moderately flooded plots than Bonita, Covington, Evangeline, Orleans or 07-146. Flooded fields led to an increase in the incidence of end rots and other diseases during sweetpotato storage.

Increased mechanization effectively reduces production costs in sweetpotato. It is estimated that increased mechanization of harvest operations can reduce labor expense by 13-25% of the total production cost (Agron, 2009) and 40% of the total cost in Louisiana (Guidry *et al*. 2015). However, increased mechanization can result in more extensive skinning damage. Rees *et al*. (2001) reported that postharvest deterioration was mostly attributed to weight loss and rotting. A plausible hypothesis is that water loss results in tissue stress, which reduces the ability of the tissue to defend itself against pathogen invasion (Rees *et al*., 2003).

Lack of curing and proper storage increase the incidence of diseases because wounds are not properly healed which can allow pathogens to enter (Aidoo, 1993). Roots must be cured immediately at 29 ^{o}C and a relative humidity of 85 to 90 percent for 4 to 7 days (Edmunds et al., 2008). During curing, the outermost parenchyma cells at the wound site desiccate slightly. Uncured roots desiccate to a greater degree. Lignification and increased sugar at the wound site has been shown to be correlated with wound healing (Rees *et al*., 2008). Arancibia *et al*. (2013) found that cured roots had lower tip rot and end rot incidence. Clark *et al*. (2013b) also reported that curing reduced the proximal end rot. Following curing, sweetpotatoes should be stored at 13°C with 85-90% relative humidity and with adequate ventilation (Edmunds et al., 2008). Storage roots of commercial cultivars, such as Beauregard and Covington can store up to 13 months and remain marketable (Smith *et al*., 2009; Edmunds *et al*., 2008).

Environmental variables affecting end rot have not been tested together in a systematic way. Thus, the objective of this research is to evaluate the influence of abiotic factors such as flooding, severity of harvest (skinning), curing, and storage conditions on the incidence and development of end rots in sweetpotato storage roots.

2.2 Materials and methods

Experiments were conducted at the Louisiana State University Agricultural Center—Sweet Potato Research Station (Chase, LA (lat. 32º6'N, long. 91º42'W)) in 2012 and 2013. The soil type was fine-silt mixed, active, thermic Typic Glossaqualfs. 'Beauregard', (B14) from foundation seed stock was used. Field preparation activities and inputs, including fertilizer applications with the rate of 1,000 lbs/acre of a 4-11-11 liquid fertilizer to equal 40 lbs of Nitrogen, and 110 lbs each of phosphorus and potassium were applied each year (Smith personal communication, 2012), herbicide, and insecticide applications were similar in each year (Boudreaux, 2005) and supplemental overhead irrigation was supplied with a traveling irrigation sprinkler if a rainfall event did not occur to maintain soil at 30-50% field capacity or 15% volumetric water content (Smith and Villordon, 2009). Between row plant spacing was 1 meter with plants spaced 30 centimeters apart within a row. Transplants were 30 centimeters long and set 8 to 10 cm deep in the soil. Planting was on 1 July 2012 and 1 July 2013. Storage roots were harvested on 7 November 2012 (130 days after planting, DAP) and 19 November 2013 (142 DAP). The experimental design for each year was randomized complete block design (RCBD) with three replications (15 meters long with 40 rows). Factorial combinations consisted of flooding/non-flooding; skinned/non-skinned; cured/non-cured; recommended storage/ambient storage (Diagram 2).

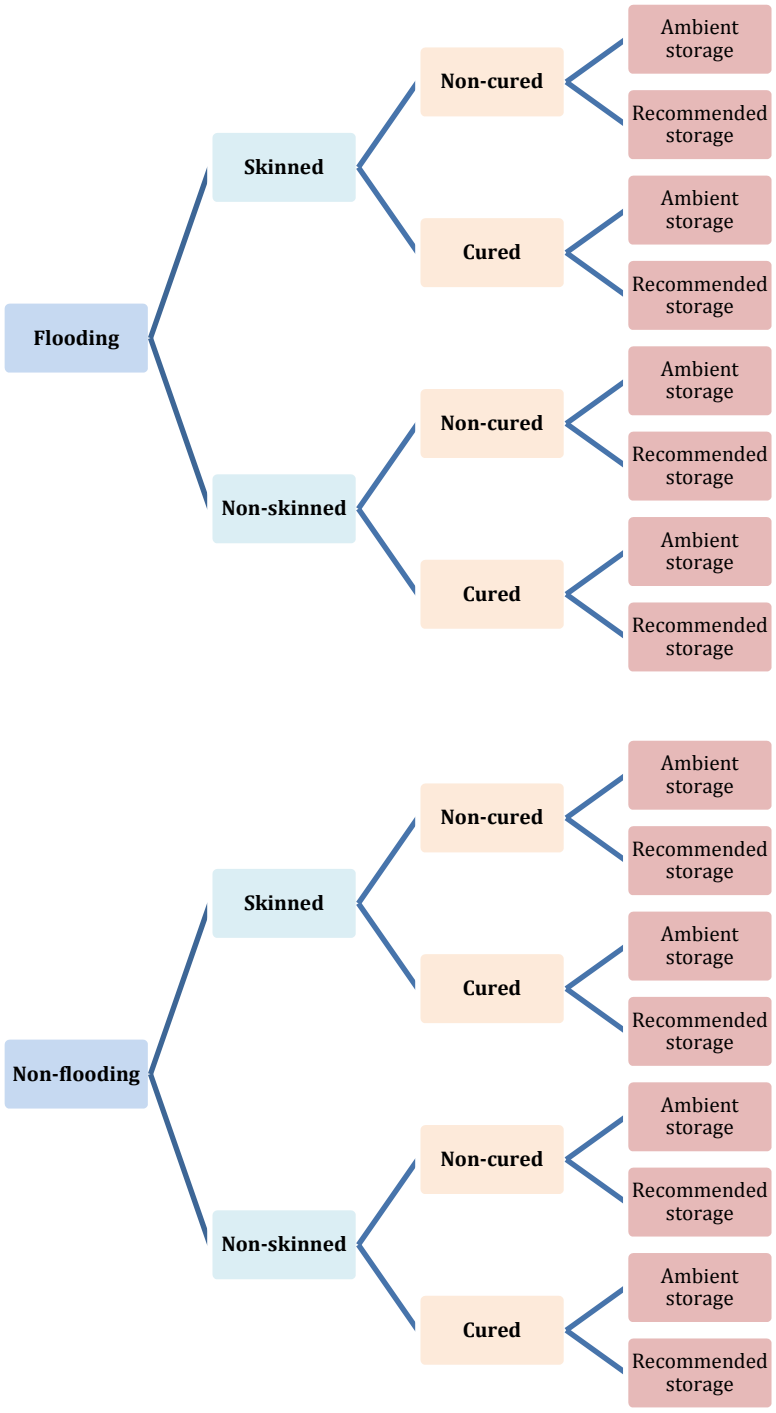

Diagram 2: Storage roots were in all possible combinations (16 treatments).

Storage roots were treated/held under all possible combinations. Main plots were divided into 2 subplots (20 rows in each subplot). I) One subplot was flooded for 2 weeks before harvesting as flooded treatment. Another subplot was harvested at the same time with no supplemental irrigation during the last 2 weeks as non-flooded treatment. II) At harvest, vines in two subplots were removed by mowing. Each subplot was divided into two parts (10 rows in each sub-subplot). Ten rows were mechanically harvested to generate skinning damage and bruises with a conventional 2 row riding harvester with a 1.5 meter drops as skinned treatment. The other ten rows were harvested using a single row mechanical chain harvester with a 0.6 meter drop to minimize skinning and bruising damage as a non-skinned treatment. 40 U.S. No. 1 size roots (from 4 to 9 centimeters diameter, 8 to 23 centimeters long) from each of four sub-subplots were stored in 240 cardboard boxes. Each sub-subplot consisted of 2,400 sweetpotato roots (60 cardboard boxes with three replications). III) After harvest, half of the storage roots in each combination (30 cardboard boxes) were cured at a temperature of 29^{o}C and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately. The remainder were not cured (30 cardboard boxes) and stored at a room temperature of 18.3^{o}C and a relative humidity of 70 percent. IV) Half of the roots (15 cardboard boxes with three replications) were stored in the recommended storage (13°C with 85-90% relative humidity and with adequate ventilation) and the remainder (15 cardboard boxes) were stored in an insulated storage building (11^{o}C Avg.; 6.8-28.7 ^{o}C and 36-98% RH in 2012, 11^{o}C Avg.; 4.1-22^{o}C and 34-96% RH in 2013) without temperature and humidity control capabilities. Heat was added during cold periods to prevent temperatures approaching 0 ^{o}C. Data from two years were analyzed similarly. Each storage treatment consisted of 600 sweetpotato roots stored in 15 cardboard boxes with three replications. Sweetpotato boxes were weighed monthly for 6 months to calculate percent weight loss. The incidence of end rot (a dry rot

progressing from either proximal and/or distal ends) of the sweetpotato root were recorded. The severity of end rot was recorded using the percentage of root length affected by end rot and was measured monthly for 6 months. For sugar content determination, 6 roots with end rot incidence and 6 roots with no end rot incidence were used for comparison. Sweetpotato root tissues near infected areas, at the end of roots that were not infected, and the tissue from the middle of both infected and non-infected roots were used for analysis. Sugar content was determined following the protocol of Picha (1985). In brief, all samples were grated with a small size grater. The soft mass from grating was squeezed and filtered through Miracloth (EMD Chemicals, San Diego, CA) and 0.45 μm Nylon mesh. 2 ml of the sweetpotato liquid was used and mixed with 18 ml of 80% ethanol. The samples were filtered with Phenomenox syringe tip filters into 1-ml shell vials and kept in the freezer. Sugars were analyzed by HPLC with a model 156 refractive index detector. Sugar were separated in the column packed with Aminex HPX-87C resin (Bio-Rad Labs, Richmond, CA). HPLC grade H_2O at a flow rate of 1.2 mL/min was used as a mobile phase (Picha, 1985).

Data were analyzed using PROC MIXED in SAS (version 9.4; SAS Institute, Cary, NC) and differences among means were compared by Fisher's LSD test ($P \leq 0.05$). For fructose, glucose and sucrose contents, data were analyzed using PROC ANOVA in SAS (version 9.4; SAS Institute, Cary, NC) and differences among means were compared by Fisher's LSD test ($P \leq 0.05$) and standard error averaged over 2 years.

2.3 Results and discussion

Flooding. Saturated soil conditions imposed 14 days prior to harvest had a significant impact on weight loss in 2012, but not in 2013 (Table 1).

Table 1: F values and probabilities for weight loss, end rot incidence, and proximal and distal end rot severity of sweetpotato in 2012 and 2013.

Year	Effects[X]	% Weight Loss		% End Rot Incidence	
		F Value[Y]	Pr>F[Y]	F Value	Pr>F
2012	Flooded system (A)[1]	17.25	*0.0007*	1.60	0.2073
	Harvest system (B)[2]	16.49	*0.0009*	6.42	*0.0120*
	Cured/Non-cured (C)[3]	169.01	*<.0001*	44.25	*<.0001*
	Recommended storage[4] /Ambient storage (D)	103.96	*<.0001*	8.95	*0.0031*
	AxB	11.48	*0.0037*	0.30	0.5817
	AxC	1.06	0.3042	1.19	0.2770
	AxD	0.38	0.5410	0.26	0.6089
	BxC	3.81	0.0523	0.30	0.5862
	BxD	1.18	0.2778	0.21	0.6479

[X]T-Test, (flooding and non-flooding; skinning and non-skinning; cured and non-cured; ambient storage and recommended storage).
[Y]F value, Mean square/Mean square error. Effects were considered significant when Pr > F was < 0.05 (Italicized probabilities).
[1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting. [2] Harvested system was skinned roots VS non-skinned roots. Skinned roots were harvested by a conventional 2 row riding harvester with a 1.5 meter drops; Non-skinned roots were harvested by a single row harvester with a 0.6 meter drop. [3] Cured/Non-cured: Cured roots were cured at a temperature of 29ºC and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3ºC and a relative humidity of 70 percent. [4] Recommended storage/ambient storage: Recommended storage at 13ºC and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2ºC Avg.; 6.8-28.7ºC in 2012, 10.8ºC Avg.; 4.1-22.0ºC in 2013) without a controlled environment.

(Table 1 continued)

Year	Effects[X]	% Proximal End Rot Severity		% Distal End Rot Severity	
		F Value	Pr>F	F Value	Pr>F
2012	Flooded system (A)[1]	0.75	0.3997	0.75	0.3997
	Harvest system (B)[2]	1.60	0.2243	1.60	0.2243
	Cured/Non-cured (C)[3]	25.27	*<.0001*	25.27	*<.0001*
	Recommended storage[4] /Ambient storage (D)	1.27	0.2619	1.27	0.2619
	AxB	1.90	0.1867	1.90	0.1867
	AxC	0.10	0.7471	0.10	0.7471
	AxD	0.10	0.7478	0.10	0.7478
	BxC	0.00	0.9787	0.00	0.9787
	BxD	0.28	0.5948	0.28	0.5948

[X]T-Test, (flooding and non-flooding; skinning and non-skinning; cured and non-cured; ambient storage and recommended storage). [Y]F value, Mean square/Mean square error. Effects were considered significant when Pr > F was < 0.05 (Italicized probabilities). [1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting. [2] Harvested system was skinned roots VS non-skinned roots. Skinned roots were harvested by a conventional 2 row riding harvester with a 1.5 meter drops; Non-skinned roots were harvested by a single row harvester with a 0.6 meter drop. [3] Cured/Non-cured: Cured roots were cured at a temperature of 29ºC and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3ºC and a relative humidity of 70 percent. [4] Recommended storage/ambient storage: Recommended storage at 13ºC and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2ºC Avg.; 6.8-28.7ºC in 2012, 10.8ºC Avg.; 4.1-22.0ºC in 2013) without a controlled environment.

(Table 1 continued)

Year	Effects[X]	% Weight Loss		% End Rot Incidence	
		F Value[Y]	Pr>F[Y]	F Value	Pr>F
2013	Flooded system (A)[1]	2.45	0.1189	0.85	0.3708
	Harvest system (B)[2]	0.24	0.6256	1.03	0.3256
	Cured/Non-cured (C)[3]	21.52	*<.0001*	70.04	*<.0001*
	Recommended storage[4] /Ambient storage (D)	56.23	*<.0001*	416.41	*<.0001*
	AxB	4.65	*0.0320*	0.83	0.3768
	AxC	2.41	0.1218	0.10	0.7557
	AxD	0.02	0.8902	1.10	0.2959
	BxC	4.19	*0.0418*	2.01	0.1583
	BxD	0.97	0.3266	2.09	0.1501
Significant 2012 x 2013		**		**	

[X]T-Test, (flooding and non-flooding; skinning and non-skinning; cured and non-cured; ambient storage and recommended storage).
[Y]F value, Mean square/Mean square error. Effects were considered significant when Pr > F was < 0.05 (Italicized probabilities).
[1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting. [2] Harvested system was skinned roots VS non-skinned roots. Skinned roots were harvested by a conventional 2 row riding harvester with a 1.5 meter drops; Non-skinned roots were harvested by a single row harvester with a 0.6 meter drop. [3] Cured/Non-cured: Cured roots were cured at a temperature of 29ºC and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3ºC and a relative humidity of 70 percent. [4] Recommended storage/ambient storage: Recommended storage at 13ºC and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2ºC Avg.; 6.8-28.7ºC in 2012, 10.8ºC Avg.; 4.1-22.0ºC in 2013) without a controlled environment.

(Table 1 continued)

Year	Effects[X]	% Weight Loss		% End Rot Incidence	
		F Value[Y]	Pr>F[Y]	F Value	Pr>F
2013	Flooded system (A)[1]	0.97	0.3267	1.61	0.2227
	Harvest system (B)[2]	2.79	0.0960	3.73	0.0714
	Cured/Non-cured (C)[3]	13.49	*0.0003*	3.93	*0.0487*
	Recommended storage[4] /Ambient storage (D)	47.36	*<.0001*	89.95	*<.0001*
	AxB	2.24	0.1360	11.61	*0.0036*
	AxC	5.91	*0.0158*	0.00	0.9604
	AxD	0.10	0.7469	0.89	0.3472
	BxC	0.00	0.9626	0.19	0.6657
	BxD	0.36	0.5493	0.30	0.5859
Significant 2012 x 2013		**		**	

[X]T-Test, (flooding and non-flooding; skinning and non-skinning; cured and non-cured; ambient storage and recommended storage).
[Y]F value, Mean square/Mean square error. Effects were considered significant when Pr > F was < 0.05 (Italicized probabilities).
[1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting. [2] Harvested system was skinned roots VS non-skinned roots. Skinned roots were harvested by a conventional 2 row riding harvester with a 1.5 meter drops; Non-skinned roots were harvested by a single row harvester with a 0.6 meter drop. [3] Cured/Non-cured: Cured roots were cured at a temperature of 29ºC and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3ºC and a relative humidity of 70 percent. [4] Recommended storage/ambient storage: Recommended storage at 13ºC and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2ºC Avg.; 6.8-28.7ºC in 2012, 10.8ºC Avg.; 4.1-22.0ºC in 2013) without a controlled environment.

The expectation was that physiological stress enhanced disease susceptibility and weight loss. Daytime high during flooding period was similar in 2012 (12ºC Avg.) and 2013 (11ºC Avg.) Rain fall was likewise similar in 2012 (0.4 cm) and 2013 (0.3 cm) during flooding periods; these factors were unlikely contributors to the differences observed. However, it is difficult to maintain a saturated environment and this may account for differences between the two years. Flooding escalates asphyxiation and thereby promotes lenticel proliferation which in turn allows an entry point for pathogens and a channel for increased water loss (Clark *et al*., 2013a). Ahn *et al*. (1980) found that sweetpotatoes flooded under high temperatures (24 to 34ºC) for 1 week showed an increase in root rotting during curing and storage. However, flooding had no impact on end rot incidence and severity in both years (Table 1). The fields were artificially flooded and it is possible that soil saturation was not great enough to mimic a severe natural inundation of water. The present study does not preclude the impact flooding might have on end rot prevalence. Using the measurement of soil moisture in the flooding comparisons may improve understanding of the flooding factor on end rot incidence.

Harvest system. A 1.5 m drop from the harvester resulted in a significant weight loss and increased incidence in 2012, but not in 2013 (Table 1). The increased abrasion and physical drop likely increased skinning and tissue bruising. Injury sites aid entry of pathogens and allow increased desiccation of storage roots (Rees *et al*., 2003) Tomlinds *et al.* (2002) also reported skinning injury was involved with an increase in weight loss and occurrence of rots. After sweetpotato was cut during harvesting or handling, new cells took several days to grow and form a protective layer to protect interior cells from infection (Sumner, 1984). The harvest systems were identical for the two years for end rot. Moreover, bacterial soft rot incidence (caused by *Dickeya dadantii* or *Clostridium* spp.) was higher in 2013 than in 2012. Mechanical damage may have

greater impact on expression of bacterial soft rot than end rot causing organisms. These results showed that a careful harvest can reduce loss; however, variability from year to year is a given. These contrasting results demonstrate how difficult it is to have consistent injuries across years; nonetheless, results showed harvest can, at times, impact storage root susceptibility to end rot. Although the different harvest systems caused the different skinning level, storage root skinning should be measured to give us the information about mechanical damage on storage roots.

Curing/Non-curing. Curing is recommended immediately after harvesting. Wounds from harvesting provide openings for water loss and pathogen attack. Curing helps to reduce weight loss and pathogen invasion by wound healing and also improves culinary characteristics (Edmunds *et al*., 2008). This study indicated that cured/non-cured factor was significant for weight loss, end rot incidence, and proximal and distal end rot severity in both years (Table 1). Curing promotes wound healing by forming suberized wound periderm resulting in reducing weight loss and preventing pathogen infection (Clark *et al*., 2013a). Results showed curing was very important to reduce weight losses and end rot incidence and severity and increase shelf life.

Recommended storage/ambient storage. Recommended storage/ambient storage factor was significant for weight loss and end rot incidence in both years. However, recommended storage/ambient storage factor was significant for proximal and distal end rot severity only in 2013 (Table 1). Storage at 13ºC with 85-90% relative humidity (RH) and adequate ventilation is recommended for storing roots and can extend storage up to 13 months (Smith *et al*., 2009). The ambient storage was in an insulated storage building without climate control capabilities (15.2ºC Avg.; 6.8-28.7ºC and 36-98% RH in 2012, 10.8ºC Avg.; 4.1-22.0ºC and 34-96% RH in 2013). The ambient storage had higher fluctuations of temperature and relative humidity during storage than the recommended storage which contributed to increase weight loss and end rot incidence. Weight

loss primarily depends on respiration and transpiration processes (Picha, 1986). Thus weight loss relates to temperature and relative humidity. Wills *et al*. (2007) stated that weight also depends on temperature of product and environment, relative humidity, and the maturity of product. Edmunds *et al*. (2008) reported that more than five degrees of temperature changes in sweetpototato can cause excessive weight loss and excessive humidity caused condensation in storage rooms, which can promote storage root decay. It is possible that fluctuations in both temperature and the humidity in the ambient storage led to enhance weight loss and end rot incidence in sweetpotato. However, proximal and distal end rot severities were significantly affected in 2013, but not in 2012. Average temperature during the ambient storage in 2013 (10.8^{o}C) was lower than in 2012 (15.2^{o}C) and low temperatures reached 4^{o}C in 2013. The environment enhanced end rot in sweetpotato roots in 2013. In addition, bacterial soft rot incidence was higher in 2013 than in 2012. Unfavorable environments may enhance weight loss and decay on storage roots in both ends. Thus, recommended storage is a primary factor to mitigate weight loss and control end rot incidence and severity.

Cured and recommended storage combinations. Among the factor combinations, data showed that the treatments that had the combinations of cured and recommended storage had lower weight loss in 2012 (Table 2 and Figure 1) and in 2013 (Table 3 and Figure 1), end rot incidence in 2012 (Table 4 and Figure 2) and in 2013 (Table 5 and Figure 2) and severity (Figure 3). Weight loss in cured and recommended combinations was only 17-21% in 2012 and 23-28% in 2013 compared to other combinations which were higher at 29% in 2012 and 70% in 2013. Booth (1974) reported that weight loss after 113 days of storage was 17% in the cured samples and 42% in uncured samples.

Table 2: Effects of flooding and non-flooding; skinning and non-skinning; cured and non-cured; ambient storage and recommended storage combinations on percent weight loss of sweetpotato in 2012.

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	Weight loss (%)		
				30 d	60 d	90 d
Flooding	Skinning	Non-cured	Ambient	$13.02\pm0.84^{A,f*}$	$17.70\pm1.27^{A,e}$	$20.03\pm1.60^{A,d}$
			Recommended	$13.31\pm0.89^{A,f}$	$16.43\pm0.83^{BC,e}$	$17.86\pm0.57^{B,d}$
		Cured	Ambient	$10.62\pm1.13^{DE,f}$	$14.22\pm0.59^{EF,e}$	$16.44\pm0.56^{BC,d}$
			Recommended	$10.44\pm0.35^{EF,f}$	$12.79\pm0.53^{GH,e}$	$14.05\pm0.51^{DE,d}$
	Non-skinning	Non-cured	Ambient	$12.99\pm1.13^{AB,f}$	$17.50\pm0.33^{AB,e}$	$19.69\pm0.28^{A,d}$
			Recommended	$11.96\pm0.17^{BC,f}$	$15.44\pm0.17^{CD,e}$	$16.91\pm0.27^{BC,d}$
		Cured	Ambient	$10.17\pm0.12^{EF,e}$	$14.00\pm0.06^{EF,d}$	$16.09\pm0.15^{C,c}$
			Recommended	$10.67\pm1.17^{DE,f}$	$12.81\pm1.42^{GH,e}$	$14.13\pm1.44^{D,d}$

*Mean and standard deviation values (N=3). Means within column followed by different capital letters are significantly different from each treatment combination and means within row followed by different lowercase letters are significantly different from each month by Fisher's LSD test ($P \leq 0.05$).

[1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting.

[2] Harvested system was skinned roots VS non-skinned roots. Skinned roots were harvested by a conventional 2 row riding harvester with a 1.5 meter drops; Non-skinned roots were harvested by a single row harvester with a 0.6 meter drop.

[3] Cured/Non-cured: Cured roots were cured at a temperature of 29°C and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3°C and a relative humidity of 70 percent.

[4] Recommended storage/ambient storage: Recommended storage at 13°C and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2°C Avg.; 6.8-28.7°C in 2012, 10.8°C Avg.; 4.1-22.0°C in 2013) without a controlled environment.

(Table 2 continued)

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	Weight loss (%)		
				120 d	150 d	180 d
Flooding	Skinning	Non-cured	Ambient	22.00±1.76^{A,c}	24.46±2.03^{A,b}	27.36±1.63^{A,a}
			Recommended	19.22±0.73^{B,c}	20.84±1.23^{B,b}	22.35±1.43^{BC,a}
		Cured	Ambient	18.19±0.35^{B,c}	20.02±0.21^{B,b}	21.40±0.23^{CD,a}
			Recommended	15.38±0.49^{CD,c}	16.74±0.53^{CD,b}	18.31±0.42^{EF,a}
	Non-skinning	Non-cured	Ambient	21.77±0.18^{A,c}	24.42±0.34^{A,b}	26.75±1.24^{A,a}
			Recommended	18.40±0.20^{B,c}	20.25±0.05^{B,b}	22.10±0.36^{BC,a}
		Cured	Ambient	18.26±0.13^{B,b}	20.60±1.45^{B,a}	21.85±1.41^{BC,a}
			Recommended	15.36±1.39^{CD,c}	17.00±1.35^{CD,b}	18.73±1.33^{EF,a}

*Mean and standard deviation values (N=3). Means within column followed by different capital letters are significantly different from each treatment combination and means within row followed by different lowercase letters are significantly different from each month by Fisher's LSD test ($P \leq 0.05$).

[1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting.

[2] Harvested system was skinned roots VS non-skinned roots. Skinned roots were harvested by a conventional 2 row riding harvester with a 1.5 meter drops; Non-skinned roots were harvested by a single row harvester with a 0.6 meter drop.

[3] Cured/Non-cured: Cured roots were cured at a temperature of 29°C and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3°C and a relative humidity of 70 percent.

[4] Recommended storage/ambient storage: Recommended storage at 13°C and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2°C Avg.; 6.8-28.7°C in 2012, 10.8°C Avg.; 4.1-22.0°C in 2013) without a controlled environment.

(Table 2 continued)

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	Weight loss (%)		
				30 d	60 d	90 d
Non-flooding	Skinning	Non-cured	Ambient	$12.29\pm0.16^{ABC,f}$	$17.05\pm0.41^{AB,e}$	$20.44\pm0.78^{A,d}$
			Recommended	$11.51\pm0.15^{CD,f}$	$14.82\pm0.45^{DE,e}$	$16.12\pm0.75^{C,d}$
		Cured	Ambient	$9.54\pm0.75^{FGH,f}$	$13.55\pm1.16^{FG,e}$	$16.68\pm2.43^{BC,d}$
			Recommended	$10.08\pm0.32^{EFG,f}$	$12.11\pm0.22^{HI,e}$	$13.38\pm0.25^{DE,d}$
	Non-skinning	Non-cured	Ambient	$10.68\pm0.44^{DE,f}$	$14.17\pm0.31^{EF,e}$	$16.90\pm0.66^{BC,d}$
			Recommended	$10.18\pm0.74^{EF,f}$	$12.78\pm0.29^{GH,e}$	$14.33\pm0.46^{D,d}$
		Cured	Ambient	$8.84\pm0.23^{H,f}$	$12.03\pm0.26^{HI,e}$	$14.27\pm0.37^{D,d}$
			Recommended	$9.06\pm0.75^{GH,f}$	$11.25\pm0.58^{I,e}$	$12.52\pm0.45^{E,d}$

*Mean and standard deviation values (N=3). Means within column followed by different capital letters are significantly different from each treatment combination and means within row followed by different lowercase letters are significantly different from each month by Fisher's LSD test ($P \leq 0.05$).

[1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting.

[2] Harvested system was skinned roots VS non-skinned roots. Skinned roots were harvested by a conventional 2 row riding harvester with a 1.5 meter drops; Non-skinned roots were harvested by a single row harvester with a 0.6 meter drop.

[3] Cured/Non-cured: Cured roots were cured at a temperature of 29°C and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3°C and a relative humidity of 70 percent.

[4] Recommended storage/ambient storage: Recommended storage at 13°C and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2°C Avg.; 6.8-28.7°C in 2012, 10.8°C Avg.; 4.1-22.0°C in 2013) without a controlled environment.

(Table 2 continued)

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	Weight loss (%)		
				120 d	150 d	180 d
Non-flooding	Skinning	Non-cured	Ambient	23.01±0.63^{A,c}	25.83±0.81^{A,b}	28.52±0.33^{A,a}
			Recommended	18.31±0.70^{B,c}	20.34±0.96^{B,b}	22.39±1.06^{BC,a}
		Cured	Ambient	18.61±2.50^{B,c}	20.75±2.75^{B,b}	22.60±2.54^{BC,a}
			Recommended	14.93±0.47^{CD,c}	16.33±0.41^{CD,b}	18.00±0.66^{EF,a}
	Non-skinning	Non-cured	Ambient	18.99±0.92^{B,c}	21.17±1.26^{B,b}	23.65±1.97^{B,a}
			Recommended	15.97±0.58^{C,c}	17.78±0.76^{C,b}	19.64±0.91^{DE,a}
		Cured	Ambient	16.23±0.46^{C,c}	17.92±0.65^{C,b}	19.75±0.74^{DE,a}
			Recommended	14.05±0.30^{D,c}	15.43±0.48^{D,b}	17.10±0.64^{F,a}

*Mean and standard deviation values (N=3). Means within column followed by different capital letters are significantly different from each treatment combination and means within row followed by different lowercase letters are significantly different from each month by Fisher's LSD test ($P \leq 0.05$).

[1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting.

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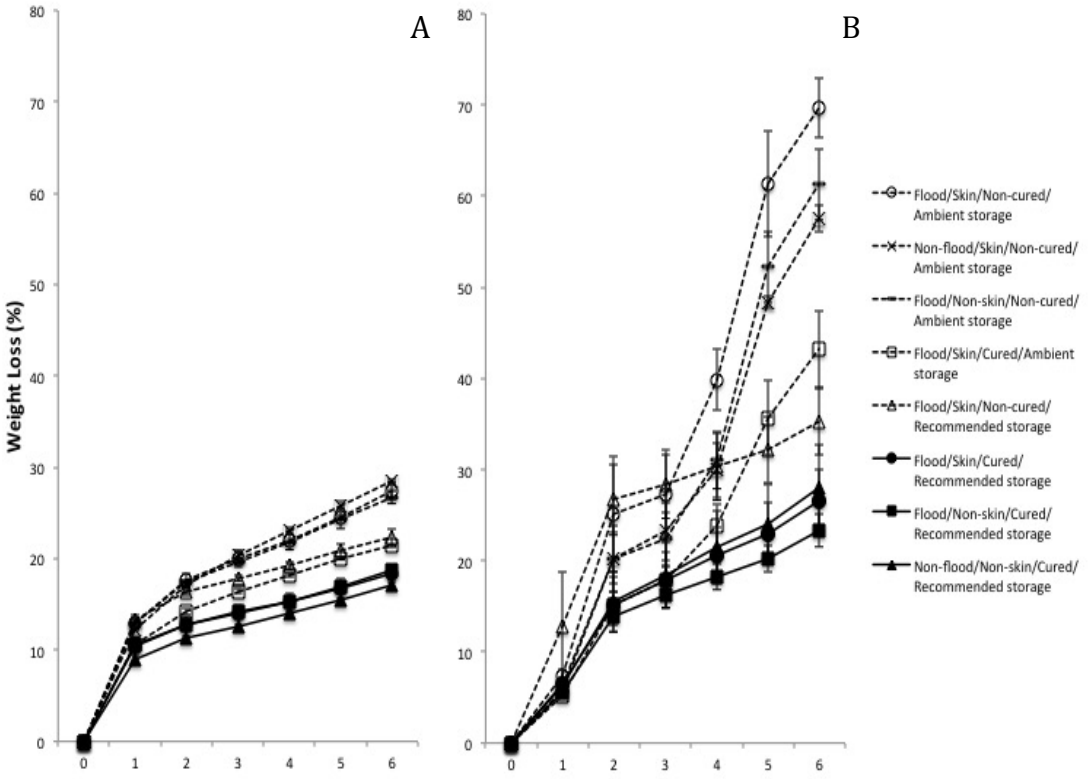

Figure 1: Percent weight loss of Beauregard sweetpotatoes during storage for 6 months after harvest as affected by: flooding and non-flooding (Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting); Skinning and non-skinning (Skinned roots were harvested by a conventional 2 row riding harvester with a 1.5 meter drops; Non-skinned roots were harvested by a single row harvester with a 0.6 meter drop); Cured and non-cured (Cured at a temperature of 29^{o}C and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3^{o}C and a relative humidity of 70 percent); Recommended storage/ambient storage (Recommended storage at 13^{o}C and a relative humidity of 85 to 90 percent and with adequate ventilation; Ambient storage was an open shed without a controlled environment) combinations on weight loss of sweetpotato in 2012 (A) and 2013 (B).

Table 3: Effects of flooding and non-flooding; skinning and non-skinning; cured and non-cured; ambient storage and recommended storage combinations on percent weight loss of sweetpotato in 2013.

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	Weight loss (%)		
				30 d	60 d	90 d
Flooding	Skinning	Non-cured	Ambient	7.34±1.4$^{B, e*}$	25.11±10.9$^{AB, d}$	27.20±7.7$^{AB, d}$
			Recommended	12.83±10.4$^{A, d}$	26.67±6.7$^{A, c}$	28.45±6.6$^{A, bc}$
		Cured	Ambient	5.28±0.5$^{B, e}$	15.21±2.4$^{C, d}$	17.86±2.7$^{CDE, d}$
			Recommended	6.38±0.8$^{B, e}$	15.24±5.3$^{C, d}$	17.83±5.4$^{CDE, cd}$
	Non-skinning	Non-cured	Ambient	5.69±0.5$^{B, e}$	20.31±4.9$^{ABC, d}$	22.31±5.3$^{ABCD, d}$
			Recommended	6.15±0.8$^{B, f}$	16.22±1.2$^{C, e}$	19.42±2.3$^{CDE, d}$
		Cured	Ambient	5.51±0.2$^{B, f}$	17.37±2.4$^{BC, e}$	20.33±1.5$^{BCDE, d}$
			Recommended	5.61±0.3$^{B, f}$	13.96±1.8$^{C, e}$	16.27±2.4$^{CDE, d}$

*Mean and standard deviation values (N=3). Means within column followed by different capital letters are significantly different from each treatment combination and means within row followed by different lowercase letters are significantly different from each month by Fisher's LSD test ($P \leq 0.05$).

[1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting.

[2] Harvested system was skinned roots VS non-skinned roots. Skinned roots were harvested by a conventional 2 row riding harvester with a 1.5 meter drops; Non-skinned roots were harvested by a single row harvester with a 0.6 meter drop.

[3] Cured/Non-cured: Cured roots were cured at a temperature of 29^{o}C and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3^{o}C and a relative humidity of 70 percent.

[4] Recommended storage/ambient storage: Recommended storage at 13^{o}C and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2^{o}C Avg.; 6.8-28.7^{o}C in 2012, 10.8^{o}C Avg.; 4.1-22.0^{o}C in 2013) without a controlled environment.

(Table 3 continued)

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	Weight loss (%)		
				120 d	150 d	180 d
Flooding	Skinning	Non-cured	Ambient	39.86±5.9$^{A, c}$	61.32±10.0$^{A, b}$	69.69±5.7$^{A, a}$
			Recommended	30.32±6.4$^{BCD, bc}$	32.21±6.4$^{DE, ab}$	35.32±6.4$^{FG, a}$
		Cured	Ambient	23.82±4.1$^{CDEF, c}$	35.68±7.1$^{D, b}$	43.14±7.5$^{EF, a}$
			Recommended	20.64±5.2^{EF,bc}	23.02±5.9$^{EF, ab}$	26.53±6.1$^{GH, a}$
	Non-skinning	Non-cured	Ambient	31.01±5.4$^{BC, c}$	52.21±6.8$^{AB, b}$	61.25±6.7$^{ABC, a}$
			Recommended	21.60±2.1$^{EF, c}$	23.77±1.8$^{EF, b}$	27.19±1.7$^{GH, a}$
		Cured	Ambient	25.85±1.0$^{BCDE, c}$	40.06±1.9$^{CD, b}$	48.69±2.5$^{DE, a}$
			Recommended	18.24±2.5$^{F, c}$	20.25±2.6$^{F, b}$	23.35±3.2$^{H, a}$

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[3] Cured/Non-cured: Cured roots were cured at a temperature of 29ºC and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3ºC and a relative humidity of 70 percent.

[4] Recommended storage/ambient storage: Recommended storage at 13ºC and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2ºC Avg.; 6.8-28.7ºC in 2012, 10.8ºC Avg.; 4.1-22.0ºC in 2013) without a controlled environment.

(Table 3 continued)

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	Weight loss (%)		
				30 d	60 d	90 d
Non-flooding	Skinning	Non-cured	Ambient	6.25±0.5$^{B, e}$	20.24±6.3$^{ABC, d}$	23.22±5.6$^{ABC, cd}$
			Recommended	6.22±1.3$^{B, e}$	16.07±5.8$^{C, d}$	18.27±5.5$^{CDE, cd}$
		Cured	Ambient	5.36±0.7$^{B, e}$	12.77±2.8$^{C, d}$	14.80±3.1$^{E, d}$
			Recommended	5.47±1.1$^{B, e}$	13.36±3.6$^{C, d}$	15.47±3.4$^{DE, d}$
	Non-skinning	Non-cured	Ambient	5.63±0.4$^{B, e}$	15.33±2.8$^{C, d}$	18.02±2.4$^{CDE, d}$
			Recommended	5.53±0.6$^{B, f}$	13.53±4.2$^{C, e}$	17.09±3.8$^{CDE, d}$
		Cured	Ambient	5.59±1.0$^{B, e}$	14.64±6.1$^{C, d}$	16.84±6.2$^{CDE, cd}$
			Recommended	6.29±1.4$^{B, e}$	15.50±5.8$^{C, d}$	18.47±6.3$^{CDE, cd}$

*Mean and standard deviation values (N=3). Means within column followed by different capital letters are significantly different from each treatment combination and means within row followed by different lowercase letters are significantly different from each month by Fisher's LSD test ($P \leq 0.05$).

[1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting.

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(Table 3 continued)

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	Weight loss (%)		
				120 d	150 d	180 d
Non-flooding	Skinning	Non-cured	Ambient	29.92±5.3$^{BCD, c}$	48.38±1.2$^{BC, b}$	57.52±2.5$^{BCD, a}$
			Recommended	20.89±5.5$^{EF, bc}$	23.06±5.9$^{EF, b}$	27.81±6.0$^{GH, a}$
		Cured	Ambient	20.87±1.3$^{EF, c}$	36.75±5.7$^{D, b}$	43.87±6.2$^{EF, a}$
			Recommended	18.03±3.6$^{F, c}$	20.16±3.7$^{F, b}$	23.53±3.5$^{H, a}$
	Non-skinning	Non-cured	Ambient	31.73±2.5$^{B, c}$	58.07±7.5$^{A, b}$	66.41±7.9$^{AB, a}$
			Recommended	21.30±3.1$^{EF, c}$	24.67±2.9$^{EF, b}$	28.89±2.6$^{GH, a}$
		Cured	Ambient	23.17±7.2$^{DEF, c}$	46.69±10.5$^{BC, b}$	54.52±9.5$^{CD, a}$
			Recommended	21.50±7.0$^{EF, bc}$	24.00±7.6$^{EF, ab}$	28.08±8.2$^{GH, a}$

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Table 4: Effects of flooding and non-flooding; skinning and non-skinning; cured and non-cured; ambient storage and recommended storage combinations on end rot incidence of sweetpotato in 2012.

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	End Rot Incidence (%)		
				30 d	60 d	90 d
Flooding	Skinning	Non-cured	Ambient	$0.00\pm0.00^{d*}$	0.00 ± 0.00^{d}	$3.13\pm1.52^{A,\ cd}$
			Recommended	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	$2.49\pm1.00^{AB,\ b}$
		Cured	Ambient	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	$0.83\pm0.76^{DE,\ cb}$
			Recommended	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	$0.71\pm0.75^{DE,\ ab}$
	Non-skinning	Non-cured	Ambient	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	$2.83\pm0.76^{A,\ c}$
			Recommended	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	$1.17\pm0.29^{CDE,\ ab}$
		Cured	Ambient	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	$0.17\pm0.29^{DE,\ b}$
			Recommended	0.00 ± 0.00	0.00 ± 0.00	$0.50\pm0.50^{DE,\ a}$

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(Table 4 continued)

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	End Rot Incidence (%)		
				120 d	150 d	180 d
Flooding	Skinning	Non-cured	Ambient	5.45±3.69$^{A, bc}$	6.77±3.06$^{A, ab}$	10.08±4.36$^{A, a}$
			Recommended	2.66±1.15$^{BCD, ab}$	2.82±1.36$^{CDE, ab}$	3.68±0.98$^{BCD, a}$
		Cured	Ambient	1.22±0.48$^{CDEFG, b}$	1.72±1.18$^{DEFG, ab}$	2.13±0.82$^{DEFG, a}$
			Recommended	0.95±0.48$^{DEFG, a}$	1.12±0.76$^{EFG, a}$	1.29±1.05$^{DEFG, a}$
	Non-skinning	Non-cured	Ambient	3.33±0.58$^{B, bc}$	4.33±1.53$^{BC, ab}$	5.17±1.61$^{BC, a}$
			Recommended	2.33±1.89$^{BCDE, a}$	2.33±1.89$^{DEF, a}$	2.83±1.89$^{CDEFG, a}$
		Cured	Ambient	0.17±0.29$^{FG, b}$	0.33±0.29$^{G, ab}$	1.17±1.26$^{EFG, a}$
			Recommended	0.83±1.04$^{DEFG, a}$	0.83±1.04$^{FG, a}$	1.00±1.32$^{FG, a}$

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(Table 4 continued)

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	End Rot Incidence (%)		
				30 d	60 d	90 d
Non-flooding	Skinning	Non-cured	Ambient	0.00±0.00^{d}	0.00±0.00^{d}	1.33±1.15$^{BCD, a}$
			Recommended	0.00±0.00^{b}	0.00±0.00^{b}	2.87±1.48$^{A, a}$
		Cured	Ambient	0.00±0.00^{c}	0.00±0.00^{c}	0.88±0.66$^{DE, bc}$
			Recommended	0.00±0.00^{b}	0.00±0.00^{b}	0.33±0.58$^{DE, ab}$
	Non-skinning	Non-cured	Ambient	0.00±0.00^{c}	0.00±0.00^{c}	2.33±1.04$^{ABC, b}$
			Recommended	0.00±0.00^{c}	0.00±0.00^{c}	0.83±0.29$^{DE, bc}$
		Cured	Ambient	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00$^{E, b}$
			Recommended	0.00±0.00	0.00±0.00	0.00±0.00^{E}

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(Table 4 continued)

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	End Rot Incidence (%)		
				120 d	150 d	180 d
Non-flooding	Skinning	Non-cured	Ambient	2.10±0.86$^{BCDEF, c}$	4.31±0.60$^{BC, b}$	5.91±0.80$^{B, a}$
			Recommended	3.04±1.76$^{BC, a}$	3.40±1.82$^{BCD, a}$	3.57±1.67$^{BCDE, a}$
		Cured	Ambient	1.38±1.12$^{BCDEFG, bc}$	1.88±1.53$^{DEFG, ab}$	3.38±2.10$^{CDEF, a}$
			Recommended	0.77±0.93$^{DEFG, a}$	0.93±0.75$^{EFG, a}$	0.93±0.75$^{G, a}$
	Non-skinning	Non-cured	Ambient	2.33±1.04$^{BCDE, b}$	4.83±2.57$^{AB, a}$	5.17±2.36$^{BC, a}$
			Recommended	1.67±1.04$^{BCDEFG, ab}$	2.00±1.00$^{DEFG, a}$	2.17±1.04$^{DEFG, a}$
		Cured	Ambient	0.38±0.33$^{EF, b}$	1.04±0.44$^{EFG, a}$	1.42±0.52$^{DEFG, a}$
			Recommended	0.00±0.00^{G}	0.50±0.87^{FG}	0.50±0.87^{G}

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[4] Recommended storage/ambient storage: Recommended storage at 13ºC and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2ºC Avg.; 6.8-28.7ºC in 2012, 10.8ºC Avg.; 4.1-22.0ºC in 2013) without a controlled environment.

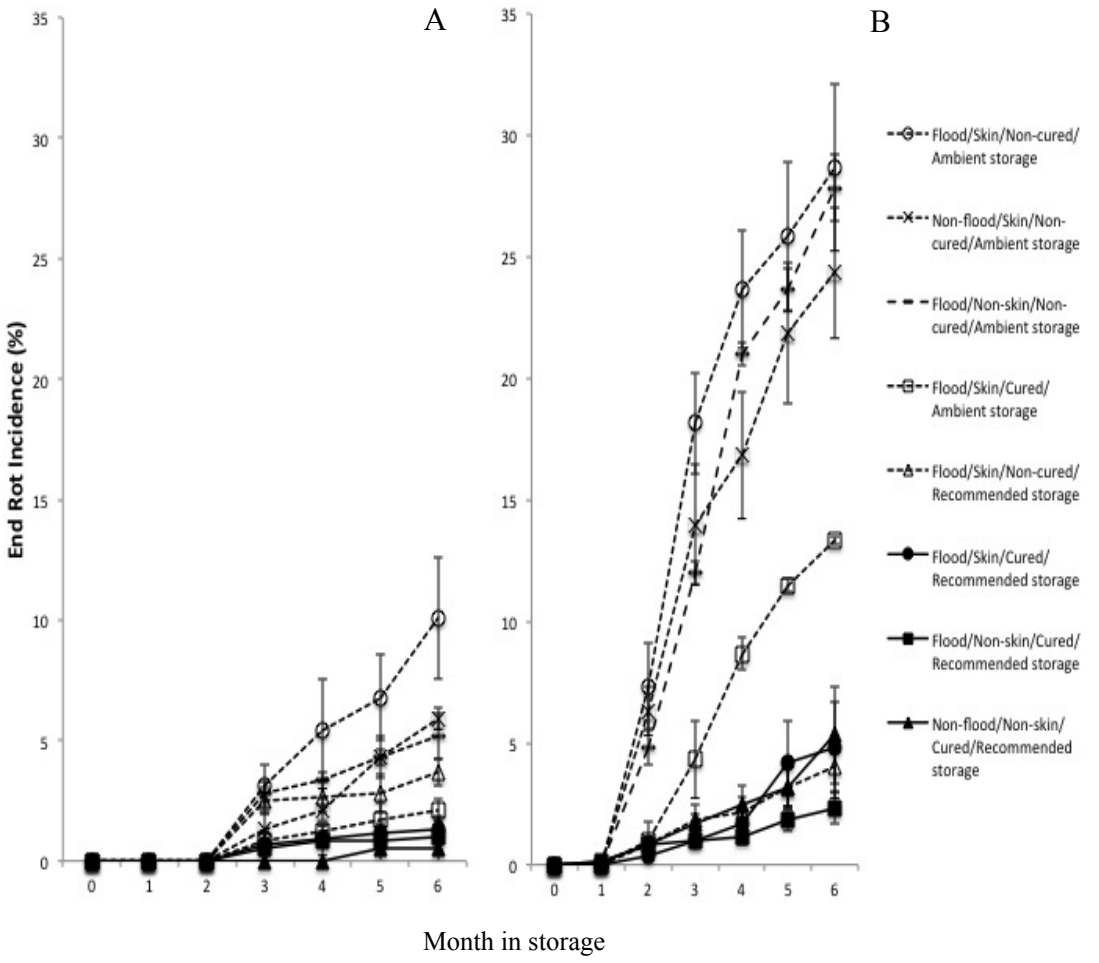

Figure 2: Flooding and non-flooding (Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting); Skinning and non-skinning (Skinned roots were harvested by a conventional 2 row riding harvester with a 1.5 meter drops; Non-skinned roots were harvested by a single row harvester with a 0.6 meter drop); Cured and non-cured (Cured at a temperature of 29^{o}C and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3^{o}C and a relative humidity of 70 percent); Recommended storage/ambient storage (Recommended storage at 13^{o}C and a relative humidity of 85 to 90 percent and with adequate ventilation; Ambient storage was an open shed without a controlled environment) combinations on end rot incidence of sweetpotato in 2012 (A) and 2013 (B).

Table 5: Effects of flooding and non-flooding; skinning and non-skinning; cured and non-cured; ambient storage and recommended storage combinations on end rot incidence of sweetpotato in 2013.

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	End Rot Incidence (%)		
				30 d	60 d	90 d
Flooding	Skinning	Non-cured	Ambient	0.00±0.00^{d*}	7.33±3.06$^{B, c}$	18.17±3.62$^{A, b}$
			Recommended	0.00±0.00^{d}	0.83±0.58$^{FG, cd}$	1.83±1.15$^{FG, bc}$
		Cured	Ambient	0.00±0.00^{d}	1.00±1.32$^{FG, d}$	4.33±2.75$^{DEF, c}$
			Recommended	0.00±0.00^{c}	0.33±0.29$^{G, c}$	1.00±0.50$^{FG, c}$
	Non-skinning	Non-cured	Ambient	0.00±0.00^{f}	4.83±1.26$^{CD, e}$	12.00±0.87$^{B, d}$
			Recommended	0.17±0.29^{d}	0.83±0.58$^{FG, c}$	1.33±1.04$^{FG, c}$
		Cured	Ambient	0.00±0.00^{e}	3.50±1.00$^{DE, d}$	8.33±2.36$^{C, c}$
			Recommended	0.00±0.00^{d}	0.83±0.29$^{FG, cd}$	1.00±0.50$^{FG, c}$

*Mean and standard deviation values (N=3). Means within column followed by different capital letters are significantly different from each treatment combination and means within row followed by different lowercase letters are significantly different from each month by Fisher's LSD test ($P \leq 0.05$).

[1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting.

[2] Harvested system was skinned roots VS non-skinned roots. Skinned roots were harvested by a conventional 2 row riding harvester with a 1.5 meter drops; Non-skinned roots were harvested by a single row harvester with a 0.6 meter drop.

[3] Cured/Non-cured: Cured roots were cured at a temperature of 29^{o}C and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3^{o}C and a relative humidity of 70 percent.

[4] Recommended storage/ambient storage: Recommended storage at 13^{o}C and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2^{o}C Avg.; 6.8-28.7^{o}C in 2012, 10.8^{o}C Avg.; 4.1-22.0^{o}C in 2013) without a controlled environment.

(Table 5 continued)

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	End Rot Incidence (%)		
				120 d	150 d	180 d
Flooding	Skinning	Non-cured	Ambient	23.67±4.19$^{A, a}$	25.83±5.35$^{A, a}$	28.67±5.92$^{A, a}$
			Recommended	2.17±1.04$^{EF, bc}$	3.17±1.44$^{D, ab}$	4.00±2.18$^{EFG, a}$
		Cured	Ambient	8.67±1.15$^{D, b}$	11.50±0.50$^{C, a}$	13.33±0.29$^{CD, a}$
			Recommended	1.67±1.26$^{EF, bc}$	4.17±3.01$^{D, ab}$	4.83±3.25$^{EFG, a}$
	Non-skinning	Non-cured	Ambient	21.00±0.87$^{A, c}$	23.67±1.53$^{A, b}$	27.83±2.36$^{A, a}$
			Recommended	1.50±1.00$^{EF, c}$	2.50±0.50$^{D, b}$	3.50±0.50$^{FG, a}$
		Cured	Ambient	14.17±2.02$^{BC, b}$	16.83±3.82$^{B, ab}$	18.83±3.62$^{B, a}$
			Recommended	1.17±0.58$^{EF, bc}$	1.83±0.76$^{D, ab}$	2.33±1.15$^{G, a}$

*Mean and standard deviation values (N=3). Means within column followed by different capital letters are significantly different from each treatment combination and means within row followed by different lowercase letters are significantly different from each month by Fisher's LSD test ($P \leq 0.05$).

[1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting.

[2] Harvested system was skinned roots VS non-skinned roots. Skinned roots were harvested by a conventional 2 row riding harvester with a 1.5 meter drops; Non-skinned roots were harvested by a single row harvester with a 0.6 meter drop.

[3] Cured/Non-cured: Cured roots were cured at a temperature of 29ºC and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3ºC and a relative humidity of 70 percent.

[4] Recommended storage/ambient storage: Recommended storage at 13ºC and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2ºC Avg.; 6.8-28.7ºC in 2012, 10.8ºC Avg.; 4.1-22.0ºC in 2013) without a controlled environment.

(Table 5 continued)

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	End Rot Incidence (%)		
				30 d	60 d	90 d
Non-flooding	Skinning	Non-cured	Ambient	0.00±0.00^{d}	6.33±1.76$^{BC, c}$	14.00±4.27$^{B, b}$
			Recommended	0.00±0.00^{d}	2.33±2.36$^{EFG, c}$	3.83±3.21$^{EFG, bc}$
		Cured	Ambient	0.00±0.00^{e}	2.17±0.58$^{EFG, e}$	6.83±2.52$^{CDE, d}$
			Recommended	0.00±0.00^{c}	0.50±0.50$^{G, c}$	0.50±0.50$^{G, c}$
	Non-skinning	Non-cured	Ambient	0.00±0.00^{e}	10.83±2.02$^{A, d}$	17.83±1.26$^{A, c}$
			Recommended	0.17±0.29^{d}	1.67±0.58$^{EFG, cd}$	3.33±1.61$^{FG, bc}$
		Cured	Ambient	0.17±0.29^{e}	3.00±1.32$^{DEF, d}$	7.50±0.50$^{CD, c}$
			Recommended	0.17±0.29^{c}	0.83±0.58$^{FG, bc}$	1.67±0.76$^{FG, bc}$

*Mean and standard deviation values (N=3). Means within column followed by different capital letters are significantly different from each treatment combination and means within row followed by different lowercase letters are significantly different from each month by Fisher's LSD test ($P \leq 0.05$).

[1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting.

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[3] Cured/Non-cured: Cured roots were cured at a temperature of 29ºC and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3ºC and a relative humidity of 70 percent.

[4] Recommended storage/ambient storage: Recommended storage at 13ºC and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2ºC Avg.; 6.8-28.7ºC in 2012, 10.8ºC Avg.; 4.1-22.0ºC in 2013) without a controlled environment.

(Table 5 continued)

Flooding system[1]	Harvest System[2]	Cured/ Non-cured[3]	Storage[4]	End Rot Incidence (%)		
				120 d	150 d	180 d
Non-flooding	Skinning	Non-cured	Ambient	16.83±4.51$^{B, b}$	21.83±5.01$^{A, a}$	24.33±4.65$^{A, a}$
			Recommended	4.67±2.57$^{E, b}$	5.83±1.53$^{D, b}$	8.50±1.73$^{DE, a}$
		Cured	Ambient	10.67±4.16$^{CD, c}$	14.50±2.78$^{BC, b}$	18.10±7.01$^{BC, a}$
			Recommended	0.67±0.29$^{F, bc}$	2.00±1.32$^{D, ab}$	2.17±1.26$^{G, a}$
	Non-skinning	Non-cured	Ambient	21.17±2.47$^{A, b}$	25.00±2.00$^{A, a}$	26.33±2.08$^{A, a}$
			Recommended	4.17±1.26$^{EF, b}$	5.17±1.89$^{D, ab}$	7.33±3.55$^{EF, a}$
		Cured	Ambient	13.00±0.87$^{C, b}$	16.67±1.26$^{B, a}$	17.17±1.44$^{BC, a}$
			Recommended	2.50±1.32$^{EF, bc}$	3.17±1.53$^{D, ab}$	5.33±3.51$^{EFG, a}$

*Mean and standard deviation values (N=3). Means within column followed by different capital letters are significantly different from each treatment combination and means within row followed by different lowercase letters are significantly different from each month by Fisher's LSD test ($P \leq 0.05$).

[1]Flooded system was flooded plot VS non-flooded plot. Flooded plot was flooded for 2 weeks before harvesting; Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting.

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[3] Cured/Non-cured: Cured roots were cured at a temperature of 29ºC and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest; Non-cured roots were stored at room temperature (18.3ºC and a relative humidity of 70 percent.

[4] Recommended storage/ambient storage: Recommended storage at 13ºC and a relative humidity of 85 to 90 percent and with adequate ventilation was used to store storage roots; Ambient storage was an open shed (15.2ºC Avg.; 6.8-28.7ºC in 2012, 10.8ºC Avg.; 4.1-22.0ºC in 2013) without a controlled environment.

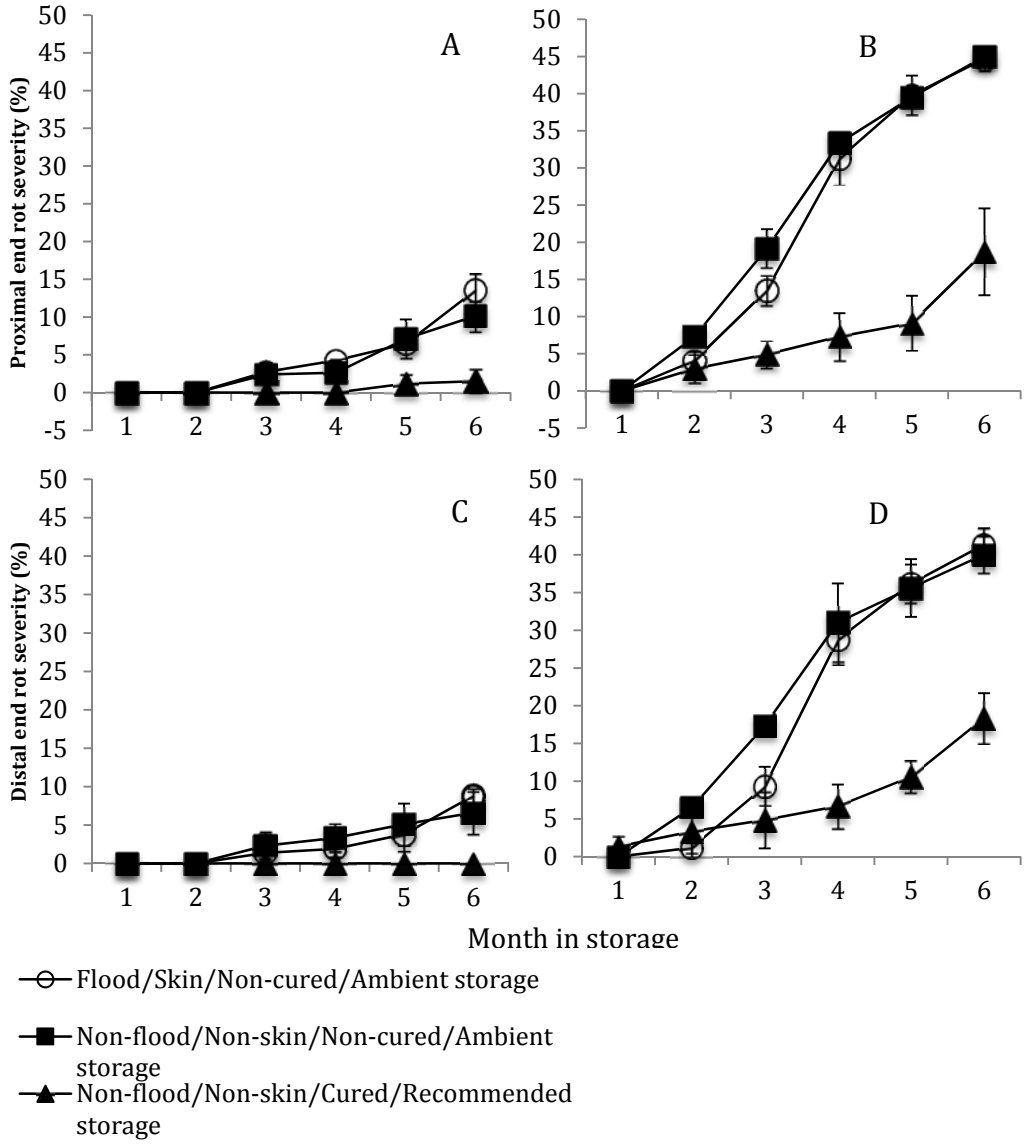

Figure 3: End rot severity in Beauregard sweetpotatoes during six months of storage as affected by: flooding/Skin/Non-cured/Ambient storage, Non-flooding/Non-skin/Non-cured/Ambient storage and Non-flooding/Non-skin/Cured/Proper storage on proximal and distal end rot severities in sweetpotatoes. Proximal end rot severity on sweetpotato during storage in 2012 (A) and 2013 (B). Distal end rot severity on sweetpotato during storage in 2012 (C) and 2013 (D). Data represents means and standard error. Flooded plot was flooded for 2 weeks before harvesting. Non-flooded plot was a plot with no supplemental irrigation during the last 2 weeks before harvesting). Skinned roots were harvested by a conventional 2 row riding harvester with a 1.5 meter drops. Non-skinned roots were harvested by a single row harvester with a 0.6 meter drop). Cured at a temperature of 29^{o}C and a relative humidity of 85 to 90 percent with proper ventilation for a five-day period immediately after harvest. Non-cured roots were stored at room temperature (18.3^{o}C and a relative humidity of 70 percent). Recommended storage at 13^{o}C and a relative humidity of 85 to 90 percent and with adequate ventilation. Ambient storage was an open shed without a controlled environment.

Curing helped to set skin after harvest (Edmunds *et al*., 2008). Recommended storage conditions should be followed when roots are stored for an extended period of time as improper storage conditions and excessive high temperature can increase respiration rate and lead to weight loss (Edmunds *et al*. 2008). Lower humidity (50%) promoted weight loss in sweetptoato (Blankenship and Boyette, 2002). It is possible that the temperature and humidity in the ambient storage was inconsistent compared to the recommended storage with controlled temperature and humidity. Curing and recommended storage reduced weight loss as individual factors. Cured storage roots stored under recommended conditions reduced weight loss to the greatest degree.

End rot incidence in cured and recommended combinations was less than 2% in 2012 and 2-5% in 2013 which was lower than all other combinations. The highest end rot incidence was 10% in 2012 and 29% in 2013 (Table 4 and 5). In addition, end rots showed incidence in the third month in 2012 whereas in 2013 by 4 months they had already leveled off. Different years had different circumstances and also individual storage root may have some defense mechanisms to against pathogens. Thus, end rot incidences inconsistently appeared between years. End rot severity was low in cured and recommended storage combination throughout the study (Figure 3). End rot is caused by several pathogens. *Fusarium* sp. *Macrophomina phaseolina*, and *Lasiodiplodia* sp. were isolated in end rot on sweetpotato (da Silva, 2013). *Fusarium solani* and *Macrophomina phaseolina* were common in end rot areas on sweetpotato (Clark, 1980; da Silva, 2013). Cavities with mycelia were often found in most treatments which were signs of *Fusarium solani*; microsclerotia normally associated with *Macrophomina phaseolina* were less common. *Furasium* sp. are primary pathogens associated with end rot; curing significantly reduces end rots. The curing environment improves skin adhesion and skin set (Smith *et al*., 2009; Villavicencio *et al*., 2007). Exclusion of pathogens by a contiguous skin with minimal wounds prevents pathogen

infection. Clark *et al*., (2013b) found that when preharvest ethephon application was not used and roots were cured immediately after harvest, then the incidence of end rots was low. In addition, after curing, storage roots stored in recommended storage helped to reduce the respiration rate which prolonged shelf life of storage roots (Ray and Ravi, 2005; Pankomera, 2015) and reduce abiotic stress. Cooley *et al*., (1952) supported both curing and proper storage (13-15^{o}C) to reduce the spoilage of sweetpotato. Thus, the combination of curing and proper storage from this study showed less end rot incidence and supports previous results.

Sugar content. Fungi can use various sugars as a source of carbon for producing mycelium and in supplying energy for living. Sugar decomposition responded to wounding and to pathogen infection. However, none of the sugar contents measured (fructose, glucose and sucrose) were significantly different in sweetpotato tissue with or without end rot comparing data averaged over both years (Figure 4).

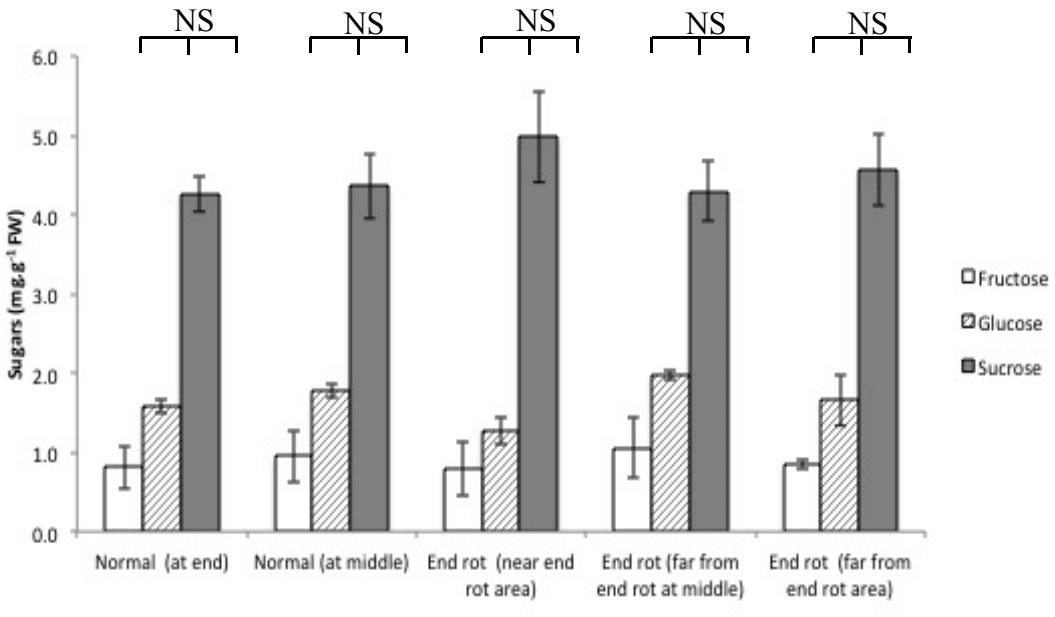

Figure 4: Fructose, glucose, and sucrose content in sweetpotatoes with and without end rot incidence in different regions. Data were means and bars represent standard error averaged over 2 years on a fresh weight (FW) basis.

Intuitively, producers recognize that sweetpotato storage roots grown under ideal conditions with little or no abiotic stress, harvested with care, and cured and stored under properly controlled environmental conditions have strong storage potential with little loss. The results of this study confirm that controlling these factors individually will improve storage quality by reducing weight loss and end rot. The present research extended the results of others by examining all of these factors at one time to determine the most critical factors involved in preventing weight loss and end rot. The most important factors were proper curing and recommended storage. Producers have little control over flooding events and often harvest operations introduce excessive skinning damage when soil conditions are dry. Given these circumstances producers should employ curing and recommended climate controlled storage facilities into their operations to help mitigate uncontrollable environmental stresses and keep end rots to a manageable level.

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CHAPTER 3. THE ROLE OF CALCIUM DEFICIENCY ON END ROT INCIDENCE

3.1 Introduction

The nutrient content of a medium orange flesh sweetpotato [*Ipomoea batatas* (L.) Lam.], baked with skin contains about 7% carbohydrate, 15% dietary fiber, 438% of the daily requirement for vitamin A and 37% of the daily requirement for vitamin C (USDA, 2012). These healthful attributes along with varied consumer products using U.S. sweetpotato has increased consumption from 4.2 pounds per capita in 2000 to 7.5 pounds per capita in 2014 (Wells *et al*., 2014). The need of continuous supply has meant product must be available year round. Reducing weight loss and disease incidence is essential to maintain profitability for a grower and provide markets with quality stock.

A number of diseases and physiological disorders afflict sweetpotato in storage. Recently, a complex of disorders referred to collectively as end rots have emerged in some production regions, most notably Mississippi (Aranciabia, *et al*., 2013). These end rots share a dry decayed, shrunken, brown to black area at either the proximal or distal or both ends of the storage roots (Clark *et al*, 2013b; da Silva and Clark, 2013). End rots can be caused by several fungal pathogens. *Fusarium* sp., *Macrophomina phaseolina*, and *Lasiodiplodia* sp. (da Silva, 2013). These fungal end rots progressively enlarge during storage and signs of the pathogen eventually appear within the affected tissue (Clark et al., 2013). However, tip rot, or restricted end rots are restricted in size, do not seem to be associated consistently with any microbial pathogen and appear to be incited by physiological causes.

Calcium is a macronutrient. It is mainly taken up in a passive pathway and disributed through the xylem. A main function of calcium is as a constituent of cell walls, membrane stability and cell integrity. Calcium is also important in root development, with roles in cell division and

cell elongation, and as a secondary messenger; e.g., regulating developmental processes and Ca^{2+}-binding proteins (Hawkesford *et al*., 2012). The primary symptom of calcium deficiency on sweetpotato is the development of necrotic leisons on young, expanding leaves, blackened newly formed leaves, root growth inhibition, and root tip death (Clark *et al*., 2013a).

Previous research found that the leaves and stems, roots and storage root weight decreased with an increase in calcium concentration. However, total sugar, crude starch and the content of calcium increased when calcium concentration was higher (Sulaiman *et al*, 2003). Calcium level had no effect on the number of roots while sweetpotato root length were varied for varieties (Sulaiman *et al*, 2004). Calcium deficiency causes blossom end rot (BER) which afflicts tomato (Adams and Ho, 1993; Taylor *et al*, 2004) and sweet pepper (Marcelis and Ho, 1999). BER can be caused by poor absorption of calcium by plants because of water stress, or low transportation of calcium to the distal fruit tissue. Ho and White (2005) reported that BER is promoted by stressful environments that limit the uptake and transport of calcium in tomato. Increased calcium and reduced BER is notable under high irrigation conditions (Bar-Tal and Aloni, 2013). However, some research indicated that BER was caused by a stress-related disorder. Saure (2001) found that BER was caused by a deterioration of the cell membrane and increased ion permeability. Palta (2010) supported that transpiration causes a high flow of calcium to accumulate in the leaves at the expense tissue. McGuire and Kelman (1984) showed that the percent surface area of the tubers decayed by *Erwinia carotovora* pv. *atroseptica* was decreased when the tuber calcium was increased. In addition, Mantsebo *et al*. (2014) showed that calcium reduced the incidence and severity of potato tuber soft rot (*Pectobacterium* and *Dickeya* species) during storage. Calcium availability to the plant is a function of the environment. The data in total demonstrates that the

soil environment around the potato can be depleted of calcium and this effects tuber calcium content to a greater extent than calcium in leaves.

In addition, healthy plants and plant organs also are affected by exposure to ethylene; disease development may occur because ethylene exposure accelerates ripening or senescence (Abeles et al., 1992). Sweetpotato roots which have been bruised or cut produce 20 times more ethylene than uninjured sweetpotato roots (Villordon, 2012). Buescher (1981) found that storage roots of four varieties of sweetpotatoes exposed to 100 ppm ethylene for 5 days at 60^{o}F had reduced storability. Clark *et al*. (2013b) showed that end rot was observed mainly in ethephon treated storage roots while end rot was less prevalent in non-treated roots.

As ethephon can stimulate end rot development on a storage root, ethephon can be used to experimentally enhance end rot incidence and thereby used to help reveal if increased calcium concentrations have a mitigating effect and reduce incidence of end rot. The understanding of effects of calcium deficiency on end rot incidence in sweetpotato have not been reported. The aim of this paper was to clarify effects of the calcium concentration and the relationship of ethephon and calcium on the incidence of end rot in sweetpotato.

3.2 Materials and methods

Experiments were conducted in the greenhouse at Louisiana State University, Baton Rouge, LA in 2014. Two tests were conducted. One was transplanted on 6 August 2014 and harvested on 13 November 2014 (100 days). Another test was transplanted on 18 August 2014 and harvested on 26 November 2014 (100 days). Beauregard (B14) from foundation seed stock was used in this study. Eleven liter pots with 9 kg of river-sand with 8 mg kg^{-1} of calcium were planted with 30 cm long transplants and set 8-10 cm deep. The plants were supplied with Hoagland solution (Hoagland and Arnon, 1950) with varying rates of calcium as $CaCl_2$ (0 to 300 ppm). Each

treatment received 500 ml of a nutrient solution per pot every week after transplanting until harvested.

Plants were arranged using a randomized complete block design (RCBD) with 12 replications (one plant per one replication). After harvest, 6 replications of each treatment of storage roots were dipped in 3.9 mM ethephon in water (determined in a preliminary study as a rate suitable for inducing end rot) in a closed container for 1 hour. The other 6 replications were dipped in water for 1 hour as control. All treatments were stored at room temperature (21^{o}C and 50% Rh) for 3 months.

The 5th fully opened leaves from each treatment were collected 55 days after transplanting and calcium content determined by the LSU AgCenter Soil Testing and Plant Analysis Laboratory (STPAL) (National Institute of Standards & Technology, 1993). Harvested roots in each treatment were divided into three parts (proximal end, middle, and distal end) and analyzed for calcium content. Total root weight, ratio of width and length of storage roots and number of roots per plant were immediately recorded after harvest. The percentage of proximal and distal end rot incidences and decayed areas were measured every month for three months. Data from the two tests were combined and analyzed by using Glimmix in SAS (version 9.4; SAS Institute, Cary, NC). Tukey's test ($P \leq 0.05$) was used to determine the differences among means. A logarithmic derivation of the ratio of width and length of storage roots were taken before analysis.

3.3 Results and discussion

Effects of calcium on sweetpotato. In the present study, calcium content in sweetpotato leaves increased with an increase in applied calcium concentration (Figure 5). Prior research showed that calcium in leaves should be between 0.8-1.6% calcium during the root enlargement stage for proper storage root development (Bryson *et al*., 2014). By this guideline, plants in the 0

and 50 ppm calcium concentration treatments were calcium deficient, at 0.2% and 0.6%, respectively, in leaves (Figure 5).

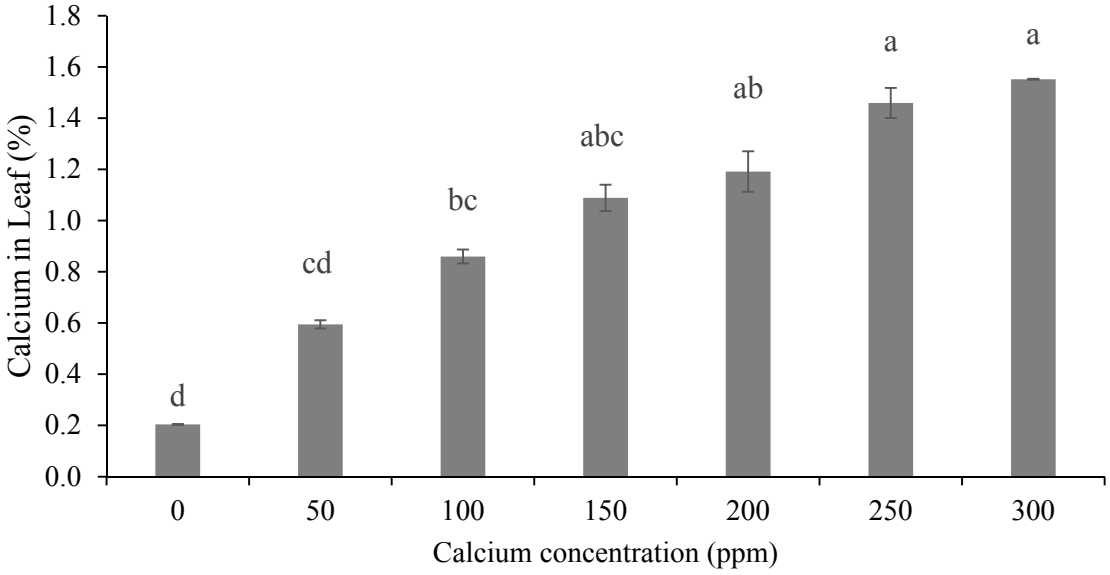

Figure 5: Calcium content in the 5th fully opened sweetpotato leaves, 55 days after transplanting at different calcium concentrations in sweetpotato.

However, only 0 ppm calcium concentration showed typical deficient symptoms on new leaves with leaf edges which were brown and scorched beginning with new leaves (Hawkesford *et al*., 2012). The calcium in the 0 ppm calcium concentration treatment is likely residual calcium in the sand media and transplants. Storage roots also showed a similar trend of increase calcium content as soil calcium content increased; however, no difference was observed in storage root regions: proximal end, middle, and distal end (Figures 6 and 7). This may be because storage roots have lateral roots along the entire length and uptake is similar in all regions. The data showed no difference among regions. Higher calcium contents were found in leaves in contrast to storage roots in all calcium concentration treatments (Figures 5 and 6). The present results are consistent with Palta (2010) who demonstrated that calcium travelled with water in xylem to leaves given higher transpiration potential than in storage organs (potato tubers) resulting in higher calcium

concentration in leaves than in tubers. In total, this supports work by Sulaiman *et al*. (2003) that sweetpotato leaves and stems, fibrous roots, and storage roots had higher calcium content at higher calcium treatment levels. The present work extends these results by demonstrating that no differences are observed in calcium concentration in different storage root regions and thus calcium translocation is equalized throughout the storage root.

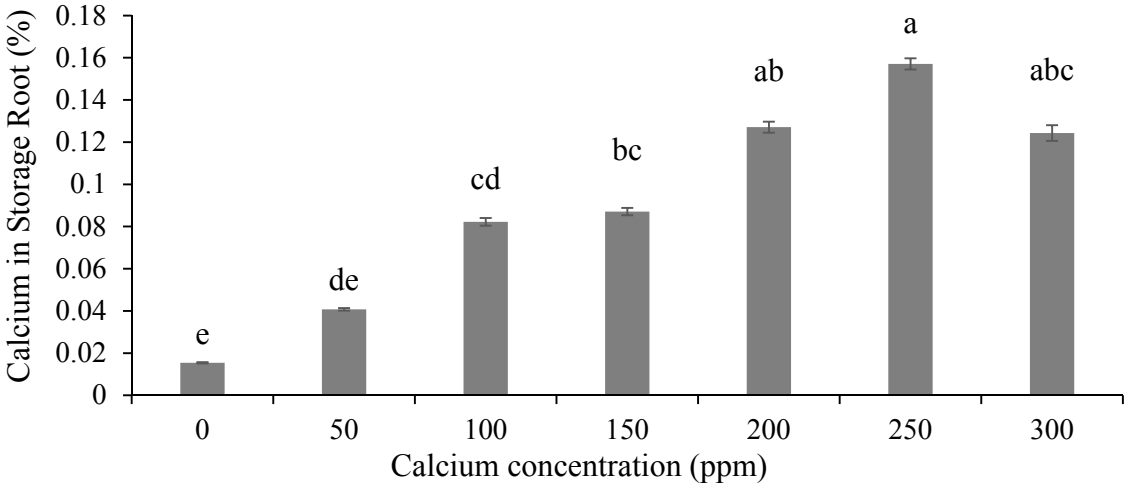

Figure 6: Calcium content in Beauregard sweetpotato storage root at different calcium concentrations.

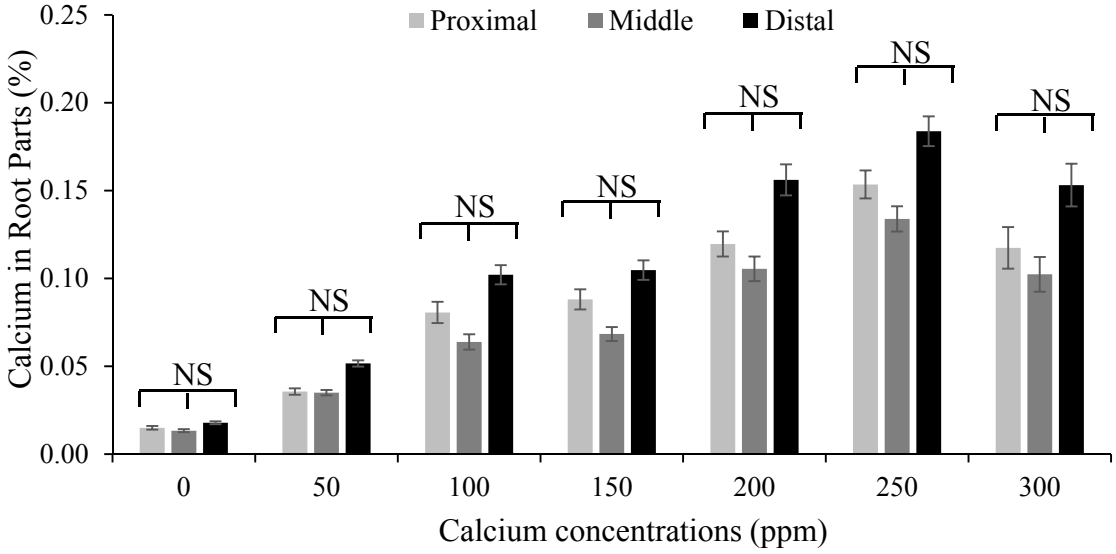

Figure 7: Calcium content in Beauregard sweetpotato storage root parts; calcium concentration in proximal, middle and distal ends of storage roots. NS = not significant (among storage root regions).

Calcium did impact shape. The width to length ratio of storage roots increased in high calcium concentration (Figure 8). This meant storage roots became rounder at higher calcium concentration. As previously described, calcium plays a role in cell division and elongation in root development. Storage roots were thin and small at lower calcium concentrations. Furthermore, Sulaiman *et al*. (2004) showed on Beniotome and Kokei No.14 varieties that the width of storage roots decreased as the calcium concentration increased in both varieties, but the root length of storage roots were varied. The storage root of Beniotome was longer at the high calcium concentration than at the low calcium concentration, while that of Kokei No. 14 was shorter at the high calcium concentration than at the low calcium concentration. Thus, varieties respond differently to calcium availability. Growers may be able to use calcium to adjust shape given low to moderate levels present in the soil for some varieties. Calcium concentration had no impact on total root weight or number of storage roots except at 0 ppm calcium concentration (Figures 9 and 10).

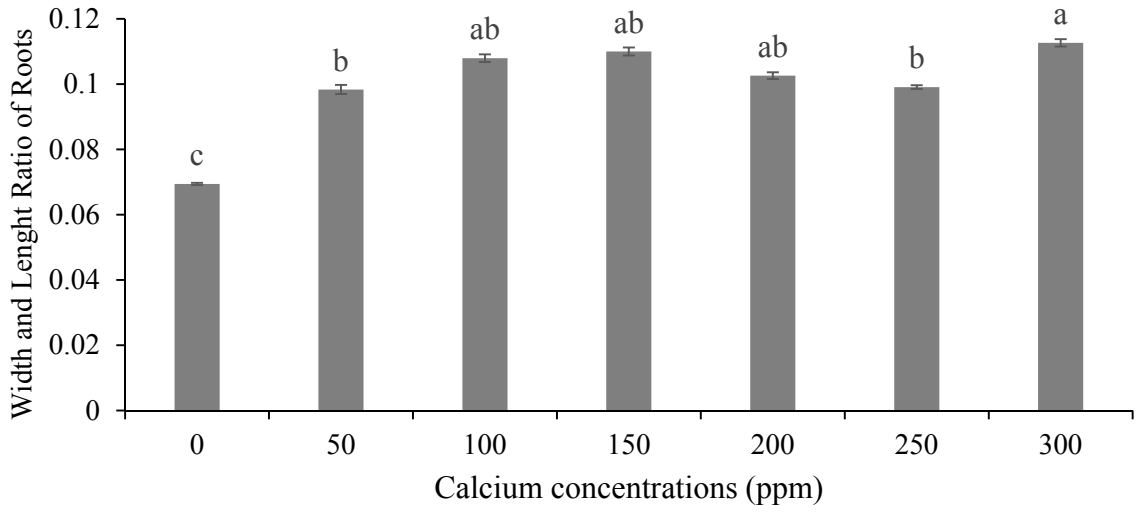

Figure 8: Width and length ratio of Beauregard sweetpotato storage roots grown at different calcium concentrations.

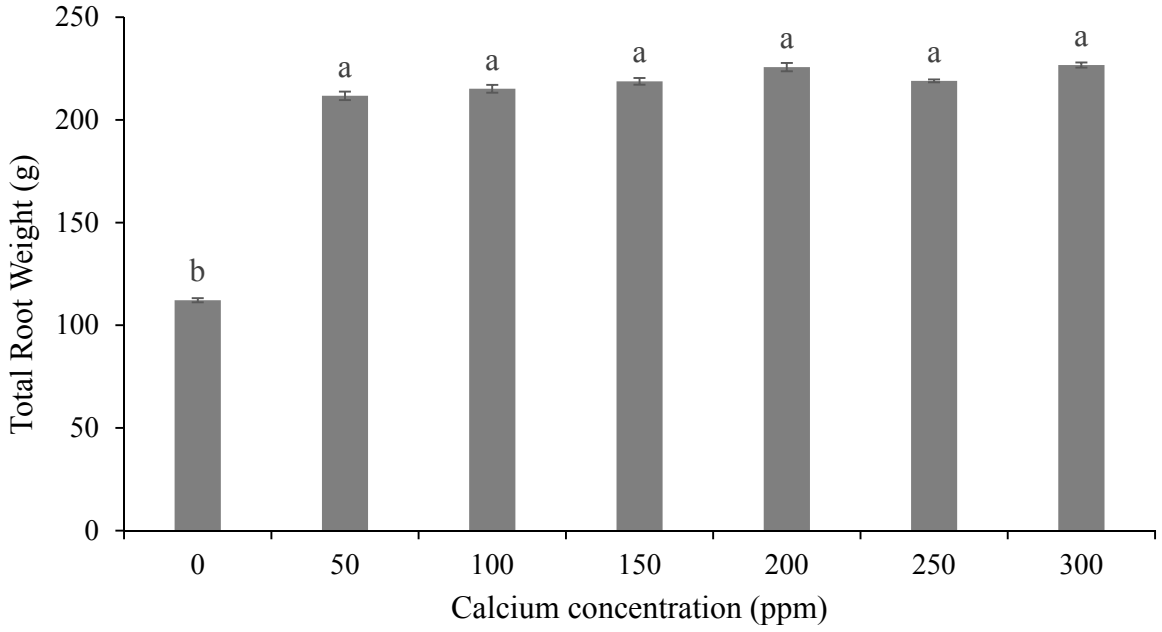

Figure 9: Total sweetpotato storage root weight at different calcium concentrations after harvest.

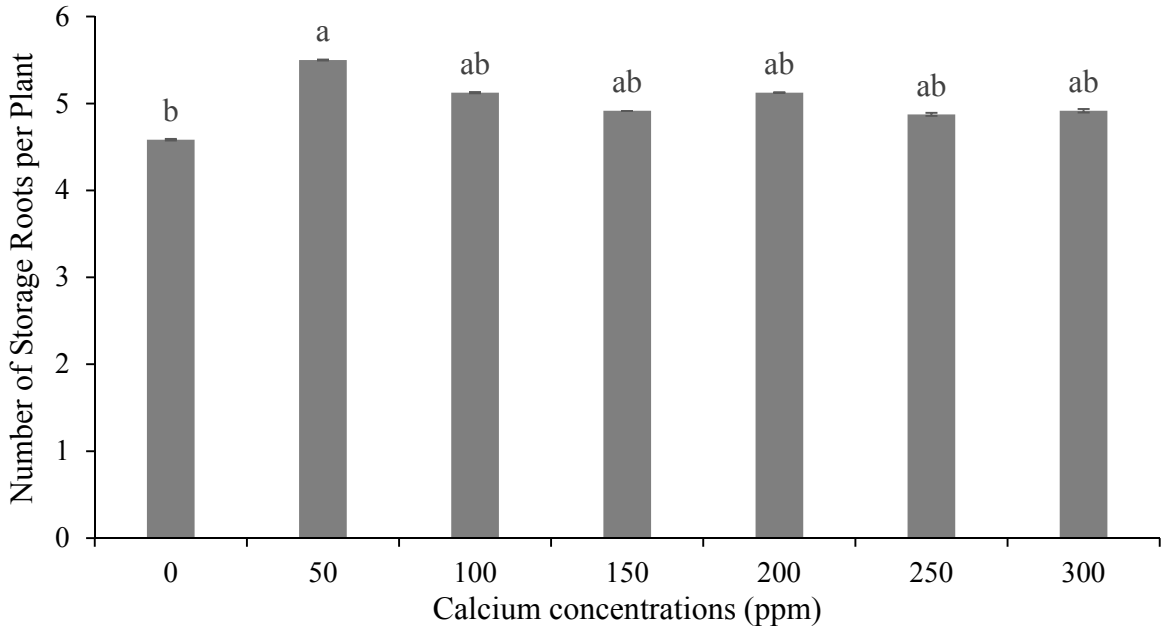

Figure 10: Number of storage roots per plant (Bottom) at different calcium concentrations after harvest.

Effects of calcium on end rot incidence and decayed areas. Calcium concentration had a significant impact on proximal and distal end rot incidences, but not on decay (Table 6).

Table 6: Incidence of proximal end rot, distal end rot, and decayed area on sweetpotato storage roots with and without ethephon treatment at various rates of calcium.treatment at various rates of calcium.

Effects[x]	% Proximal End Rot Incidence		% Distal End Rot Incidence		% Decayed area	
	F Value[Y]	Pr>F[Y]	F Value	Pr>F	F Value	Pr>F
Calcium concentration (A)	8.11	*<0.0001*	8.92	*<0.0001*	0.08	0.9978
With ethephon/without ethephon (B)	168.81	*<0.0001*	173.78	*<0.0001*	382.54	*<0.0001*
AxB	1.86	0.0922	0.44	0.8496	0.55	0.7701
Month (C)	67.48	*<0.0001*	69.37	*<0.0001*	0.08	0.9190
AxC	0.76	0.6953	1.16	0.3081	0.08	1.0000
BxC	4.34	*0.0138*	1.04	0.3550	13.18	*<0.0001*
AxBxC	1.21	0.2720	1.65	0.0762	0.81	0.6448

[X]ANOVA, Analysis of variance (0 to 300 ppm calcium concentrations; 3.9 ethephon and non-ethephon treatment)
[Y]F value, Mean square/Mean square error. Effects were considered significant when Pr > F was < 0.05 (Italicized probabilities)

Only 0 ppm calcium treatment differed with other calcium concentrations. Calcium deficiency in fruit is usually the result of poor distribution of calcium and ends of fruits are markedly low in calcium in the distal ends, where blossom end rot develop. (Keiser and Mullen, 1993; Adams and Ho, 1993). Calcium movement in potato is preferential to leaves leaving tubers at lower calcium levels that lead to calcium deficiency in tubers (Palta, 2010; McGuire and Kelman, 1984). Localized tissue calcium deficiencies induce cell death and necrosis (Kleinhenz et al., 1999). In the present study, calcium deficiency had an impact on proximal and distal end rot incidence rates. Calcium concentration treatments 50 ppm and higher had lower proximal end

rot incidence at 30 d, 60 d, and 90 d (Table 7). The trend was similar for distal end rot (Table 8), however, the threshold was higher (100 ppm) in comparison to 0 ppm calcium treatment. Data showed calcium deficiency had impact on end rot incidence, but no effect to the percent of decayed areas (Table 9). Therefore, calcium is involved with end rot incidence in sweetpotato, but does not restrict decay. Sand substrate was used for understanding the role of calcium deficiency on end rot incidence. This study was under conducted controlled under greenhouse conditions in sand media. Experiment should be conducted in soil to assess the potential of mitigating end rot incidence rates in soils with marginal calcium availability.

Effects of ethephon on end rot incidence and decayed areas. Ethephon had a significant impact on proximal and distal end rot incidences and increased decay (Table 6). This is consistent with Clark *et al*. (2013b) that end rots were observed on storage roots arising from plants treated in ethephon compared to non-treated plants. In the present study storage roots were dipped in ethephon solution and it is likely distal and proximal openings, as well as lenticels, allow for easy entry of the solution and optimize tissue exposure. Ethephon is as an ethylene generator. Ethylene inhibits cell division and growth in the meristems of roots and accelerates ripening and senescence (Burg, 1973; Clark *et al*., 2013a). Thus, damaged tissues are primed for infection by pathogens. These results suggest that ethephon induced proximal and distal end rot incidences and encouraged an environment of senescent cells susceptible to pathogen growth.

Calcium and ethephon interaction. Calcium had no mitigating effect on proximal and distal end rot when storage roots were subjected to ethephon (Table 6). This data suggests that high calcium levels in storage roots cannot mitigate environmental conditions which support expression of end rot in sweetpotato under higher ethylene conditions.

Table 7: Incidence of proximal end rot on Beauregard sweetpotato storage roots with and without ethephon treatment at various rates of calcium applied during development.

Calcium concentration[1] (ppm)	Dip after harvest[2]	Proximal End Rot Incidence (%)		
		30 d	60 d	90 d
0	Ethephon (-)	55.4±30.0^{ab}	62.4±23.2^{a}	70.6±19.7^{a}
	Ethephon (+)	76.0±26.4^{a}	86.6±17.9^{a}	86.6±17.9^{a}
50	Ethephon (-)	7.0±10.6^{cd}	15.3±19.1^{b}	18.4±17.7^{b}
	Ethephon (+)	40.5±25.4^{bc}	61.8±31.8^{a}	70.7±27.8^{a}
100	Ethephon (-)	5.5±10.9^{cd}	5.5±10.9^{b}	14.5±19.1^{b}
	Ethephon (+)	52.2±38.8^{ab}	61.2±27.9^{a}	68.2±23.5^{a}
150	Ethephon (-)	1.2±4.0^{d}	8.6±16.4^{b}	22.5±31.3^{b}
	Ethephon (+)	56.3±35.8^{ab}	61.9±38.5^{a}	65.2±37.9^{a}
200	Ethephon (-)	9.3±15.9^{cd}	11.4±22.1^{b}	18.9±27.5^{b}
	Ethephon (+)	48.5±23.3^{ab}	69.0±24.4^{a}	72.1±24.0^{a}
250	Ethephon (-)	10.4±29.1^{cd}	23.9±32.1^{b}	26.0±31.2^{b}
	Ethephon (+)	61.3±28.2^{ab}	77.8±26.2^{a}	79.9±24.7^{a}
300	Ethephon (-)	7.0±16.6^{cd}	9.7±18.0^{b}	17.3±31.0^{b}
	Ethephon (+)	62.3±31.2^{ab}	75.2±24.2^{a}	81.0±26.8^{a}

*Mean and standard deviation values (N=12). Means within column followed by different letters are significantly different from each treatment combination by Tukey's test ($P \leq 0.05$).

[1]Plants were supplied by Hoagland solution with varying rates of calcium as $CaCl_2$ (0 to 300 ppm).

[2]Ethephon treatment after harvest: storage roots were dipped with 3.9 mM ethephon in a closed container for 1 hour; storage roots were dipped with water for 1 hour as control.

Table 8: Incidence of distal end rot on sweetpotato storage roots with and without ethephon treatment at various rates of calcium during development.

Calcium concentration[1] (ppm)	Dip after harvest[2]	Distal End Rot Incidence (%)		
		30 d	60 d	90 d
0	Ethephon (-)	27.3±19.7^{bc}	43.8±26.2^{bcd}	54.5±21.5^{ab}
	Ethephon (+)	77.0±22.7^{a}	79.1±23.7^{a}	84.9±19.5^{a}
50	Ethephon (-)	0.0±0.0^{c}	18.1±24.1^{cde}	21.2±22.6^{bc}
	Ethephon (+)	42.2±15.7^{b}	59.9±23.2^{a}	62.0±25.7^{a}
100	Ethephon (-)	0.0±0.0^{c}	0.0±0.0^{e}	7.7±11.5^{c}
	Ethephon (+)	36.6±32.9^{b}	49.8±37.6^{abc}	51.8±35.2^{ab}
150	Ethephon (-)	2.8±9.5^{c}	6.8±12.7^{e}	7.0±15.1^{c}
	Ethephon (+)	42.0±29.3^{b}	55.8±27.3^{ab}	55.8±27.3^{a}
200	Ethephon (-)	5.8±15.1^{c}	10.0±22.3^{e}	16.5±23.9^{c}
	Ethephon (+)	26.5±17.5^{bc}	47.4±33.3^{abc}	54.9±32.4^{ab}
250	Ethephon (-)	0.0±0.0^{c}	12.8±22.8^{de}	17.0±24.7^{c}
	Ethephon (+)	45.3±33.5^{b}	59.3±34.4^{ab}	66.2±38.3^{a}
300	Ethephon (-)	0.0±0.0^{c}	7.6±13.9^{e}	10.4±21.1^{c}
	Ethephon (+)	46.9±30.0^{b}	54.4±28.1^{ab}	59.8±29.4^{a}

*Mean and standard deviation values (N=12). Means within column followed by different letters are significantly different from each treatment combination by Tukey's test ($P \leq 0.05$).

[1]Plants were supplied by Hoagland solution with varying rates of calcium as $CaCl_2$ (0 to 300 ppm).

[2]Ethephon treatment after harvest: storage roots were dipped with 3.9 mM ethephon in a closed container for 1 hour; storage roots were dipped with water for 1 hour as control.

Table 9: Decayed area on sweetpotato storage roots with and without ethephon treatment at various rates of calcium during development.

Calcium concentration[1] (ppm)	Dip after harvest[2]	Decayed Areas (%)		
		30 d	60 d	90 d
0	Ethephon (-)	4.52±3.3^{bcd}	7.18±4.3^{ab}	17.09±15.6^{a}
	Ethephon (+)	6.65±4.8^{abcd}	9.41±6.6^{ab}	19.45±15.7^{a}
50	Ethephon (-)	1.46±1.4^{cd}	1.68±1.3^{b}	1.79±1.3^{d}
	Ethephon (+)	10.15±7.2^{ab}	11.53±9.8^{a}	12.08±9.8^{abcd}
100	Ethephon (-)	1.95±2.5^{cd}	2.29±2.8^{b}	2.60±3.0^{cd}
	Ethephon (+)	8.79±7.9^{abc}	9.00±7.87^{ab}	10.08±9.6^{abcd}
150	Ethephon (-)	2.35±1.6^{cd}	2.91±2.2^{b}	3.42±2.4^{bcd}
	Ethephon (+)	8.83±6.6^{abc}	9.28±6.5^{ab}	9.75±7.2^{abcd}
200	Ethephon (-)	1.94±2.5^{cd}	2.45±2.7^{b}	2.94±2.8^{cd}
	Ethephon (+)	8.76±6.0^{abcd}	9.23±6.1^{ab}	10.17±6.3^{abcd}
250	Ethephon (-)	2.28±1.6^{cd}	2.54±1.5^{b}	2.68±1.6^{cd}
	Ethephon (+)	12.98±9.4^{a}	13.8±9.9^{a}	14.86±10.7^{ab}
300	Ethephon (-)	1.38±1.2^{d}	1.67±1.5^{b}	1.95±1.5^{cd}
	Ethephon (+)	12.07±8.0^{a}	13.03±8.9^{a}	13.60±9.0^{abc}

*Mean and standard deviation values (N=12). Means within column followed by different letters are significantly different from each treatment combination by Tukey's test ($P \leq 0.05$).

[1]Plants were supplied by Hoagland solution with varying rates of calcium as $CaCl_2$ (0 to 300 ppm).

[2]Ethephon treatment after harvest: storage roots were dipped with 3.9 mM ethephon in a closed container for 1 hour; storage roots were dipped with water for 1 hour as control.

The present research clarifies the role of calcium on storage root shape, weight, and number, end rot incidence, and decay on sweetpotato storage roots which reduced calcium content in sweetpotato leaves and storage roots, total root weight, number, and size of storage roots. Calcium had an impact on end rot. Ethephon induced proximal and distal end rot incidences and other decays. Results showed that tissue damage via ethephon has a pronounced impact on onset of end rot. The results of the present study and those presented in chapter 2 demonstrate that the environment in which sweetpotato storage roots are handled after harvest has a profound effect on end rot incidence. Storage roots cured at 29ºC and 85-90% RH for five days and stored at 13ºC and 85-90% RH were far less susceptible to end rot. Moreover, high calcium had no mitigating effect on end rot symptoms.

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CHAPTER 4. EXPRESSED GENES IN STORAGE ROOTS TREATED WITH ETHEPHON AND 1-MCP

4.1 Introduction

Sweetpotato, [*Ipomoea batatas* (L.) Lam.], is an important food crop and highly nutritious. The nutrient content of a medium orange flesh sweetpotato baked with skin contains about 7% carbohydrate, 15% dietary fiber, 438% of the daily requirement for vitamin A and 37% of the daily requirement for vitamin C (USDA, 2012). Sweetpotato production in the U.S. has increased to meet consumer demands. The crop also needs to be made available year round and thus stored up to 10 months. This long storage subjects the crop to postharvest diseases (Edmunds *et al*., 2008).

Stored sweetpotato often succumbs to many diseases and physiological disorders; a more recent disorder termed end rot has emerged in some production regions (Mississippi) (Aranciabia *et al*., 2013). End rots of sweetpotato storage roots are commonly caused by *Fusarium solani* (Clark, 1980; da Silva, 2013) and *Macrophomina phaseolina* (da Silva, 2013). Flooding is a common abiotic stress associated with end rot incidence. Clark (2012) showed that flooding increased end rot occurrence during storage. Ethephon and non-cured sweetpotato storage roots were the factors that enhance end rot (Arancibia *et al*., 2013; Clark *et al*., 2013) the most.

End rot is a symptom with multiple causes. Yet expression can be induced by the application of ethephon, a compound which generates ethylene gas. Villordon (2012) found that sweetpotato roots which have been bruised or cut produce 20 times more ethylene than uninjured sweetpotato roots. Buescher (1981) found that storage roots exposed to 100 ppm ethylene for 5 days at 60^{o}F was detrimental to storability of the four varieties of sweetpotatoes studied. Arancibia *et al*. (2013) showed that end rot was observed mainly in ethephon treated storage roots while end rot was less prevalent in non-treated roots. Several reports showed that ethylene induced many enzymes for plant responses. Ethylene at low concentration increased the activity of peroxidase

and polyphenoloxidase and resisted infection by *Ceratocystis fimbriata* in sweetpotato tissue (Stahmann *et al*., 1966). Haga *et al*. (1988) found that exogenous ethylene induced phenylalanine ammonia lyase (PAL) in rice. de Jong *et al*. (2002) found that ethylene highly stimulated camptothecin-induced hydrogen peroxide production and cell death. L-α-(2-aminoethoxyvinyl) glycine inhibited ethylene synthesis and silver thiosulphate inhibited ethylene perception. This blocked camptothecin-induced hydrogen peroxide production and programmed cell death (PCD) (de Jong *et al*., 2002). Ethylene thus impacts different enzymatic pathways.

An ethylene inhibitor, 1-methylcyclopropene (1-MCP), (Sisler *et al*., 1996) functions by interfering with ethylene receptor sites. It is widely used for agricultural applications such as the inhibition of ripening or senescence. Villordon (2012) applied 1 ppm 1-MCP to uninjured and injured sweetpotato roots (2.5-3.8 cm proximal and distal end) and found that 1-MCP reduced breakdown in sweetpotato roots. Lippert and Blanke (2004) also reported that 0.5 $\mu l\, l^{-1}$ 1-MCP treated plums prevented or retarded bruising after 4 weeks of cold storage. However, different concentrations of 1-MCP affect quality of strawberries. At low concentration of 1-MCP (5 to 15 $nL \cdot L^{-1}$) postharvest life was prolonged by 35% at 20 °C and 150% at 5 °C, but at high concentration (500 $nL \cdot L^{-1}$) quality declined at both 20 and 5 °C (Ku *et al*., 1999).

End rot is not traceable to any one given stress or pathogenic event which compounds our ability to breed for resistance in sweetpotato. The species is already inherently difficult to breed given that it is a hexaploid, demonstrates incompatibility, and traits are quantitatively inherited. Molecular markers can be used to screen and select for cultivars to facilitate breeding. Ramanarao *et al.* (2012) reported annealing control primer system was designed to identify differentially expressed genes in biological process pathways. Molecular mechanisms triggered by the onset of end rot are unknown and may provide insight into plant protective mechanisms to exploit in a

breeding program. The beneficial aspect of this technique provides identification of rarely expressed and transient transcripts. Differentially expressed genes can be used to further trait breeding.

End rot is a symptom with multiple causes. Expression of end rot can be induced by the application of ethephon. The gene expression of sweetpotato storage roots treated with ethylene which induced end rot has not been tested. To identify similarly and differentially expressed genes, sweetpotato storage roots with end rot incidence and sweetpotato storage roots treated with ethylene and 1-MCP were used to find candidate genes involved with end rot incidence.

4.2 Materials and methods

Plant materials, ethephon and 1-MCP treatments. Freshly harvested U.S.#1 size roots (5 to 9 cm diameter, 8 to 23 cm long) of sweetpotato cv. Beauregard were washed and dipped in three treatments in a closed container for 1 hour with three replications: 1) 3.9 mM ethephon, 2) 1 ppm 1-MCP, and 3) water as a control. The storage roots were then stored at room temperature (21^0C and 50% RH). Three replications of storage root tips and transverse section of middle sections were collected 1 day, 3 days, 7 days, and 14 days after treatment. In addition, storage roots with end rot incidence (2-3 cm of end rot severity) were sampled from healthy tissue near the infected area ("tip section") and middle sections after 3-months storage with three replications (Figure 11). The samples were immediately frozen in liquid nitrogen and stored at -80^{o}C for RNA extraction.

RNA Isolation, cDNA analysis and ACP-based gene-fishing PCR. Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA) following the vendor's manual, but Trizol (Invitrogen, Carlsbad, CA) was used instead of the RLT buffer to break down the starch in the samples. RNA quality and quantity were determined using a ND-1000 spectrophotometer

(Nanodrop Technologies, Wilmington, DE). Five µg of RNA of each treatment extracted from storage root regions and different time points were used for later analysis.

Figure 11: Beauregard sweetpotato storage roots with end rot incidence (2-3 cm of end rot severity) after 3-months storage.

First strand cDNA was synthesized using a GeneFishing™ DEG premix kit (Seegene, Rockville, MD; Effendy *et al*., 2013; Ramanarao *et al*., 2012) according to the manufacturer's instructions. Three µg of RNA of each treatment extracted from storage root tissues and different time point as described earlier was reverse transcribed to first strand DNA by adding 10 µM dT-ACP1, 5X RT buffer, 2 mM dNTP, 20 u RNase inhibitor, 200u M-MLV reverse transcriptase in 20 µl at 42ºC for 90 min. The first strand cDNA was diluted with 80 µl of DNase-free water for further analysis. For second strand cDNA synthesis, a PCR tube on ice was prepared with 3 µl of 50 ng of the diluted first-strand cDNA, 2 µl of 5 µM arbitrary ACP, 1 µl of 10 µM dT-ACP2, 10 µl of 2X SeeAmp™ ACP™ Master Mix to a final volume of 20 µl (Seegene, Rockville, MD; Effendy *et al*., 2013; Ramanarao *et al*., 2012). The PCR mixture was placed in a preheated thermal cycler at 94ºC. Cycle times and temperature were according to GeneFishing™ DEG premix kit's recommendations (Seegene, Rockville, MD). The amplified PCR products were run in a 2.5% agarose gel at 80 volts for 4 hours. Twenty ACP primers were captured from gels for identifying the differentially expressed genes.

Cloning and Sequencing of DEGs. Seventy-six fragments were collected from gels based on their differential expression or intensity between control and treatment. Gel fragments were extracted using a Qiaquick gel extraction kit (Qiagene, Valencia, CA). The 76 DEGs were cloned into pGEM®-T Easy vector (Promega, Madison, WI) using protocol by Baisakh *et al*. (2006) and using M13F/R primers to confirm that PCR fragment was in the plasmid. Plasmids were isolated from 65 independent clones and sequenced with T7 primer in an ABI 3730x1 genetic analyzer (Applied Biosystems, Foster city, CA) as described by Effendy *et al*. (2013). DNA sequences were cleaned and functional annotation of the DEGs was searched from the non-redundant nucleotide and protein database of NCBI using BLASTN and BLASTX interface (http://www.ncbi.nlm.nih.gov/BLAST).

Semi-quantitative reverse transcription polymerase chain reaction (sqRT-PCR) analysis and quantitative reverse transcription PCR (qRT-PCR) of DEGs. For sqRT-PCR, five DEGs with known function annotation (Table 10) were quantified using sqRT-PCR. One µl of total RNA isolated from storage root regions and different time points was reverse transcribed by iScript™ cDNA synthesis kit with an half reaction for reverse transcription process (Bio-Rad, Hercules, CA) following the manufacturer's recommendations. Two µl of the first-stand cDNA were further used with DEG-specific primers for PCR analysis (Table 10) and primers designed by using Primer 3.0 web resource (http://bioinfo.ut.ee/primer3-0.4.0/). The PCR program was used as described by Ramanarao *et al*. (2012). For qRT-PCR, the protocol for cDNA synthesis was the same as sqRT-PCR. iTaq™ Universal SYBR® Green supermix was used for PCR analysis with three replications (Bio-Rad, Hercules, CA), 1 µl of cDNA and 10 ng DEG-specific primer (Table 10) in a MyiQ real-time PCR analysis system (Bio-Rad, Hercules, CA). The relative expression of the

sweetpotato elongation factor gene (Solis, 2012) was used as a reference gene and expression calculated with the $2^{-\Delta\Delta Ct}$ method (Ramanarao *et al*., 2012; Effendy *et al*., 2013).

Table 10: Differentially expressed genes (DEGs) induced in response to ethephon and 1-MCP in sweetpotato storage roots and the corresponding primer sequences.

DEG	Similarity	Length	Forward primer (5'-3')	Reverse primer (5'-3')	Product length (bp)
IbEM05	Thioredoxin H2 *(Ipomoea batatas)*	306	CCCAAAATTTGCT GCTTGAT	CTGTGAATGCTG CGACTACG	199
IbEM06	Osmotin *(Nicotiana tabacum)*	345	ACCACCTTTGGAG GACAACA	ACTTGATCATGG GCAGAAGG	183
IbEM07	Autophagy related gene8 *(Ipomoea nil)*	496	CAAATTGCCCAAC AGTCAGA	GTCTACCAGGCA TTCGCTTC	157
IbEM12	Expressed protein (*Oryza sativa* Japonica Group)	484	ATAGGGGCTGGAG TTGAGGT	CAAGCCATCATG CTTCTTCA	150
IbEM16	Transcription initiation factor (*Vitis vinifera)*	113	GATCGTCGTCGGA GCTGTA	GTGCCGCACTTG TTGCAG	106
IbEF1a	Elongation factor (EF) (*Ipomoea batatas)*	213	CCAAGATTGATAG ACGGTCTGG	CAGTTGGGTCCT TCTTGTCAAC	100

bp = base pair

4.3 Results and discussion

Twenty annealing control primers (ACPs) were used to identify the expression of differentially expressed genes (DEGs) under ethephon and 1-MCP treatments. The present study found amplification for 5 DEGs with upregulated expression. The DEGs selected were *IbEM05, IbEM06, IbEM07, IbEM12,* and *IbEM16* and were amplified using 5 ACPs, ACP2, ACP3, ACP4,

ACP5, and ACP7, respectively (Figures 12 and 13). The functional annotation of the DEGs were similar to genes involved in protective mechanisms, transcription regulation, and an expressed protein (unknown) (Table 10).

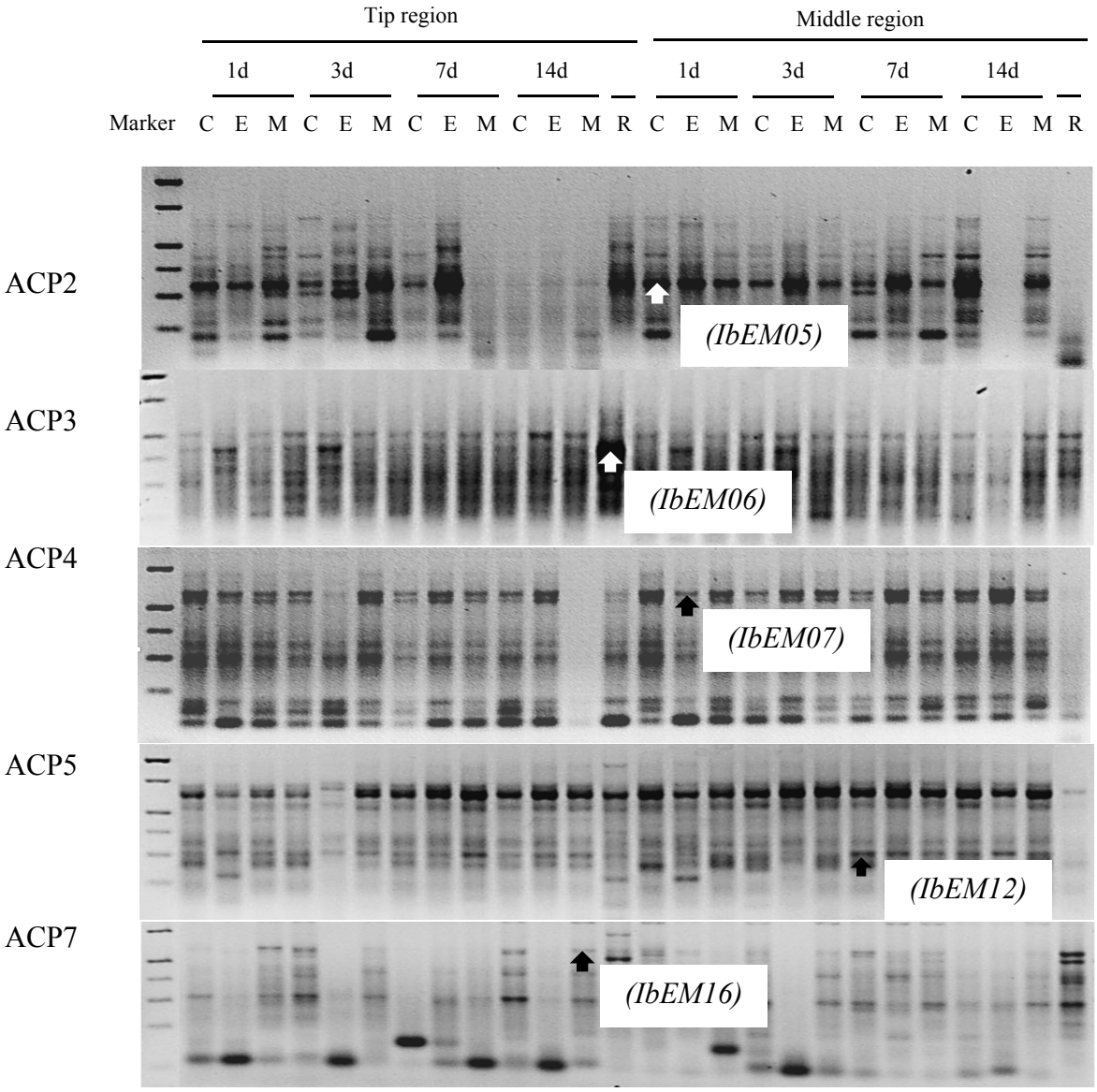

Figure 12: Representative gels from PCR product using annealing control primers (ACP) showing differentially expressed genes (DEGs) in sweetpotato storage root treated with 3.9 mM ethephon (E), 1 ppm 1-MCP (M) and water as a control (C) from two different regions (both tips and middle) at 1 day, 3 days, 7 days, 14 days and storage roots with end rot (R). Upward arrows expressed upregulated DEGs, respectively. Marker, 1-kb DNA size marker.

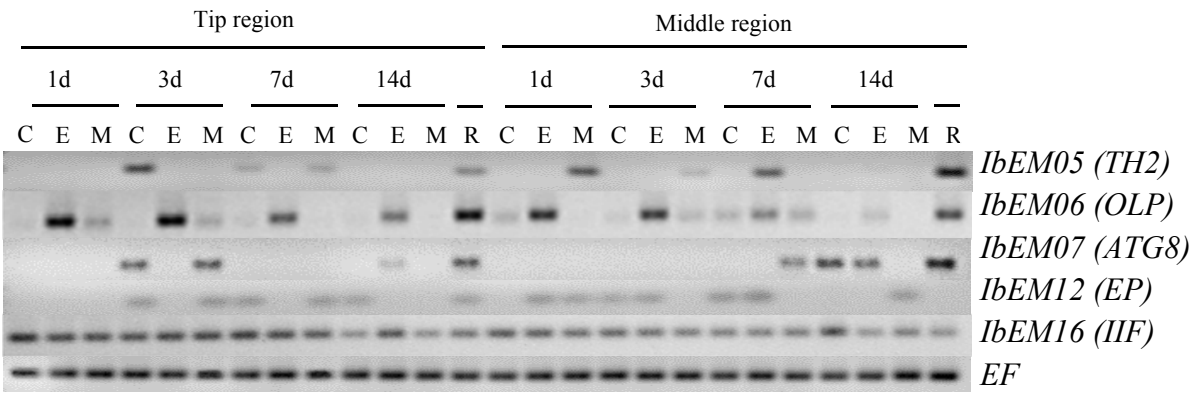

Figure 13: Semiquantitative reverse transcription PCR analysis of differentially expressed genes in sweetpotato storage root treated with 3.9 mM ethephon (E), 1 ppm 1-MCP (M) and water as a control (C) from two different regions (tips and middle) at 1 day, 3 days, 7 days, 14 days, and storage roots with end rot (R).

IbEM05 is similar to Thioredoxin H2 (*TH2*). *TH2* functions as a protective gene by catalyzing dehydroascorbate reductase and monodehydroascorbate reductase to ascorbate. These enzymatic reactions play an important role in preventing cell damage from reactive oxygen species (Huang *et al*., 2008). Effendy *et al*. (2013) found that *TH2* transcripts have been induced by skinning injury, and it is also responsible for developmental and environmental cues in sweetpotato (Huang *et al*., 2004). *TH2* had higher expression in 1-MCP treated storage roots comparing to control than in ethephon treated storage roots comparing to control in every region and time point except in the middle region at 7d. *TH2* showed significant up-regulation in sweetpotato storage roots treated with 1-MCP at 3d in the tip region and at 1d in the middle region. Expression was moderate in ethephon treatment at 7d in middle region (Figure 14). *TH2* showed elevated transcription in end rot storage roots in healthy tissue near the infection area and middle regions in semiquantitative PCR analysis. The mRNA level was lower in healthy tissue near the infection area than middle region (Figures 13 and 14). Stahmann *et al*. (1966) found ethylene at low concentrations increased activity of peroxidase, an antioxidant enzyme. Moreover, Birecka and Miller (1974) found that ethylene stimulated peroxidase reactions and peroxidase levels in

different sweetpotato storage tissue zones. *TH2* was expressed in 1-MCP treated storage roots comparing to control. 1-MCP blocked ethylene, but new ethylene receptors may be formed and cells become sensitive to ethylene (Blankenship, 2001). This may lead to 1-MCP treated storage roots comparing to control having low ethylene concentration and higher *TH2* expression which may protect storage roots. Storage roots did not show end rot in 1-MCP treated storage roots. While ethylene treated storage roots showed some *TH2* expression, the moderate *TH2* expression may not be high enough to protect storage roots and thus end rot appears. The transcripts of infected end rot storage roots were less intense in healthy tissue near infected area than in the middle region. *TH2* activity is highest in poor ethylene environments and is thus hypothesized as having limited protective activity in high ethylene environments.

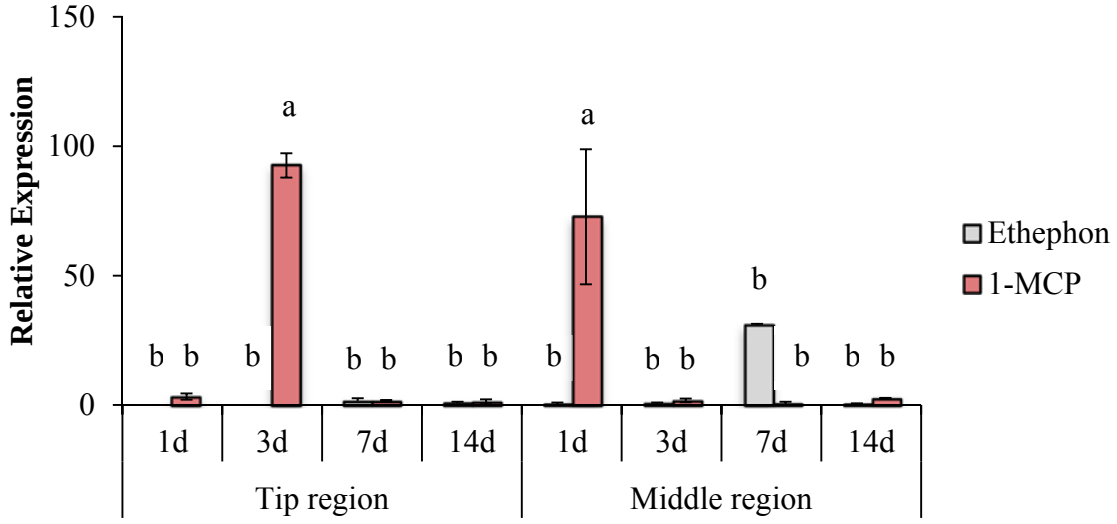

Figure 14: Relative expression of *IbEM05* which was similar to thioredoxin H2 in sweetpotato storage root treated with 3.9 mM ethephon and 1ppm 1-MCP from two different regions (tips and middle) at different time points.

IbEM06 is a DEG similar to osmotin or osmotin-like protein (*OLP*). *OLP* functions as a stress responsive antifungal protein (Kumar et al., 2015). It has been found that osmotin is elevated under cold temperatures (Patade *et al*., 2013). Neale *et al*. (1990) reported further that osmotin was induced by water stress, viral infection and wounding. It was also considered as a pathogenesis-

related protein (*PR* protein) inducible by ethylene (Wang *et al*., 2002). *OLP* showed greater up-regulation in ethephon treated storage roots comparing to control than in 1-MCP treated storage roots comparing to control for all regions and time points. Transcripts were found in tips and middle regions, peaked at 1d, and declined over time. Semiquantitative PCR analysis showed that transcription was higher in healthy tissue near infected area than in the middle region of end rot afflicted sweetpotato storage roots. (Figure 13). This study showed that osmotin has high expression in ethephon treated storage roots and storage roots with end rot (Figures 13 and 15). However, Diaz et al. (2002) found that *PR* proteins (glucanase and chitinase) were not active against *Botrytis cinera*. This is consistent with the present study. Although osmotin had high expression in ethylene treated storage roots and sweeptotato with end rot infection (Figures 13 and 15), storage roots still showed end rot incidence.

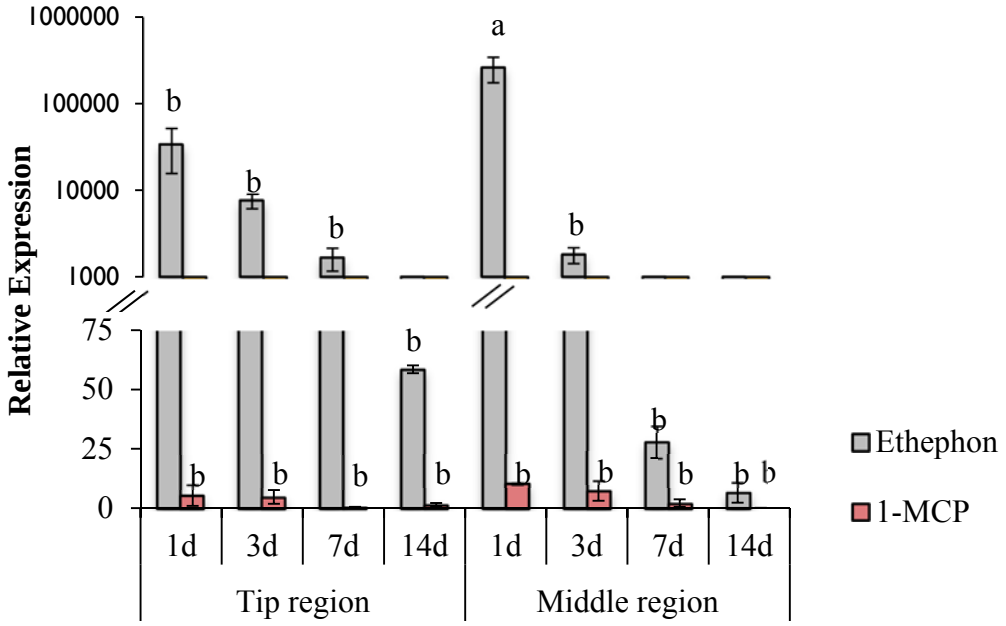

Figure 15: Relative expression of *IbEM06* which was similar to osmotin in sweetpotato storage root treated with 3.9 mM ethephon and 1ppm 1-MCP from two different regions (tips and middle) at different time points.

The DEG *IbEM07*, is similar to autophagy related gene 8 (*ATG8*). Autophagy is involved in cell degradation and homeostasis. This plays an important role in cell survival in eukaryotic species

(Thompson *et al*., 2005; Pyo *et al*., 2012). Shibuya *et al*. (2009) suggested that autophagy delays programmed cell death (PCD). In contrast, ethylene highly stimulated PCD (de Jong *et al*., 2002). Moreover, expression of autophagy during petal senescence was associated with several members of the *InATG8* gene family (Shibuya *et al*., 2011). *ATGs* in *Arabidopsis* were differently expressed in distinct tissues (Slavikova *et al*., 2005). *ATG8* showed a slight increase in mRNA accumulation. Expression was higher in 1-MCP treated storage roots compared to control than in ethephon treated storage roots compared to control for most of the time points (Figure 16). The peak for the 1-MCP treated storage roots was at 7d in both regions. The ethephon treatment showed similar expression trends to the 1-MCP treatment. The transcription of storage roots with end rot by semiquantitative analysis showed higher expression in the middle region than in healthy tissue near end rot area (Figures 13 and 16). *ATG8* expression may delay PCD in ethylene treated storage roots which is a defense mechanism of plant. Therefore, ethylene treated storage roots showed end rot incidence. Moreover, healthy tissue near infected area showed lower expression of *ATG8* than in the middle regions. Thus, end rot may suppress expression of ATGs. High *ATG8* expression may protect tissue and lessen end rot expression.

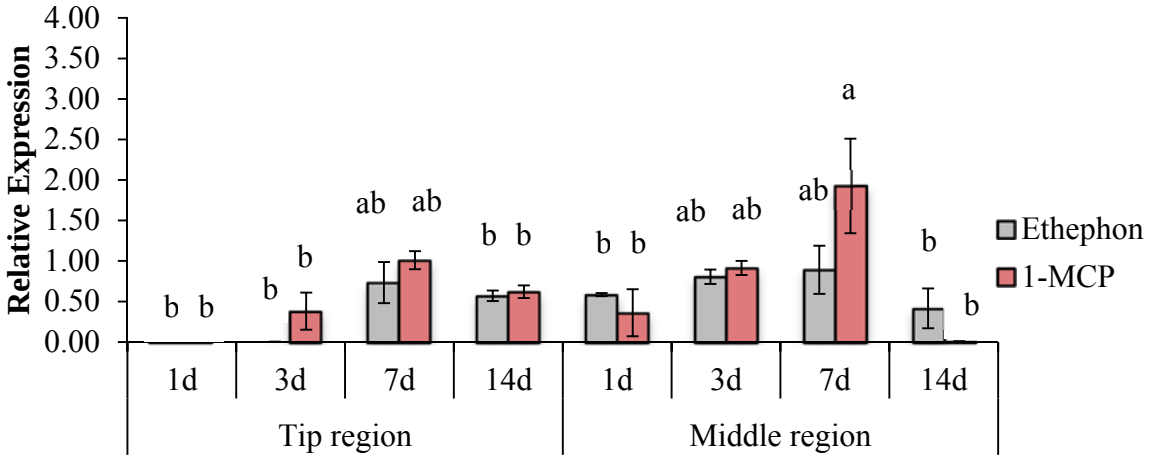

Figure 16: Relative expression of IbEM07 which was similar to autophagy related gene 8 in sweetpotato storage root treated with 3.9 mM ethephon and 1ppm 1-MCP from two different regions (tips and middle) at different time points.

The cDNA (*IbEM12*) is similar to expressed protein (*EP*). Expressed protein (*EP*) is a gene of unknown function. Ethephon treated storage roots showed elevated *EP* transcript expression in comparison to 1-MCP in the middle region for the time points (Figure 17). In contrast the ethephon treated storage roots showed a decrease in *EP* in the tip regions compared to 1-MCP treated storage roots. Sweetpotato with end rot showed elevated transcription of *EP* in healthy tissue near the infected area, and not in the middle (Figures 13 and 17). This gene was found in the Rice Annotation Project (2007), but the function is still not clear and based on our current study, no clear trends exist as to its importance in end rot incidence.

The transcript of *IbEM16* was similar to transcription initiation factor (*IIF*). The transcription factor is a protein that binds to DNA sequences and controls expression of other genes (Taiz and Zeiger, 2002). Results were inconsistent in the present study. *IIF* showed higher expression in 1-MCP treated storage roots than in ethephon treated storage roots in the tip regions at 1d. Ethephon treated storage roots showed elevated *IIF* expression in the middle region at 3d (Figure 18).

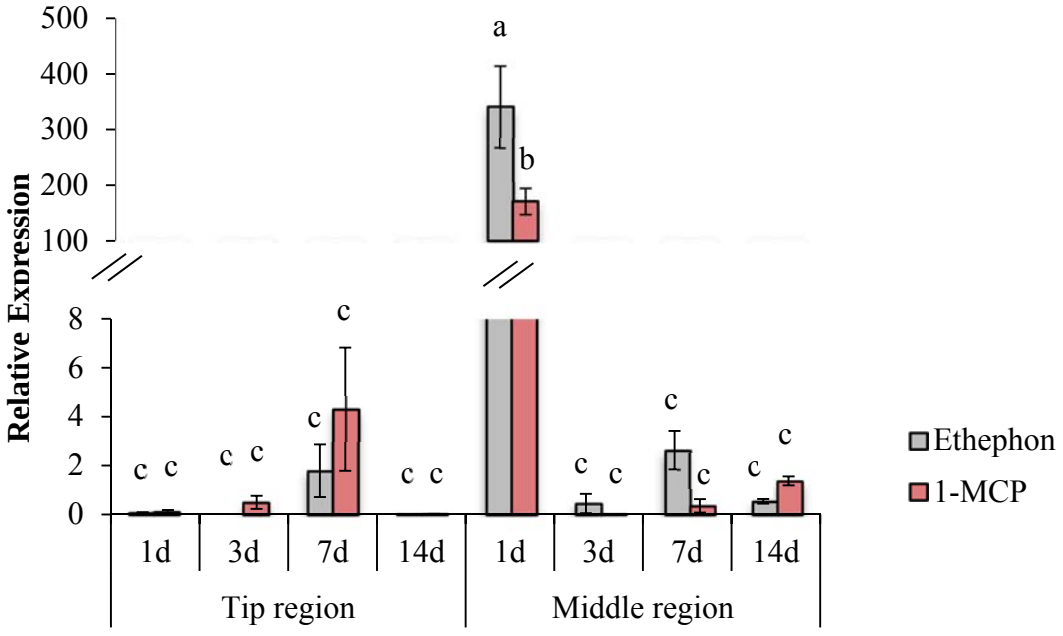

Figure 17: Relative expression of *IbEM012* which was similar to expressed protein in sweetpotato storage root treated with 3.9 mM ethephon and 1ppm 1-MCP from two different regions (tips and middle) at different time points.

Semiquantitative PCR showed sweetpotato with end rot had elevated *IIF* expression in both regions (Figures 13 and 18). However, *IIF* had low expression with no statistical differences in treatments and regions of storage roots. Little is known about the gene other than it being a transcription factor for initiation of other genes. It has value in response to ethylene and end rot.

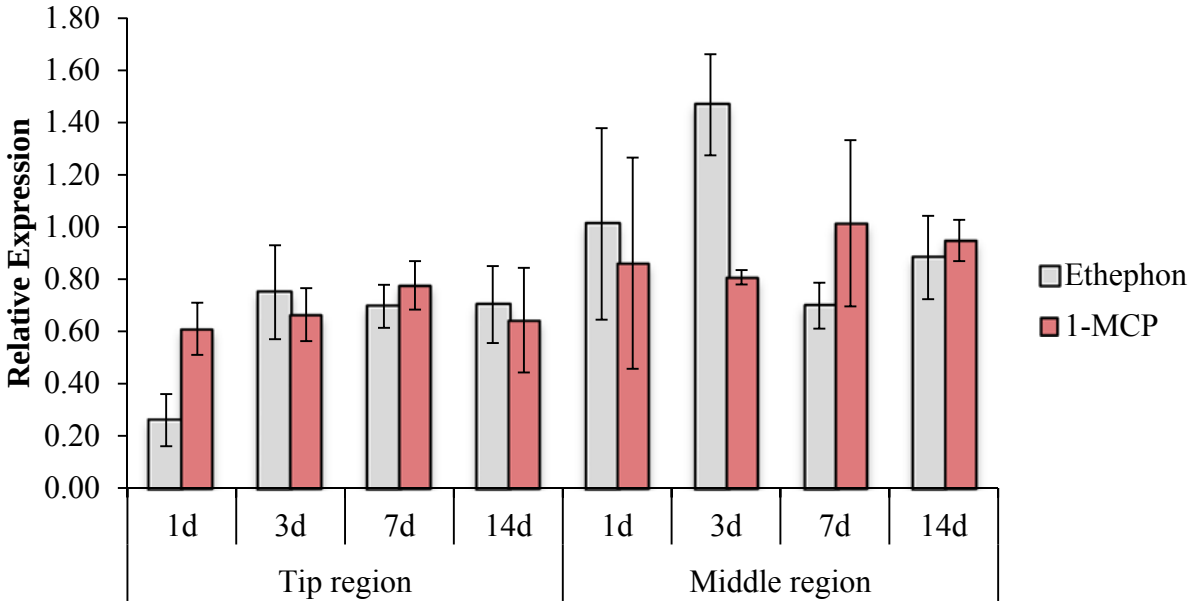

Figure 18: Relative expression of *IbEM16* which was similar to transcription initiation factor in sweetpotato storage root treated with 3.9 mM ethephon and 1ppm 1-MCP from two different regions (tips and middle) at different time points. No statistically significant differences among treatments and regions.

Ethylene treated storage roots. Genes were divided into 3 time responses: early, middle, and late (Table 11). The early response gene including *IIF*, *OLP*, and *EP*. The middle time points involved protective mechanism (*TH2* and *ATG*). The late response genes consisted of genes in a protective mechanism (*ATG*) and transcriptional gene (*IIF*). These indicated that ethylene treated storage roots had delayed expression of protective genes resulting in end rot incidence on sweetpotato storage roots.

Table 11: Upregulated expression of 5 DEGs in sweetpotato storage root treated with 3.9 mM ethephon and 1ppm 1-MCP from two different regions (tips and middle) at different time responses.

Time response	Ethephon		1-MCP	
	Tip	Middle	Tip	Middle
Early	*OLP*	*OLP, EP, IIF*	*TH2, IIF*	*TH2, EP, IIF*
Middle	*IIF*	*TH2, ATG, IIF*	*EP, IIF*	*ATG, IIF*
Late	*ATG, IIF*	*IIF*	*ATG, IIF*	*IIF*

1-MCP treated storage roots (Table 11). *IIF* was expressed in both regions at all-time points. *ATG* and *TH2* were early and middle time genes involved in protective mechanisms, transcription regulation (*IIF*) and expressed protein (*EP*). *ATG* and *IIF* was found in the late response. Results showed that some genes (*ATG*, and *EP*) expressed in the middle region before the tip regions (Table 11), but the mechanism is unknown.

Physiology observation. The present study found that end rot appeared in sweetpotato storage roots 2-3 weeks after treatment with ethephon. No end rot was observed in 1-MCP treated storage roots. It is possible that 1-MCP treated storage roots induced genes such as *TH2* and *ATG* that are involved in protective mechanisms in contrast to ethephon treated storage roots.

In conclusion, all five genes (*IbEM05, IbEM06, IbEM07, IbEM12,* and *IbEM16*) were expressed in sweetpotato with end rot. The functional annotation of the DEGs were similar to genes involved in protective mechanisms, transcription regulation, and an expressed protein (unknown). The five genes showed different levels of mRNA transcription response to ethephon treated storage roots and 1-MCP treated storage roots compared to control. 1-MCP induced higher expression of *TH2* and *ATG* in storage roots than the ethephon treated storage roots and those free of end rot incidence. Ethephon treated storage roots had higher mRNA transcription of *OLP* than 1-MCP in storage roots and showed greater end rot incidence. *IIF* showed low expression in all treatments in both regions. *EP* expression was inconsistent among treatments and regions. It may be possible to

lessen end rot through breeding by increasing expression of protective mechanism genes (*TH2* and *ATG*) which are enhanced in the presence of ethylene. This study provides new information on protective mechanisms in sweetpotato as a response to ethylene and 1-MCP treated storage roots, and demonstrates putative genes useful as potential molecular markers for breeding.

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CHAPTER 5. SUMMARY AND CONCLUSIONS

5.1 Summary and conclusions

Sweetpotato end rots have a complex etiology. Little is known about what factors in the environment trigger end rot development, genetics behind end rot incidence, and how to manage the crop to minimize end rots. Understanding the induction factors and genetics involved in end rot will enable better strategies to avoid end rot incidence.

Among four environmental factors including flooded/non-flooded; skinned/non-skinned; cured/non-cured; recommended storage/ambient storage showed that sweetpotato storage roots undergoing curing at 29^{o}C and 85-90% RH for five days and storage at 13^{o}C and 85-90% RH were most important to reduce end rot in comparison to other factor combinations.

Calcium deficiency had impact on end rot incidence. High concentration of calcium in storage root tissue lessened end rot incidence. No relationship was observed between calcium and ethephon. However, lack of calcium as a plant macronutrient affected total root weight, number and size of storage roots. Ethephon application enhanced proximal and distal end rot incidences and other decays. It caused tissue damage and had a pronounced impact on onset of end rot.

Differentially expressed genes (DEGs) involved in end rot induced by ethephon and 1-MCP and storage roots with end rot incidence were identified using 20 ACPs. All 5 DEGs were functionally annotated as similar to genes known to be involved in protective mechanisms, transcriptional regulation, and one was an expressed protein of unknown function. Genes induced by 1-MCP may be involved in protective mechanisms mitigating incidence of end rot after treatment with ethephon. Up-regulated in protective mechanism genes may be used as markers to further sweetpotato breeding.

5.2 Future research

Recommendations for future research.

1. Postharvest technology may enhance storage longevity and quality. Waxing or 1-MCP may be candidate treatments to reduce end rot and extend storage.
2. Measure soil moisture in the flooding treatments and measure storage root skinning to better understand the flooding factor and mechanical damage on end rot incidence in storage roots.
3. The role of calcium on sweetpotato storage root shape. Other nutrients such as potassium, sodium, or ammonium may affect calcium uptake (Geraldson *et al*., 1956). Therefore, a balance of nutrients may be used to generate an elliptical shape desired in produce markets.
4. Study calcium deficient soils as a means of mitigating end rot incidence.
5. Genes which are up-regulated in the presence of 1-MCP (*TH2* and *ATG*) may aid in countering the effect of ethylene induced physiological reactions. These up-regulated genes may be candidate genes for screening and selecting lines for breeding traits.
6. Ethylene is important as an inducer/enhancer of end rots. Studies on genes up-regulated in response to ethylene may lead to genetic mechanisms to counteract tissue response to ethylene injury.

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APPENDICES

Appendix A. SAS code for F values and probabilities for weight loss in the study of effect of environmental factors on expression of end rot in sweetpotato roots.

```
data Exp1;

input Rep $ Flooding $ Harvest $ Cured $ Storage $ Box $ Month $ %Weightloss;

datalines;

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1 1 1 1 1 1 6 25.79
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1	2	2	1	1	3	5	21.56
1	2	2	1	1	3	6	23.32
1	2	2	1	1	4	1	10.95
1	2	2	1	1	4	2	15.49
1	2	2	1	1	4	3	17.84
1	2	2	1	1	4	4	20.50
1	2	2	1	1	4	5	22.85
1	2	2	1	1	4	6	26.76
1	2	2	1	1	5	1	10.80

1	2	2	1	1	5	2	16.05
1	2	2	1	1	5	3	18.61
1	2	2	1	1	5	4	20.74
1	2	2	1	1	5	5	24.15
1	2	2	1	1	5	6	25.85
1	2	2	1	2	1	1	9.24
1	2	2	1	2	1	2	12.32
1	2	2	1	2	1	3	14.01
1	2	2	1	2	1	4	15.97
1	2	2	1	2	1	5	18.07
1	2	2	1	2	1	6	20.31
1	2	2	1	2	2	1	11.40
1	2	2	1	2	2	2	14.61
1	2	2	1	2	2	3	16.37
1	2	2	1	2	2	4	19.10
1	2	2	1	2	2	5	21.67
1	2	2	1	2	2	6	24.08
1	2	2	1	2	3	1	9.66
1	2	2	1	2	3	2	13.32
1	2	2	1	2	3	3	14.79
1	2	2	1	2	3	4	16.54
1	2	2	1	2	3	5	18.45
1	2	2	1	2	3	6	20.35

1	2	2	1	2	4	1	8.72
1	2	2	1	2	4	2	11.13
1	2	2	1	2	4	3	12.48
1	2	2	1	2	4	4	13.98
1	2	2	1	2	4	5	15.19
1	2	2	1	2	4	6	16.69
1	2	2	1	2	5	1	9.19
1	2	2	1	2	5	2	12.30
1	2	2	1	2	5	3	13.92
1	2	2	1	2	5	4	15.41
1	2	2	1	2	5	5	17.16
1	2	2	1	2	5	6	18.65
1	2	2	2	1	1	1	8.69
1	2	2	2	1	1	2	11.63
1	2	2	2	1	1	3	13.55
1	2	2	2	1	1	4	14.87
1	2	2	2	1	1	5	16.20
1	2	2	2	1	1	6	19.29
1	2	2	2	1	2	1	9.03
1	2	2	2	1	2	2	11.59
1	2	2	2	1	2	3	13.61
1	2	2	2	1	2	4	15.36
1	2	2	2	1	2	5	16.98

1	2	2	2	1	2	6	18.06
1	2	2	2	1	3	1	8.71
1	2	2	2	1	3	2	11.94
1	2	2	2	1	3	3	14.61
1	2	2	2	1	3	4	16.71
1	2	2	2	1	3	5	18.54
1	2	2	2	1	3	6	19.66
1	2	2	2	1	4	1	8.97
1	2	2	2	1	4	2	11.58
1	2	2	2	1	4	3	14.36
1	2	2	2	1	4	4	15.82
1	2	2	2	1	4	5	17.94
1	2	2	2	1	4	6	22.68
1	2	2	2	1	5	1	9.10
1	2	2	2	1	5	2	12.54
1	2	2	2	1	5	3	14.93
1	2	2	2	1	5	4	17.46
1	2	2	2	1	5	5	19.10
1	2	2	2	1	5	6	21.49
1	2	2	2	2	1	1	8.99
1	2	2	2	2	1	2	11.31
1	2	2	2	2	1	3	13.35
1	2	2	2	2	1	4	14.99

1	2	2	2	2	1	5	16.35
1	2	2	2	2	1	6	17.98
1	2	2	2	2	2	1	9.30
1	2	2	2	2	2	2	11.47
1	2	2	2	2	2	3	12.71
1	2	2	2	2	2	4	14.11
1	2	2	2	2	2	5	15.19
1	2	2	2	2	2	6	17.21
1	2	2	2	2	3	1	9.42
1	2	2	2	2	3	2	11.41
1	2	2	2	2	3	3	12.55
1	2	2	2	2	3	4	13.98
1	2	2	2	2	3	5	14.98
1	2	2	2	2	3	6	16.55
1	2	2	2	2	4	1	8.19
1	2	2	2	2	4	2	10.79
1	2	2	2	2	4	3	11.91
1	2	2	2	2	4	4	13.40
1	2	2	2	2	4	5	14.39
1	2	2	2	2	4	6	16.50
1	2	2	2	2	5	1	8.97
1	2	2	2	2	5	2	11.03
1	2	2	2	2	5	3	12.35

1	2	2	2	2	5	4	13.97
1	2	2	2	2	5	5	15.44
1	2	2	2	2	5	6	16.91

.

.

.

;

run;

```
Proc mixed;
Class Rep F M C St Box Month;
  Model Percent_proximal = F|M|C|St|Month/ddfm=KR;
  Random Rep Box*F*M Box(Rep F M C St);
  LSMEANS F|M|C|St|Time;
  Run;
```

Flooding column was represented by flooding = 1; non-flooding = 2,

Harvest column was represented by skinning = 1; non-skinning = 2,

Cured column was represented by non-cured = 1; cured = 2,

Storage column was represented by ambient storage = 1; recommended storage = 2

The data output showed the table of Type 3 tests of Fixed Effects and provided F value and Pr>F of effect (Table A.1.). F value and Pr>F as F value and probabilities was used in Table 1.

Table A.1. F values and probabilities for weight loss in the study of effect of environmental factors on expression of end rot in sweetpotato roots.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
F	1	16.2	17.25	0.0007
M	1	16.2	16.49	0.0009
F*M	1	16.2	11.48	0.0037
C	1	203	169.01	<.0001
F*C	1	203	1.06	0.3042
M*C	1	203	3.81	0.0523
St	1	204	103.96	<.0001
F*St	1	204	0.38	0.5410
M*St	1	204	1.18	0.2778
C*St	1	203	2.35	0.1265

F value and probabilities were used in Table 1

Appendix B. SAS code for Proc ANOVA in the study of effect of environmental factors on expression of end rot in sweetpotato roots.

```
Data physio_crop1;

Do tr = 'a','b','c','d','e','f','g','h','i','j','k','l','m','n','o','p';

Do block = 1 to 3;

Input weightloss_month1@@;

Output;

End;

End;

Cards;

28.55  28.02  25.51

22.97  20.72  23.36

21.44  21.16  21.61
```

17.95 18.77 18.21

28.08 26.54 25.62

22.51 21.97 21.82

21.50 23.41 20.65

18.10 17.83 20.25

28.65 28.15 28.77

23.29 21.22 22.64

21.98 20.44 25.40

18.73 17.86 17.42

25.62 21.67 23.67

20.02 18.60 20.29

20.24 20.10 18.90

17.03 16.51 17.78

;

```
PROC ANOVA;
class block tr;
Model weightloss_month6 = block tr;
Means tr/LSD;
Means tr;
run;
```

There were 16 combination treatments in the study of effect of environmental factors on expression of end rot in sweetpotato roots.

1. Treatment a was referred to flooding/skinning/non-cured/ambient storage.
2. Treatment b was referred to flooding/skinning/non-cured/recommended storage.
3. Treatment c was referred to flooding/skinning/cured/ambient storage.
4. Treatment d was referred to flooding/skinning/cured/recommended storage.
5. Treatment e was referred to flooding/non-skinning/non-cured/ambient storage.
6. Treatment f was referred to flooding/non-skinning/non-cured/recommended storage.
7. Treatment g was referred to flooding/non-skinning/cured/ambient storage.
8. Treatment h was referred to flooding/non-skinning/cured/recommended storage.
9. Treatment i was referred to non-flooding/skinning/non-cured/ambient storage.
10. Treatment j was referred to non-flooding/skinning/non-cured/recommended storage.
11. Treatment k was referred to non-flooding/skinning/cured/ambient storage.
12. Treatment l was referred to non-flooding/skinning/cured/recommended storage.
13. Treatment m was referred to non-flooding/ non-skinning/non-cured/ambient storage.
14. Treatment n was referred to non-flooding/non-skinning/non-cured/recommended storage.
15. Treatment o was referred to non-flooding/non-skinning/cured/ambient storage.
16. Treatment p was referred to non-flooding/non-skinning/cured/recommended storage.

The data were analyzed by month and by treatment. Each analysis was showed in the table of Means with the same letter are not significantly different. The means and standard deviation values were used in Table 2, 3, 4, and 5.

Table B.1. Means of weight loss at the 6th month in the study of effect of environmental factors on expression of end rot in sweetpotato roots.

Means with the same letter are not significantly different.				
t Grouping		Mean	N	tr
	A	28.5233	3	i
	A	27.3600	3	a
	A	26.7467	3	e
	B	23.6533	3	m
C	B	22.6067	3	k
C	B	22.3833	3	j
C	B	22.3500	3	b
C	B	22.1000	3	f
C	B	21.8533	3	g
C	D	21.4033	3	c
E	D	19.7467	3	o
E	D	19.6367	3	n
E	F	18.7267	3	h
E	F	18.3100	3	d
E	F	18.0033	3	l
	F	17.1067	3	p

Appendix C. SAS code for Proc Glimmix in the study of the role of calcium deficiency on end rot incidence.

```
data Proximal_END_ROT;

input Trial NON_DIP_or_DIP Rep Tr Month Proximal_END_ROT;

datalines;

1 1 1 0 1 0

1 1 1 0 2 50
```

1	1	1	0	3	50
1	1	2	0	1	50
1	1	2	0	2	50
1	1	2	0	3	50
1	1	3	0	1	33
1	1	3	0	2	67
1	1	3	0	3	67
1	1	4	0	1	100
1	1	4	0	2	100
1	1	4	0	3	100
1	1	5	0	1	20
1	1	5	0	2	20
1	1	5	0	3	40
1	1	6	0	1	80
1	1	6	0	2	80
1	1	6	0	3	80
1	2	7	0	1	100
1	2	7	0	2	100
1	2	7	0	3	100
1	2	8	0	1	83
1	2	8	0	2	100
1	2	8	0	3	100
1	2	9	0	1	80

1	2	9	0	2	80
1	2	9	0	3	80
1	2	10	0	1	25
1	2	10	0	2	75
1	2	10	0	3	75
1	2	11	0	1	100
1	2	11	0	2	100
1	2	11	0	3	100
1	2	12	0	1	50
1	2	12	0	2	50
1	2	12	0	3	50
1	1	1	50	1	0
1	1	1	50	2	0
1	1	1	50	3	0
1	1	2	50	1	17
1	1	2	50	2	17
1	1	2	50	3	17
1	1	3	50	1	0
1	1	3	50	2	25
1	1	3	50	3	25
1	1	4	50	1	0
1	1	4	50	2	25
1	1	4	50	3	25

1	1	5	50	1	25
1	1	5	50	2	50
1	1	5	50	3	50
1	1	6	50	1	0
1	1	6	50	2	0
1	1	6	50	3	0
1	2	7	50	1	20
1	2	7	50	2	40
1	2	7	50	3	40
1	2	8	50	1	29
1	2	8	50	2	29
1	2	8	50	3	29
1	2	9	50	1	20
1	2	9	50	2	60
1	2	9	50	3	60
1	2	10	50	1	80
1	2	10	50	2	100
1	2	10	50	3	100
1	2	11	50	1	50
1	2	11	50	2	83
1	2	11	50	3	83
1	2	12	50	1	75
1	2	12	50	2	100

1	2	12	50	3	100
1	1	1	100	1	0
1	1	1	100	2	0
1	1	1	100	3	0
1	1	2	100	1	0
1	1	2	100	2	0
1	1	2	100	3	0
1	1	3	100	1	33
1	1	3	100	2	33
1	1	3	100	3	33
1	1	4	100	1	20
1	1	4	100	2	20
1	1	4	100	3	20
1	1	5	100	1	0
1	1	5	100	2	0
1	1	5	100	3	0
1	1	6	100	1	0
1	1	6	100	2	0
1	1	6	100	3	0
1	2	7	100	1	50
1	2	7	100	2	50
1	2	7	100	3	50
1	2	8	100	1	17

1	2	8	100	2	17
1	2	8	100	3	17
1	2	9	100	1	83
1	2	9	100	2	83
1	2	9	100	3	83
1	2	10	100	1	71
1	2	10	100	2	71
1	2	10	100	3	71
1	2	11	100	1	0
1	2	11	100	2	50
1	2	11	100	3	50
1	2	12	100	1	100
1	2	12	100	2	100
1	2	12	100	3	100
1	1	1	150	1	0
1	1	1	150	2	0
1	1	1	150	3	0
1	1	2	150	1	0
1	1	2	150	2	0
1	1	2	150	3	0
1	1	3	150	1	0
1	1	3	150	2	0
1	1	3	150	3	25

1	1	4	150	1	0
1	1	4	150	2	20
1	1	4	150	3	20
1	1	5	150	1	0
1	1	5	150	2	40
1	1	5	150	3	60
1	1	6	150	1	0
1	1	6	150	2	0
1	1	6	150	3	0
1	2	7	150	1	67
1	2	7	150	2	83
1	2	7	150	3	83
1	2	8	150	1	0
1	2	8	150	2	0
1	2	8	150	3	0
1	2	9	150	1	67
1	2	9	150	2	83
1	2	9	150	3	83
1	2	10	150	1	100
1	2	10	150	2	100
1	2	10	150	3	100
1	2	11	150	1	67
1	2	11	150	2	100

1	2	11	150	3	100
1	2	12	150	1	67
1	2	12	150	2	83
1	2	12	150	3	83
1	1	1	200	1	0
1	1	1	200	2	0
1	1	1	200	3	0
1	1	2	200	1	0
1	1	2	200	2	0
1	1	2	200	3	0
1	1	3	200	1	25
1	1	3	200	2	25
1	1	3	200	3	50
1	1	4	200	1	0
1	1	4	200	2	0
1	1	4	200	3	0
1	1	5	200	1	0
1	1	5	200	2	0
1	1	5	200	3	0
1	1	6	200	1	0
1	1	6	200	2	0
1	1	6	200	3	0
1	2	7	200	1	33

1	2	7	200	2	33
1	2	7	200	3	33
1	2	8	200	1	50
1	2	8	200	2	50
1	2	8	200	3	50
1	2	9	200	1	50
1	2	9	200	2	100
1	2	9	200	3	100
1	2	10	200	1	40
1	2	10	200	2	100
1	2	10	200	3	100
1	2	11	200	1	40
1	2	11	200	2	80
1	2	11	200	3	80
1	2	12	200	1	75
1	2	12	200	2	75
1	2	12	200	3	50
1	1	1	250	1	100
1	1	1	250	2	100
1	1	1	250	3	100
1	1	2	250	1	0
1	1	2	250	2	0
1	1	2	250	3	0

1	1	3	250	1	25
1	1	3	250	2	25
1	1	3	250	3	25
1	1	4	250	1	0
1	1	4	250	2	17
1	1	4	250	3	17
1	1	5	250	1	0
1	1	5	250	2	20
1	1	5	250	3	20
1	1	6	250	1	0
1	1	6	250	2	75
1	1	6	250	3	75
1	2	7	250	1	100
1	2	7	250	2	100
1	2	7	250	3	100
1	2	8	250	1	43
1	2	8	250	2	100
1	2	8	250	3	100
1	2	9	250	1	100
1	2	9	250	2	100
1	2	9	250	3	100
1	2	10	250	1	100
1	2	10	250	2	100

1	2	10	250	3	100
1	2	11	250	1	50
1	2	11	250	2	75
1	2	11	250	3	75
1	2	12	250	1	33
1	2	12	250	2	67
1	2	12	250	3	67
1	1	1	300	1	0
1	1	1	300	2	0
1	1	1	300	3	25
1	1	2	300	1	0
1	1	2	300	2	0
1	1	2	300	3	0
1	1	3	300	1	0
1	1	3	300	2	0
1	1	3	300	3	0
1	1	4	300	1	33
1	1	4	300	2	33
1	1	4	300	3	33
1	1	5	300	1	0
1	1	5	300	2	0
1	1	5	300	3	0
1	1	6	300	1	0

1	1	6	300	2	33
1	1	6	300	3	100
1	2	7	300	1	50
1	2	7	300	2	75
1	2	7	300	3	75
1	2	8	300	1	75
1	2	8	300	2	100
1	2	8	300	3	100
1	2	9	300	1	100
1	2	9	300	2	100
1	2	9	300	3	100
1	2	10	300	1	20
1	2	10	300	2	20
1	2	10	300	3	20
1	2	11	300	1	100
1	2	11	300	2	100
1	2	11	300	3	100
1	2	12	300	1	67
1	2	12	300	2	67
1	2	12	300	3	67

.

.

.

```
;
run;
Proc Glimmix;
Class Trial DIP Rep Tr;
Model Proximal_END_ROT = DIP|Tr|ddfm = KR;
Random Trial Rep(Trial*DIP);
LSmeans DIP|Tr;
Run;
Proc Glimmix; where month=3;
Class Trial DIP Rep Tr;
Model Proximal_END_ROT =DIP|Tr / CL ddfm=KR;
Random Trial Rep(Trial*DIP);
LSmeans DIP|Tr / Lines adjust=Tukey;
Run;
Proc means data= Proximal_END_ROT; where month=3;
Class Tr DIP;
var Proximal_END_ROT;
Run;
```

NON_DIP_or_DIP was referred to storage roots did not dipped with ethephon = 1; storage roots dipped with ethephon = 2.

The data were showed in the table of Type III Tests of Fixed Effects (Table C.1.). F values and probability were used in Table 6. The data were analyzed by month. Each analysis was showed in the table of Tukey-Kramer Grouping for DIP*Tr Least Squares Means

(Alpha=0.05) (Table C.2.). The means and standard deviation values were used in Table 7, 8 and 9 (Table C.3.).

Table C.1. F value and probability in the study of the role of calcium deficiency on end rot incidence.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
DIP	1	22.01	186.81	<.0001
TRT	6	132	8.11	<.0001
DIP*TRT	6	132	1.86	0.0922
MTH	2	308	67.48	<.0001
DIP*MTH	2	308	4.34	0.0138
TRT*MTH	12	308	0.76	0.6953
DIP*TRT*MTH	12	308	1.21	0.2720

Table C.2. Means with the different significant of proximal end rot at the 3rd month in the study of the role of calcium deficiency on end rot incidence.

Tukey Grouping for DIP*Tr Least Squares Means (Alpha=0.05)			
DIP	Tr	Estimate	
2	0	86.5833	A
2	300	81.0000	A
2	250	79.9167	A
2	200	72.0833	A
2	50	70.6667	A
1	0	70.5833	A
2	100	68.1667	A
2	150	65.1667	A
1	250	26.0000	B
1	150	22.5000	B
1	200	18.9167	B
1	50	18.4167	B
1	300	17.3333	B
1	100	14.5000	B

Table C.3. Means and standard deviations of proximal end rot at the 3rd month in the study of the role of calcium deficiency on end rot incidence.

Analysis Variable : Prox							
Tr	DIP	N Obs	N	Mean	Std Dev	Minimum	Maximum
0	1	12	12	70.5833333	19.6675013	40.0000000	100.0000000
	2	12	12	86.5833333	17.9466803	50.0000000	100.0000000
50	1	12	12	18.4166667	17.7018660	0	50.0000000
	2	12	12	70.6666667	27.7630668	29.0000000	100.0000000
100	1	12	12	14.5000000	19.0668680	0	50.0000000
	2	12	12	68.1666667	23.5442845	17.0000000	100.0000000
150	1	12	12	22.5000000	31.2976909	0	100.0000000
	2	12	12	65.1666667	37.8941908	0	100.0000000
200	1	12	12	18.9166667	27.5432167	0	75.0000000
	2	12	12	72.0833333	24.0282347	33.0000000	100.0000000
250	1	12	12	26.0000000	31.1914907	0	100.0000000
	2	12	12	79.9166667	24.7035453	25.0000000	100.0000000
300	1	12	12	17.3333333	31.0551806	0	100.0000000
	2	12	12	81.0000000	26.7683667	20.0000000	100.0000000

Appendix D. SAS code for Proc Glimmix in the study of expressed genes in storage roots treated with ethephon and 1-MCP

```
data Primer;

input Region_1_T_2_M $ rep $ treatment $ time $ Primer2;

datalines;

                1               1               1               1d              0.00

                1               2               1               1d              0.00

                1               3               1               1d              0.00
```

1	1	1	3d	0.00
1	2	1	3d	0.08
1	3	1	3d	0.01
1	1	1	7d	1.06
1	2	1	7d	0.00
1	3	1	7d	3.58
1	1	1	14d	0.49
1	2	1	14d	0.93
1	3	1	14d	1.48
2	1	1	1d	0.19
2	2	1	1d	1.35
2	3	1	1d	0.02
2	1	1	3d	0.66
2	2	1	3d	0.49
2	3	1	3d	1.15
2	1	1	7d	3.76
2	2	1	7d	2.99
2	3	1	7d	2.55
2	1	1	14d	0.73
2	2	1	14d	0.43
2	3	1	14d	0.46
1	1	2	1d	1.83
1	2	2	1d	.

1	3	2	1d	4.76
1	1	2	3d	98.36
1	2	2	3d	.
1	3	2	3d	86.82
1	1	2	7d	.
1	2	2	7d	2.01
1	3	2	7d	1.16
1	1	2	14d	.
1	2	2	14d	2.41
1	3	2	14d	0.06
2	1	2	1d	104.69
2	2	2	1d	.
2	3	2	1d	40.79
2	1	2	3d	3.20
2	2	2	3d	1.19
2	3	2	3d	0.93
2	1	2	7d	0.00
2	2	2	7d	0.00
2	3	2	7d	0.02
2	1	2	14d	2.36
2	2	2	14d	2.95
2	3	2	14d	2.46

Region_1_T_2_M was referred to tip region = 1; middle region = 2.

Treatment was referred to ethephon = 1; 1-MCP =2.

The significance of data were showed in the table of Type III Tests of Fixed Effects. The means with the different letter were significantly different represented in the table of Tukey-Kramer Grouping for Region_*treatme*time Least Squares Means (Alpha=0.05) (Table D.1).

Table D.1. Tukey-Kramer Grouping for Region_*treatme*time Least Squares Means in the study of expressed genes in storage roots treated with ethephon and 1-MCP.

Tukey-Kramer Grouping for Region_*treatme*time Least Squares Means (Alpha=0.05) LS-means with the same letter are not significantly different.				
Region_1_T_2_M	treatment	time	Estimate	
1	2	3d	92.5867	A
2	2	1d	72.7367	A
1	2	1d	3.2917	B
2	1	7d	3.1000	B
2	2	14d	2.5900	B
1	2	7d	1.8741	B
2	2	3d	1.7733	B
1	1	7d	1.5467	B
1	2	14d	1.5241	B
1	1	14d	0.9667	B
2	1	3d	0.7667	B
2	1	14d	0.5400	B
2	1	1d	0.5200	B
1	1	3d	0.03000	B
2	2	7d	0.006667	B
1	1	1d	-161E-15	B

VITA

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