ABSTRACT

JIANG, CHEN. Sweetpotato Root Quality in Response to Abiotic Factors and Maximizing Greenhouse Plant Production by Adjusting Fertilizer Application Rates. (Under the direction of Jonathan Schultheis.)

A two-year greenhouse experiment was conducted in 2011 and 2012 on varieties 'Covington', 'Beauregard' and 'Evangeline' to evaluate nitrogen (N) fertilizer rate on greenhouse sweetpotato plant production. Plant cuttings were planted with Fafard 2 soilless media in pots in the greenhouse. A 20-10-20 fertilizer was used for all treatments. Five N fertilizer rates ranging from 50 to 400 ppm and two application methods (fertilizer applied every irrigation versus tap water irrigation after every 2 fertigation applications) were evaluated. Plants were harvested 9 times over the season in both years from 14 April to 15 August in 2011 and from 5 March to 19 June in 2012. Total nodes, fresh vine weight and number of live plants were recorded after each harvest. 'Evangeline' produced more nodes than 'Beauregard' and 'Covington'; 'Covington' produced the most vine mass, followed by 'Beauregard' and 'Evangeline'. Regardless of variety, the 50 and 400 ppm rates significantly reduced plant growth and production; fertilizer rates from 100 ppm to 300 ppm resulted in similar plants. The effect of fertilizer rate was the most pronounced in the late season compared with early or middle season. High EC and acidity as well as low calcium were observed primarily at high N rates (300 ppm and 400 ppm), and the most plant death occurred at 400 ppm. Tissue N level could be monitored during production and 7% N can be used as an indicator of N toxicity. Leaching was helpful in mitigating against toxicity. Nitrogen rate above 100 ppm strongly inhibited storage root formation while the highest root

yield was obtained at the 50 ppm rate; ‘Covington’ produced the most number of storage roots among the three varieties.

A series of studies was undertaken to understand the occurrence and causes of internal necrosis (IN) in ‘Covington’ sweetpotato. A survey was conducted for two seasons across nearly 25 NC growers or businesses per season to investigate the occurrence of IN in ‘Covington’ during storage. Results indicated the problem was widely spread in facilities throughout the state but both incidence and severity were generally low. There were some exceptions in which a few businesses were impacted by a high percentage of IN with severe root symptoms. Storage temperature and relative humidity were monitored in the surveyed commercial facilities, with no relationship found between IN and storage temperature and relative humidity conditions. Internal tissue of symptomatic roots was cultured in both moisture chambers and acidified PDA medium and no consistent pathogen types were isolated that could be associated with IN. Laboratory studies which stored sweetpotato roots in air-tight barrels with 100 ppm ethylene did not find any relationship between ethylene gas in storage and IN. Effects of Prep (Ethephon (2-Chloroethyl) phosphonic acid) and other insecticides and herbicides were evaluated to detect the occurrence of IN. Only Prep induced IN. IN symptoms were not detectable at harvest; the earliest significant incidence was found 8 days after harvest, and symptoms became more prevalent and severe 30 days after harvest. Curing enhanced the incidence and severity of IN. Anatomical work was done using DAPI (4', 6-diamidino-2-phenylindole) fluorescent dye to detect cell death in roots which had been exposed to Prep but prior to the presence of visual IN symptoms. This approach was not successful as the specific region on the root where IN occurs varied.

Sweetpotato Root Quality in Response to Abiotic Factors and Maximizing Greenhouse Plant Production by Adjusting Fertilizer Application Rates

by
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DEDICATION

To my family.

BIOGRAPHY

Chen Jiang was born December 16, 1987 to Hanzhong Jiang and Qingji Shi. Born and raised in the center of metropolis Beijing, she had no idea what agriculture was until she went to college. She graduated from Experimental High School Attached to Beijing Normal University in 2006. With a strong interest in science (especially life science), and with the encouragement of her father Hanzhong, who believed agriculture is very important for the development of China and the rest of the world, Chen decided to go to Nanjing Agricultural University (NAU) and majored in Agronomy. While at NAU, she was fortunate to be taught by fun and knowledgeable professors who opened her mind to agriculture and aroused her passion to work with plants. As a junior, she joined a crop physiology lab on campus to work on a cotton fiber proteomics project, with the guidance of Dr. Zhiguo Zhou and Dr. Youhua Wang. While there she enjoyed both the field and lab work and decided to go to graduate school later on for more agricultural research training and become a scientist one day. Chen graduated from NAU in 2010 summer and moved shortly thereafter to Raleigh, NC to pursue a Master of Science degree at North Carolina State University. Under the direction of Dr. Jonathan Schultheis, Chen worked on research projects studying sweetpotato root quality in response to abiotic factors and maximizing greenhouse plant production by adjusting fertilizer application rates.

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Approx. 25 North Carolina sweetpotato growers / businesses

Specialty Crop Research Initiative

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CHAPTER I. NITROGEN (N) FERTILIZATION RATES AND APPLICATION TIMING EFFECTS ON GREENHOUSE SWEETPOTATO PLANT PRODUCTION

Chen Jiang, Zvezdana Pesic-VanEsbroeck, Jason A. Osborne and Jonathan R. Schultheis

A two-year greenhouse experiment was conducted in 2011 and 2012 on varieties 'Covington', 'Beauregard' and 'Evangeline' to evaluate nitrogen (N) fertilizer rate on greenhouse sweetpotato plant production. Plant cuttings were planted with Fafard 2 soilless media in pots in the greenhouse. A 20-10-20 fertilizer was used for all treatments. Five N fertilizer rates ranging from 50 to 400 ppm and two application methods (fertilizer applied every irrigation versus tap water irrigation after every 2 fertigation applications) were evaluated. Plants were harvested 9 times over the season in both years from 14 April to 15 August in 2011 and from 5 March to 19 June in 2012. Total nodes, fresh vine weight and number of live plants were recorded after each harvest. 'Evangeline' produced more nodes than 'Beauregard' and 'Covington'; 'Covington' produced the most vine mass, followed by 'Beauregard' and 'Evangeline'. Regardless of variety, the 50 and 400 ppm rates significantly reduced plant growth and production; fertilizer rates from 100 ppm to 300 ppm resulted in similar plants. The effect of fertilizer rate was the most pronounced in the late season compared with early or middle season. High EC and acidity as well as low calcium were observed primarily at high N rates (300 ppm and 400 ppm), and the most plant death occurred at 400 ppm. Tissue N level could be monitored during production and 7% N can be used as an indicator of N toxicity. Leaching was helpful in mitigating against toxicity. Nitrogen rate above 100 ppm strongly inhibited storage root formation while the highest root

yield was obtained at the 50 ppm rate; ‘Covington’ produced the most number of storage roots among the three varieties.

Key words: sweetpotato, sweet potato, nitrogen, fertilizer rate, greenhouse, plant production, node, tissue analysis, media analysis, storage root.

Introduction

Sweetpotato [*Ipomoea batatas* (L.) Lam.] is mainly grown in the southeastern part of the United States (US) with national production of 2,385 million pounds (USDA-ESMIS, 2010). North Carolina (NC) ranked first in the production of US, with 54,000 acres of crop harvested and a production value of $173 million in 2010.

Sweetpotato planting stock is increased by vegetative propagation. Sweetpotato true seeds are only used for breeding purposes while organs such as storage roots, shoot tips and stem cuttings can be used for asexual propagation (Loebenstein and Thottappilly (eds.), 2009). In the US, all planting material is obtained by slips or sprouts that are either cut or pulled from storage roots grown in nursery beds. The nursery beds are also called "hotbeds" or simply "beds", with either a black or clear plastic mulch cover to increase soil temperature, which promotes root sprouting.

Because sweetpotatoes are asexually propagated, mixtures with other clones, roots harboring disease, mutation and insects are concerns of the industry which decrease the positive attributes for which a variety was released. In the US, sweetpotato foundation and certification programs exist in multiple states such as NC, Alabama, Louisiana, Mississippi and California; these programs have served the industry seed stocks that maintain the characteristics and quality of released varieties since the 1940s (Dangler, 1994 a; Dangler, 1994 b; Mulkey and Hernandez, 1994; Schultheis et al., 1994; Sloan, 1994). Before the development and usage of tissue culture, elimination of off-type plants and roots, as well as elimination of insects and diseases were done by careful management and true-to-type hill selections. Starting in the 1960s, California was the first state to use meristem-tip tissue

culture in foundation seed production (Dangler et al., 1994). The protocol was originally designed to overcome problems with russet crack, which was caused by Sweet potato feathery mottle virus (SPFMV), and later on used to exclude other systemic pathogens through meristem-tip culture. The California program served as a model for other states in the use of tissue culture in foundation sweetpotato seed programs. North Carolina researchers documented that by using hill selected, virus-indexed micropropagated (VIM) plants, yield can be increased up to 20% (Schultheis, 1994) and russet crack symptoms in 'Beauregard' can be significantly reduced (Jester et al., 1993). It was also documented that high yield and good storage quality of VIM materials could decline in generations due to the accumulation of virus, pathogens and mutations (Bryan et al., 2003). Generation 1(G1) plants produced consistently higher yield and more uniform storage roots than G2-G5 plants.

The Micropropagation Unit and Repository (MPUR) at NC State University was founded in 1996. The MPUR provides growers with the highest quality virus-indexed Nuclear plants and serves as a source of propagation material for the sweetpotato certified seed industry in the state and out-of-state. Nuclear plants are obtained from MPUR in October through January by Certified Sweetpotato Seed Growers and these elite materials are multiplied in their greenhouses as fast as possible by asexual vine cuttings. Once the greenhouse is filled to near or at capacity, plants grow into vine cuttings which are cut and transplanted to the field to produce first generation seed stock (G1).

Sweetpotato is a dicotyledonous plant native to Central America (Austin, 1977; Austin, 1978). As a tropical plant, sweetpotato rarely flowers and when it does it is usually during short days (Edmond and Ammerman, 1971). For breeders in temperate areas, grafting,

girdling or day-length control is needed in order to induce flowering during long days. Fast plant growth generally requires high temperature, high light intensity and good soil moisture (Lebot, 2009). Optimal day/night temperature for sweetpotato plant growth is 30/22 ℃ (Reddy et al., 2010). Sweetpotato plant exposure to brief periods of low temperature can have a significant effect on the carbon transport system, and for very young plants, prolonged exposure to 10 ℃ can cause plant death (Kays et al., 1982). Under low light intensity (i.e., nurseries), sweetpotato plants can only grow vigorously if they are attached to the storage roots which provide nutrients (Lebot, 2009). Regular irrigation promotes plant growth, but moisture in the media needs to be utilized by the plants in the pot which results in some drying between irrigations. Under very high humidity and low light intensity, water is absorbed at a faster rate in plant roots than it is transpired through leaf cells (Averre and Jones, 1991). Enlarged leaf cells will divide, rupture, and form blisters, which is called Intumescence (also called "Edema" or "Oedema"). This is a physiological disorder that is not related to any disease.

Fast plant growth also requires adequate nutrients, among which, nitrogen (N) is considered one of the most important. The literature on N application to sweetpotato is focused on the role it plays on maximizing storage root yield and quality (Constantin et al., 1974; Bellinder and Morse, 1982; Constantin et al., 1984). Identifying an optimum N rate for field production is difficult as both environmental conditions and genotype characteristics vary in N uptake and assimilation efficiency. The current NC recommendation is to apply 45 to 67 kg N/ha after 28 days of planting (http://www.ncsweetpotatoes.com/sweet-potato-industry/growing-sweet-potatoes-in-north-carolina/soils-and-fertilization/), but Phillips et al.

(2005) emphasized that the most predominant sweetpotato variety 'Beauregard' required less N than other varieties, and a range between 28 and 56 kg N/ha would result in optimal yield, depending on precipitation and soil moisture. Nitrogen requirement that maximizes vine or plant production for various edible commercial sweetpotato varieties grown to produce field transplants was not found in the literature. However, for ornamental sweetpotato plants, which are propagated exclusively for their foliage and vegetation, Syngenta has a recommendation for one of their varieties Sidekick™ (http://www.syngentaflowersinc.com/pdf/cultural/SidekickIpomoeaCulture.pdft).They recommend that plants should be given 200 ppm N and they grow best when a mix of Cal-Mg (i.e. 15-5-15, 14-4-14, etc.) and ammonium-containing (i.e. 20-10-20, 15-15-15, etc.) fertilizers are used. They also mentioned that electrical conductivity (EC) level in the media needs to be monitored and the media should be leached with water as needed to avoid salt building up. However, they did not provide any detailed fertilization schedule that growers can follow directly. Commercial sweetpotato plant producers in NC have been determining fertilizer need empirically. A N rate between 100 ppm and 200 ppm has generally been used by many growers, with variation due to the stage of plant growth and development, as well as the weather conditions (Jim Jones, Jones Farms, Bailey, NC; Sonny Scott, Scott Farms, Lucama, NC, personal communication).

Nitrogen is a component of many organic compounds in plants, including amino acids and chlorophyll, and its availability promotes rapid growth and increases leaf size and quality (Tucker, 1999). Greenhouse nutrition studies on various species have indicated that N fertilizer rates affected plant growth, especially shoot growth. Texas mountain laurel

(*Sophora secundiflora*) seedlings cultured in a media of 1:1 Sunshine mix No. 4 (SunGro Hort., 15831 N.E. 8th Street, Suite 100, Bellevue, WA 98008): composted mulch (Western Organics, Inc., 2807 S 27th Ave, Phoenix, AZ 85009) achieved higher shoot height when watered with a range of 100-250 $mg \cdot L^{-1}$ (equal to 100-250 ppm) N solution than watered with 50 $mg \cdot L^{-1}$ N solution (Niu et al., 2011). *Anthurium* (*Anthurium andraeanum* Lind.) plants grown in coir medium receiving constant 105 $mg \cdot L^{-1}$ or 158 $mg \cdot L^{-1}$ N fertilization had higher dry weight, leaf area and flower numbers than plants receiving 79 $mg \cdot L^{-1}$ or 210 $mg \cdot L^{-1}$ N fertilization, the later rates were either too low or too high (Chang et al., 2012). Hydroponic grown bell pepper (*Capsicum annuum* L.) stem and leaf growth response was quadratic to increasing N concentration from 0.25 to 14 mmol L^{-1} (equal to 3.5 to 196 ppm), with the peak stem length and stem dry matter at 7.3 mmol L^{-1} (102.2 ppm) and 8.0 mmol L^{-1} (112 ppm) of N concentrations, respectively (Bar-Tal et al., 2001). The above studies also suggest that the limited growth of plants under low N supply could be related to the low leaf chlorophyll content while the reduced growth under high N rates could be due to the antagonistic effects of N to K, Ca, and Mg. The reduced plant growth at high N levels was also attributed to high salt and low pH conditions around the root zone which killed the roots.

When growing sweetpotato plants for propagation increase, there is competition between foliage and roots for carbohydrate supply. The goal of sweetpotato greenhouse plant producers is to maximize vegetative growth and avoid production practices that favor storage root formation. The formation of sweetpotato storage roots can reduce foliage/plant production, and storage root enlargement can make it difficult to fertilize and irrigate containerized plants, and in extreme circumstances can become so enlarged that they break

the pots. Sweetpotato roots develop from the nodal part of the stem and thereby are considered as adventitious roots (Togari, 1950). Wilson and Lowe (1973) have observed the relation between stele lignification and the inability of adventitious roots to form storage roots. Togari (1950) also emphasized that in the first 20 days of planting, environmental conditions can affect the balance between cambium development and lignification, and in turn determining how many roots can become storage roots. Fertilizing plants with potassium-bearing fertilizer, such as potash, can accelerate cambium activities and thus help form storage roots, while fertilizing plants with high N (210 ppm), resulted in few storage roots (Wilson, 1973a and 1973b). During the early stage of storage root development, respiration rate of the roots amounted to 25% of the whole plant; frequent irrigation, which benefits shoot growth may lead to inadequate oxygen in the soil, which will inhibit cell division and expansion and thereby prevent storage root formation (Watanabe et al., cited in Goldsworthy and Fisher (eds.), 1984). Other factors including temperature and day length also influence storage root formation (Togari, 1950; Lebot, 1986). Low temperature (maximum 23.4 ℃ during the day) and long night conditions promote storage root development, otherwise cambium activities can be inhibited and storage roots do not develop.

When considering some of the previous work and the needs of the commercial sweetpotato plant propagation industry, the goals of this study were to 1) determine the N fertilization rates that resulted in the most sweetpotato plant production, 2) determine if there were differences in plant production between sweetpotato varieties in response to N rates, 3) determine the effects of pure tap water leaching on mitigating plant death caused by constant fertigation, and 4) determine the effects of N fertilizer levels on storage root formation.

Methods

A greenhouse study was conducted in 2011 and 2012 at Method Greenhouses, Raleigh, NC. Plastic pots with diameter 25.4 cm (HFO1000, Hummert Int., Earth City, MO 63045) were filled with 1.5 kg soilless mix (Fafard 2 Mix, Conrad Fafard Inc., P.O. Box 790, Agawam, MA 01001). Cut plants (2 node cuttings) were obtained by taking cuttings from elite nuclear plants maintained at the Method Greenhouses by the MPUR. Ten plants per pot were planted approximately 5 cm distance from each other on 17 March 2011 and 31 January 2012.

The experiment was subjected to a randomized complete block split plot design (RCBSPD) with repeated measures overtime. Three sweetpotato varieties (Covington, Beauregard and Evangeline) and five N fertilizer rates (50, 100, 200, 300, and 400 ppm) were evaluated. Two fertilization/ irrigation application methods were used; either fertilizer applied every irrigation (fertigation) (FEI), or tap water irrigation after every 2 times of fertigation (short as "leach"). Fertilizer applied every irrigation (FEI) application was tested at all five fertilizer rates, while the "leach" application was only at the 200, 300 and 400 ppm fertilizer rates. Each treatment combination (fertilizer rate * application method * variety) was replicated four times, with each replication being a block. Due to the limitation of the drip irrigation system, every fertilizer treatment (combination of fertilizer rate and application methods) was a whole-plot factor while every variety was a sub-plot factor. To eliminate possible positional or micro-environmental effects of the whole-plot factor, pots in each row (whole plot) on greenhouse benches were moved to the adjacent row after every plant harvest. In each year, there were a total 9 harvests. For evaluation convenience and to

simplify response comparisons throughout the growing season, the 9 harvests were divided into three groups as “Early” (harvests 1, 2 and 3), “Middle” (harvests 4, 5, and 6) and “Late” (harvests 7, 8 and 9).

Plants were fertigated/ irrigated as needed (ranging from one to four days). Time between fertigation/ irrigation varied, depending on the stage of plant growth and environmental conditions (i.e., sunny vs. cloudy). Greenhouse temperature ranged between 32 to 38 ℃ during the day and 21 to 27 ℃ at night. The fertilizer used for this experiment was 20-10-20 Greenhouse Premium with 12% nitrate and 8% ammonium (Agri-B, 1102 Third Avenue Albany, GA 31707). All rows on the same bench shared one PVC main line which was connected to a dosatron (DI16, Dosatron Int., 2090 Sunnydale Blvd., Clearwater, FL 33765). The dosatron pumped the fertilizer solution (or pure water when leaching) and combined it with tap water in a 1:5 ratio. A spaghetti tube was plumbed into the PVC main line to distribute the given fertilizer solution or irrigation water into given treatment rows. A ring-shape emitter (PCR10-12, Dramm, 2000 North 18 St, Manitowoc, WI 54220) was connected to the spaghetti tube to distribute the solution into every part of each pot evenly. Each time a treatment was administered, fertigation was applied until all pots in the same treatment started to leach nutrient solution/water from the bottom of the pot. For “leach” treatments, when tap water was used, irrigation needed to be applied until water was drained from all treatment pots for at least five minutes.

Time of each plant harvest was determined when vines from the slowest growing plants hung over the lip of the pots and were approximately 0.6 m in length. Once this occurred, in order to make comparison easier and to have a consistent timing for harvest,

plants in all pots were harvested. All cut vines were weighed and total fresh mass was determined for each pot; total number of nodes on all vines per pot was recorded, as 2-3 nodes could usually make a plant, and the record of node number can be used to predict the number of plants. The interval between each harvest was between 10 and 14 days, and primarily varied due to weather conditions. In 2011, the initial plant cutting was on 14 April and concluded with the ninth cutting on 15 August. In 2012, the same number of harvests was obtained with the first harvest beginning 5 March and the ninth harvest 19 June.

In both 2011 and 2012, three soilless media samples were collected prior to beginning the experiment to document the pH, EC and nutrition levels (especially N) of the substrate. Soilless media samples for each pot (96 per experiment) were taken at the end of each year's experiment. Tissue samples were obtained three times throughout both experimental seasons (14 April, 15 June and 15 August, 2011; 5 March, 3 May and 19 June, 2012). The recommended method for tissue analysis is to collect the most recent mature leaf in a quantity that provides sufficient material for chemical processing (Mills and Jones, 1996). Based on this recommendation, the first and second fully expanded sweetpotato leaves from the tip growing point were sampled. All samples (both media and tissue) were submitted to the NCDA&CS (4300 Reedy Creek Road, Raleigh NC 27607) for nutrient analysis. Once the final soilless media samples were collected, the media in each pot was sifted through and the number and weight of storage roots were recorded in order to gain information on the effects of N and sweetpotato variety on storage root formation.

The effect that fertilizer rate had on plant quality was evaluated with field plantings, by obtaining cuttings from two greenhouse plant harvests (5 and 8) in 2012. Five plants were

cut and collected for each treatment of the FEI application (variety * fertilizer rate) and transplanted to the field the following day (May 4 and Jun 8, 2012). Both plant stand and yield data were recorded as a measure of plant quality. Roots were harvested 28 August 2012 for the earlier planting (116 days after planting) and 11 September 2012 for the later planting (95 days after planting). Roots were graded according to USDA standards (USDA, 2005) and weighed.

Statistical analysis for all data was coenducted by SAS 9.3 and JMP Pro 9 (100 SAS Campus Dr. Cary, NC 27513). Data for two different fertilization/ irrigation application methods were analyzed separately. Emphasis was focused on the FEI application data. "Leach" treatments data were only used to form a T- test comparison between leaching and no leaching treatments using the same N rates.

1. Node data for FEI application treatments were fitted into a model:

$$Node_{ijkpq} = var_i + trt_j + eml_k + (var \times trt)_{ij} + (var \times eml)_{ik} + (trt \times eml)_{jk} + (var \times trt \times eml)_{ijk} + REP(YEAR)_{pq} + REP_p \times trt_j \times var_i \times (YEAR)_q + E_{ijkpq}$$

Where Node= the number of nodes, var= sweetpotato varieties (i= Beauregard, Covington, Evangeline), trt= fertilizer rate (j= 50, 100, 200, 300, 400), eml= harvest time (k= early, middle, late), REP= replication (p= 1, 2, 3, 4), YEAR= year being evaluated (q= 2011, 2012), E= errors generated in repeated measures. Among them, effects written by lower case letters are fixed effects, and effects written by upper case letters are random effects.

2. Tissue N data for FEI application treatments were analyzed by harvest time (eml) and year. Soilless media data for FEI application treatments were analyzed by year. Both tissue and soilless media N data were fitted into the model:

$$Nitrogen_{ijp} = var_i + trt_j + (var \times trt)_{ij} + (REP \times trt \times var)_{ijp} + E_{ijp}$$

Where Nitrogen= the amount of nitrogen, var= sweetpotato varieties (i= Beauregard, Covington, Evangeline), trt= fertilizer rate (j= 50, 100, 200, 300, 400), REP= replication (p= 1, 2, 3, 4), E= errors generated in repeated measures. Among them, effects written by lower case letters are fixed effects, and effects written by upper case letters are random effects.

3. All data otherwise were analyzed by Tukey HSD multiple comparisons using Least Square Means.

Results

1. Node production for FEI applications

Results from both 2011 and 2012 showed that node production was significantly affected by fertilizer rate (trt), variety (var) and harvest time (eml), and there was a strong two-way interaction between fertilizer rate and harvest time (Table 1). Nodal response differed significantly between years. Interaction between variety and harvest time was significant in the model; however this appeared to be an artifact effect. The significance is likely due to the large differences in the main effect between cultivars.

Regardless of variety and harvest time, node production was the highest in both 2011 and 2012 when 100 to 300 ppm fertilizer rates were applied (Table 2). When 50 ppm N was used, node production was reduced and fresh plant weight was low. The ratio of fresh weight to nodes (W/N) was also significantly lower than it was in all other fertilizer rate treatments. The highest N rate (400 ppm) resulted in an overall node number reduction of 15% and 20% in 2011 and 2012 respectively, compared to plants grown at 200 ppm.

Variety growth response differed in terms of node production, regardless of fertilizer rate and harvest time (Table 1). Results were consistent between the two years; 'Evangeline' always had the highest node production which was 52% and 50% higher than 'Covington', the least productive variety, in 2011 and 2012 respectively (Table 3). On the other hand, 'Covington' had the highest W/N ratio among the three varieties and the vines seemed to be thicker and stronger. Thus, comparing node to node, 'Covington' gained more weight on the stem and leaf tissue than the other two varieties 'Beauregard' and 'Evangeline'.

Harvest time had a significant effect on node production (Table 1) but the effect varied across years (Table 4). In 2011, node production in "early" season was significantly lower than it was in "middle" and "late" season; while in 2012, node production in "early" and "middle" season was statistically the same, with the "late" season lower than "middle" season.

There was a strong interaction between fertilizer rate and harvest time in both years (Table 1), which means that plants responded to fertilizer treatments in a different manner at the various periods of production season. For both years, fertilizer rate consistently had the greatest differences between highest and lowest production in the "late" season; during which periods medium rates of fertilizer between 100 and 300 ppm produced the most number of nodes while both 50 ppm and 400 ppm resulted in reduced number of nodes (Table 5). Node production in response to different fertilizer rates did occur occasionally in the "early" and "middle" seasons but the occurrence was not as consistent as it was in the "late" season. In 2011, "early" season node production was similar across all fertilizer rates; the exception was at 50 ppm in which node production was reduced 19% compared when all other treatments

were averaged (100 to 400 ppm). In 2012, “middle” season node production was similar across all rates; the only exception was at 400 ppm in which node number dropped 27% compared to the average of all other treatments (50 to 300 ppm).

2. Nutritional analysis for FEI applications

Tissue and soilless media samples were collected and analyzed to determine the nutrition status in response to fertilizer treatments. For both years, as the fertilizer rate increased, N levels increased both in the soilless media and in plant tissue (Figures 1 and 2). For both years, N level in the soilless media increased linearly as the fertilizer rate increased, with the r-square value at least 97% (Figure 1). The more N fertilizer was applied, the more N was accumulated in the substrate. By the end of the 2011 season, applications with 50 ppm or 400 ppm N rate accumulated either very low (1 ppm for 50 ppm treatment) or very high (241 ppm for 400 ppm treatment) levels of N in the soilless media. The N concentration in soilless media was 127 ppm when 200 ppm fertilizer was applied in 2011. Fertilizer effects followed the same order in the 2012 soilless media samples, though the absolute values were all greater than 2011, with the 400 ppm treatment having an extraordinarily high amount of N accumulation (532 ppm).

Instead of a linear relationship between N fertilizer levels and N soilless media concentration, N levels in leaf tissue increased logarithmically as fertilizer rate increased when averaged over three sampling periods (Figure 2). During both years, as N fertilizer rate increased, N level in plant tissue first increased rapidly when its value was relatively low (below 7%). After tissue N level neared or exceeded 7%, though these values still increased

as N fertilizer rate increased, the increase was much less rapid. This information indicated that when tissue N was approximately 7%, it might be injurious or lethal. Data from this study showed that plants grew slower and produced fewer nodes whenever tissue N exceeded 7% level (Figure 3, Table 5). For example, in 2011, only in the "late" season for treatments 300 ppm and 400 ppm, did tissue N levels exceed 7%, which corresponded to when node production was reduced. In 2012, treatments and periods with tissue N levels above 7% occurred more often and included "middle" season 200 ppm to 400 ppm treatments, and the "late" season 400 ppm treatment. Node production decreased at both these two periods for each of the previously mentioned treatments. Slower growth and less production was an initial sign of N toxicity. In time these N levels could result in plant death. This happened in both years when N levels ranged from 300 ppm to 400 ppm, with the 400 ppm killing the most number of plants, which was 28% and 50% at the end of 2011 and 2012 seasons, respectively (Table 6).

Less growth was clearly shown when the fertilizer rate was high and tissue N level exceeded 7%; this also occurred when plants did not receive enough fertilizer. In the "late" seasons of both years, depleted media N supply (Figure 1) plus low tissue N level at 50 ppm treatment (Figure 3, late season) resulted in significantly lower production of nodes than medium rate treatments (Table 5).

There were some differences between varieties in their N levels in the leaf tissue (Table 7). In the 2011 study, 'Evangeline' always had the lowest tissue N concentration, numerically or statistically, among the three varieties over the entire duration of the experiment. In 2012, N levels were similar among varieties, and the only exception was the

lower level in ‘Evangeline’ during the “late” season.

3. Effect of year

There were significant differences in node production in 2011 and 2012. This would be expected due to differences between years/season growing conditions. The year factor was taken into account in the statistical model as a random effect. Node production in 2012 was lower than 2011 across all varieties, fertilizer rates and harvest times (Tables 2, 3, 4), while N concentration from plant tissue and soilless media were both higher in 2012 than they were in 2011 (Figures 1 and 2). In terms of plant mortality, plants died earlier and in greater numbers during the production season of 2012 than 2011 (Table 6). Overall, 39% more plants died in 2012 than in 2011, which was mainly due to the higher mortality of ‘Beauregard’ and ‘Evangeline’ in 2012 (Figure 4).

4. Effect of leaching

Leaching reduced both media and tissue N concentration (Table 8) and increased nodal yield (Table 9). Yield comparisons were made between the leaching and non-leaching treatments at various N rates. In both years, leaching increased yield when fertilizer rate was 300 or 400 ppm. Leaching also improved node yield at the 200 ppm rate in 2012 but not in 2011. Tissue and media N concentration was lowered when pots applied with the same fertilizer rate were leached than if not leached (Table 8), with one exception in 2011 in which tissue levels were similar at the 300 ppm treatment.

5. Storage root evaluation

The effects of fertilizer rate and variety on storage root formation were evaluated at the end of each year's experiment. Results were consistent across both years; high fertilizer rates strongly inhibited sweetpotato storage root formation while the highest root yield was obtained at the 50 ppm N rate for all three varieties (Figures 5 and 6). 'Covington' produced significantly more roots with greater weight than 'Beauregard' and 'Evangeline' (Table 10). The overall storage root yield in 2012 was lower than 2011 across all varieties.

6. Field transplanting- stand and yield

The effects that N fertilization had on sweetpotato plant stand and yield were evaluated in 2012 when transplants were cut from the greenhouse and planted in the field. Cuttings were obtained from the greenhouse at harvest 5 and harvest 8. Under favorable soil moisture conditions there were no differences in stand or yield between varieties so stand and yield data were combined across varieties. Stands were similar across all treatments and there were no significant differences in yield (Table 11).

Discussion

Results of node production suggest that optimal yield can be reached when N fertilizer rate is between 100 and 300 ppm (Table 2), with some variation between years or differences in growing season. However, if plant toxicity and deficiency are being considered, a narrower range of N fertilizer rate between 100 and 200 ppm seems more favorable.

Soil pH and EC (Electronic conductivity) are important values to manage plant- soil nutrition and plant production (Whipker and Cavins, 2000; Camberato et al., 2009). Electronic conductivity (EC) measures the total dissolved salts in the substrate. Recommended EC range for greenhouse vegetative cuttings is between 0.76 and 2.0 mS/cm (equal to 76 to 200 dS/m) (Camberato et al., 2009). A report from the Queensland government demonstrated that, depending on the soil types, EC threshold for field sweetpotato crops is between 100 and 300 dS/m, above which yield reduction could occur (http://www.derm.qld.gov.au/factsheets/pdf/water/w55.pdf). Similarly, but in a narrower range, Syngenta's ornamental sweetpotato variety Sidekick™ was recommended to be grown in a media with EC value between 180 and 220 dS/m (http://www.syngentaflowersinc.com/pdf/cultural/SidekickIpomoeaCulture.pdft). Compared with these reports, EC levels appear high in some treatments of this study (Figure 7 A and B). Data showed that in both years, as N rate increased, EC level increased, which exceeded 220 dS/m at the 400 ppm treatment in 2011, and 200 to 400 ppm treatments in 2012. Soilless media pH affects the amount of nutrients available to plants (Camberato et al., 2009). Though the optimal range is different from species to species, pH level between 5.4 and 6.2 is generally considered as good, within which range all nutrients are readily available. For sweetpotato, Syngenta suggested that media pH for Sidekick™ should be between 5.8 and 6.2 (http://www.syngentaflowersinc.com/pdf/cultural/SidekickIpomoeaCulture.pdft). In our study, the overall pH levels in both years appeared lower than this recommendation, and they changed as N rate changed (Figure 7A and B). When N fertilizer rate increased, pH value first decreased and then leveled off at 4.3- 4.6. Interestingly, in both years, this happened at

the 200 ppm N rate. It has been well documented that low pH can lead to increased plant availability of micronutrients such as aluminum (Al), manganese (Mn), zinc (Zn), copper (Cu), and iron (Fe) (Prasad and Sinha, 1982; Sarkar and Wyn Jones, 1982; Sims, 1986; Melakeberhan et al., 1995; Mortveldt, 2012). In this study, the concentration of Al was not reported, but tissue levels of Fe, Mn, Cu and Zn all appeared high in some treatments when pH was low (Appendix A, Table 2 and 3), which could be toxic to plants.

In addition, Calcium (Ca) deficiency seemed to occur at the high fertilizer rate treatments. Data showed (Figure 8) that tissue Ca concentration in the "late" season decreased significantly when N fertilizer rate increased. Critical range of Ca in plant tissue has been documented in some literature. One report suggested that Ca critical range for the most recently mature sweetpotato leaf is between 0.7% and 1.2% (Uchida (ed.), 2000). Another report suggested Ca concentration in plant tissue should be around 12.5% of N concentration (Stevens et al., 2002). When considering the Stevens et al. (2002) report, N level in this study was between 5% and 7.5% for a substantial portion of the study (Figures 2 and 3), therefore Ca level should be between 0.6% and 0.9%. However, our data showed that in the late season of both years, Ca level dropped to below 0.7% when 200 ppm N rate was used, and it kept decreasing to below 0.6% when fertilizer rate increased to 300 ppm and 400 ppm (Figure 8). Compared with both reports above (Uchida (ed.), 2000; Stevens et al., 2002), Ca level was low when N fertilizer rate was at 300 ppm. As an essential component in plant tissue, Calcium plays a structural role in cell walls and membranes; functions as a counter-cation in the vacuole; and serves as an intracellular messenger in the cytosol (Marschner, 1995). With Ca level being so low in the plant tissue, plant health and

productivity appeared to have been compromised.

Considering all factors (EC, pH, and availability of other mineral nutrients) mentioned above, N fertilizer rate between 100 and 200 ppm seemed to be favorable for plant growth and production in both years. This result is supported by other greenhouse N studies conducted on other species, such as Texas mountain laurel (*Sophora secundiflora*), *Anthurium* (*Anthurium andraeanum* Lind.) and bell pepper (*Capsicum annuum* L.), which all reported a similar range of N fertilization rate for optimal shoot (stem and leave) growth (Bar-Tal et al., 2001; Niu et al, 2011; Chang et al., 2012).

Growth reduction usually happens when a plant nutrient reaches a toxic range (Smith, 1962). In this study, almost every time when tissue N level was higher than 7%, growth was retarded and node production was reduced (Figure 3, Table 5). This indicated 7% tissue N might be an indicator of plant toxicity. Toxic levels of N for edible sweetpotato grown in the greenhouse have not been documented. However, a sufficient range for field grown sweetpotatoes in the middle of the growing season was reported between 3.3 and 4.5% when the most recent fully developed leaves were sampled (Mills and Jones, 1996). Sufficiency range of nutrients usually varies considerably with the stage of plant/crop growth. For N, highest concentrations were found in new leaves and N content became less with the age of plant. Compared with field grown sweetpotato, leaves obtained from greenhouse shoot production were much younger and therefore should have been expected to have a higher N content. This case has been documented in other crops. Cucumber plants grown in the greenhouse need a higher range of N than those grown in the field, which is 4.5 - 6.0% versus 4.0 - 5.0% (Campbell, 2000). Greenhouse grown lettuce can tolerant an even higher N

level, which is from 4.5 to 6.5%. Based on these reports, the relatively high N tissue levels in this study seemed to be reasonable, while 7% can be used as an indicator of sweetpotato plant toxicity in greenhouses.

The results in 2012 were quite different in many ways from what we found in 2011. The overall nodal yield in 2012 was much lower than 2011(Tables 2, 3, 4) while N concentration from plant tissue and soilless media were both higher in 2012 than 2011 (Figures 1 and 2). In terms of plant mortality, overall mortality was higher and plants died at earlier harvest times in 2012 than in 2011 (Table 6). A key reason for these differences was likely due to the abnormally high levels of N and EC in the initial Fafard 2 mix soilless media in 2012 (Table 12). The reason why these values were high in the 2012 soilless media versus the 2011 is unclear. This serves as a reminder that commercial soilless mixes may not have the consistency in media properties from one batch to another. Before starting greenhouse production, growers should always check their media composition by taking soil samples and adjust their fertilization schedule based on their media conditions.

The interaction between light and nitrate assimilation in plants has been documented for years but the mechanism behind it is still illusive (Chen and Ries, 1969; Canvin and Atkins, 1974; Aslam, 1979). Light has been observed to promote nitrate uptake in rye and oat seedlings (Chen and Ries, 1969) and the effect of light on the activity of nitrate reductase has been known in many species, including wheat, barley, corn, tobacco, and even apple roots (Hageman and Flesher, 1960; Travis, 1970; Zielke and Filner, 1971; Frith, 1974; Aslam, 1979). In our experiment, a very high tissue N was observed once at the 400 ppm treatment in the “middle” season of 2012 (Figure 3), which reached 9%. Corresponding negative

growth responses for this 400 ppm treatment during this middle season were also measured, which included decreased node production (Table 5) and increased plant mortality (Table 6). These events, based on the literatures above (Hageman and Flesher, 1960; Chen and Ries, 1969; Travis, 1970; Zielke and Filner, 1971; Frith, 1974; Aslam, 1979), seemed to be related to the effect of light. As the tissue samples were taken on harvest 5 (May 3rd 2012), data showed that there was a period before harvest 5, which was from harvest 2 to harvest 3 (March 27th to April 9th), when solar radiation was much stronger and more constant than any other time. More specifically, the average solar radiation during this period was 208 W/m^2 versus 141 W/m^2 for the entire 2012 season (Table 13). Since the substrate N level was higher than usual at the beginning of the experiment in 2012, it was possible that plants, assisted by the constant and more intense radiation, took up more N than they actually needed. If tissue samples could have been collected at harvest 3, when the highest light intensity was shown, we suspect that the tissue N would be even higher than it was at harvest 5. This discussion above serves as a possible explanation for the high tissue N level during harvest 5 in 2012, and at the same time is a reminder that light intensity in greenhouses need to be monitored as high light intensity can interact with high nitrogen supply and cause more severe nitrogen toxicity.

Summary

This study provided some useful information for sweetpotato plant growers on greenhouse fertilization. Regardless of variety, a range between 100 ppm and 200 ppm N fertilizer rate favors plant growth and results in optimal production. There were differences between varieties with respect to plant production. ‘Evangeline’ grew the fastest and produced the most plants/nodes, followed by ‘Beauregard’, then ‘Covington’. Additionally, ‘Covington’ produced a stockier, heavier plant cutting than ‘Beauregard’ or ‘Evangeline’. Awareness of the nutrient status of the initial soil media is critical, and fertilizer application rate should be adjusted accordingly. Tissue analysis should be done during production and the percentage of N should be monitored. If a 7% N level is approached, plant toxicity is likely to happen and leaching with water should be used to mitigate against toxicity. Maintaining at least 100 ppm N levels with constant fertigation resulted in minimal storage root formation. Plants produced in the greenhouse at various N levels and set in the field had similar plant stands and yields when growing conditions were generally favorable for plant establishment.

References

Aslam, M., R.C. Huffaker, D.W. Rains, and K.P. Rao. 1979. Influence of light and ambient carbon dioxide concentration on nitrate assimilation by intact barley seedlings. Plant Physiol. 63(6):1205-1209.

Austin, D.F. 1977. Hybrid polyploids in *Ipomoea* section Batatas. J. Hered 68(4):259-260.

Austin, D.F. 1978. The *Ipomoea batatas* complex. I. Taxonomy. Bull. Torrey Bot. Club 105(2):114-129.

Averre, C.W. and R.K. Jones. 1991. Edema. General principles information note 3, NC State Univ. www.ces.ncsu.edu/depts/pp/notes/oldnotes/gp3.htm

Bar-Tal, A., B. Aloni, L.Karni, and R. Rosenberg. 2001. Nitrogen nutrition of greenhouse pepper. II. Effects of nitrogen concentration and NO_3: NH_4 ratio on growth, transpiration, and nutrient uptake. HortScience 36(7):1252-1259.

Bellinder, R. and R. Morse. 1982. Effect of nitrogen and precipitation on yield of sweetpotato. Veg. Growers News 36(10):1-4.

Bryan, A.D., Z. Pesic-VanEsbroeck, J.R. Schultheis, K.V. Pecota, W.H. Swallow, and G.C. Yencho. 2003. Cultivar decline in sweetpotato I. Impact of micropropagation on yield, storage root quality and sweetpotato feathery mottle virus incidence in 'Beauregard'. J. Amer. Soc. Hort. Sci. 128(6):846-855.

Camberato, D.M., R.G. Lopez, and M.V. Mickelbart. 2009. pH and Electrical Conductivity measurements in soilless substrates. Purdue Univ. Extension Publication H0-237-W. http://www.extension.purdue.edu/extmedia/ho/ho-237-w.pdf

Campbell, C.R., ed. 2000. Reference sufficiency ranges for plant analysis in the southern region of the United States. NC Department of Agriculture and Consumer Services. Southern Cooperative Series Bulletin 394, Raleigh, NC. www.ncagr.gov/agronomi/saaesd/scsb394.pdf (verified 2011 Jun 27).

Canvin, D.T. and C.A. Atkins. 1974. Nitrate, nitrite and ammonia assimilation by leaves: effect of light, carbon dioxide and oxygen. Planta. 116(3):207-224.

Chang, K.H., R.Y. Wu, G.P. Chang, T.F. Hsieh, and R.S. Chung. 2012. Effects of nitrogen concentration on growth and nutrient uptake of *Anthurium andraeanum* Lind. cultivated in coir under different seasonal conditions. HortScience 47(4):515-521.

Chen, T.M. and S.K. Ries. 1969. Effect of light and temperature on nitrate uptake and nitrate reductase activity in rye and oat seedlings. Can. J. Bot. 47(2):341-343.

Constantin, R.J., L.G. Jones, H.L. Hammett, T.P. Hernandez, and C.G. Kahlich. 1984. The response of three sweetpotato cultivars to varying levels of nitrogen. J. Amer. Soc. Hort. Sci. 109(5):610-614.

Constantin, R.J., T.P. Hernandez, and L.G. Jones, 1974. Effects of irrigation and nitrogen fertilization on quality of sweetpotatoes. J. Amer. Soc. Hort. Sci. 99:308-310.

Dangler, J.M, R.W. Scheuerman, R.N. Campbell, and C.A. Clark. 1994. Meristem-tip culture and California's clean foundation sweetpotato program. HortTech. 4(3):227-228.

Dangler, J.M. 1994a. Sweetpotato foundation programs provide sweetpotato growers with high quality pathogen-free seedstock. HortTech. 4(3):224-224.

Dangler, J.M. 1994b. Alabama's foundation sweetpotato program maintains root quality. HortTech. 4(3):224-227.

Edmond, J.B and G.R. Ammerman. 1971. Sweet potatoes: production, processing, marketing. AVI Pub. Co., Westport, CT.

Frith, G.J.T. 1974. Light stimulated activity of nitrate reductase in apple roots. Plant Cell Physiol. 15(1):153-155.

Goldsworthy, P.R., and N.M. Fisher (eds.). 1984. 'Studies on the effects of soil physical conditions on the growth and yield of crop plants'. The physiology of tropical field crops. Wiley, London, UK. p. 555.

Hageman, R.H. and D. Flesher. 1960. Nitrate reductase activity in corn seedlings as affected by light and nitrate content of nutrient media. Plant Physiol. 35(5):700-708.

Jester, W.R.(Bill), C.W. Averre, and J.R. Schultheis, 1994. Russet crack-like symptoms in Beauregard sweetpotato reduced by virus indexing and micropropagation. HortScience 29(7):727 (Abstr.).

Kays, S.J., C.E. Magnuson, and Y. Fares. 1982. Assimilation patterns of carbon in developing sweet potatoes using ^{11}C and ^{14}C. p. 95-118. In: R.L. Villareal and T.D. Griggs (eds.). Sweet Potato. Processing of the first international symposium. Asian vegetable research and development center, Tainan, Taiwan.

Lebort, V. (ed). 2009. Sweet potato: developmental physiology. p.127-137. In: Tropical root and tuber crops: cassava, sweet potato, yams and aroids. CABI, London, UK.

Lebort, V. 1986. Evaluation of local and introduced cultivars of sweet potato (*Ipomoea batatas* (L.) Lam.) in Vanuatu. J. South Pacific Agr. 11(3):25-31.

Loebenstein, G. and G. Thottappilly (eds.). 2009. The Sweetpotato. Springer-media, Houten, Netherlands.

Marschner, H. 1995. Mineral nutrition of higher plants, 2nd ed. Academic Press, London. pp. 285-298.

Melakeberhan, H., A.L.Jones, E. Hanson, and G.W. Bird. 1995. Effect of low soil pH on aluminum availability and on mortality of cherry seedlings. Plant Dis. 79(9):886-892.

Mills, H.A. and J.B. Jones Jr. 1996. Plant analysis handbook II: a practical sampling, preparation, analysis, and interpretation guide (revised). MicroMacro Publ., Athens, GA.

Mortvedt, J.J. 2012. Bioavailability of micronutrients, p. 73. In: M.E., Sumner (ed.). Handbook of soil science. CRC Press, Boc Raton, FL.

Mulkey, W.A and J.H. Hernandez. 1994. Louisiana's sweetpotato foundation seed program. HortTech. 4(3):228-230.

Niu, G., D. Rodriguez, and M. Gu. 2011. Response of *Sophora secundiflora* to nitrogen form and rate. HortScience. 46(9):1303-1307.

Phillips, S.B., J.G. Warren, and G.L. Mullins. 2005. Nitrogen rate and application timing affect 'Beauregard' sweetpotato yield and quality. HortScience 40(1):214-217.

Prasad, B., P. Sinha. 1982. Change in the status of micronutrients in soil with long term application of chemical fertilizers, lime and manure. Plant and Soil. 64(3):437-441.

Reddy, K.R., B. Gajanayake, M. Shankle, R. Arancibia, and A. Villordon. 2010. Temperature effects on sweetpotato storage root growth and development. National Sweetpotato Collaborators Group, Annul. Conf., Orange Beach, Alabama.

Sarkar, A.N. and R.G. Wyn Jones. 1982. Effect of rhizosphere pH on the availability and uptake of Fe, Mn and Zn. Plant and Soil 66(3):361-372.

Schultheis, J.R. 1994. Virus-free micropropagated plants and clone effects on sweetpotato yield. HortScience 29(7):727 (Abstr.).

Schultheis, J.R., C.E. Collins, and C.W. Averre. 1994. The certified sweetpotato seed program in North Carolina. HortTech. 4(3):232-236.

Sims, J.T. 1986. Soil pH effects on the distribution and plant availability of manganese, copper, and zinc. Soil Sci. Soc. Am. J. 50(2):367-373.

Sloan, R.C., Jr. 1994. Mississippi sweetpotato foundation seed program. HortTech. 4(3):230-232.

Smith, P.F. 1962. Mineral analysis of plant tissues. Ann. Rev. Plant Physiol. 13.81-108.

Stevens, G., P. Motavalli, P. Scharf, M. Nathan, and D. Dunn. 2002. Integrated pest management; crop nutrient deficiencies & toxicities. UM Extension, Univ. Missouri-Columbia. http://ipm.missouri.edu/ipm_pubs/ipm1016.pdf

Togari, Y. 1950. A study of tuberous root formation in sweet potato. Bul. Nat. Agr. Expt. Sta. 68:1-96.

Travis, R.L., W.R. Jordon, and R.C. Huffaker. 1970. Light and nitrate requirements for induction of nitrate reductase activity in *Hordeum vulgare*. Physiol. Plant. 23(4):678-685.

Tucker, M.R. 1999. Essential plant nutrients: their presence in North Carolina soils and role in plant nutrition. NCDA&CS-Agronomic Division, Raleigh, NC.

Uchida, R. ed. 2000. Recommended plant tissue nutrient levels for some vegetable, fruit, and ornamental foliage and flowering plants in Hawaii. In: J.A. Silva and R. Uchida (eds.). Plant nutrient management in Hawaii's soils, approaches for tropical and subtropical

agriculture. College of Tropical Agriculture and Human Resources, Univ. Hawaii, Manoa, HI.

Whipker, B.E. and T.J. Cavins. 2000. Electrical conductivity (EC): units and conversions. Commercial Floriculture Extension & Research, NC State Univ.

Wilson, L.A. 1973a. Stimulation of adventitious bud production in detached sweet potato leaves by high level of nitrogen supply. Euphytica 22(2):324-326.

Wilson, L.A. 1973b. Effect of different levels of nitrogen supply on early tuber growth of two sweet potato cultivars. Trop. Agr. 50:53-54.

Wilson, L.A. and S.B. Lowe. 1973. The anatomy of the root system in West Indian sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars. Ann. Bot. 37(3):633-643.

Zielke, H.R. and P. Filner. 1971. Synthesis and turnover of nitrate reductase-induced by nitrate in cultured tobacco cells. J. Biol. Chem. 246(6):1772-1779.

[USDA] U.S. Dept. Agr. 2005. U.S. Standard for grades of sweet potatoes. U.S. Dept. Agr., Washington, D.C.

[USDA-ESMIS] U.S. Dept. Agr. - Economics, Statistics and Market Information System. 2010. U.S. Dept. Agr., Washington, D.C.

Table 1. Results of type 3 analysis of variance for node production of FEI application.

Source of effects	Type of effect [w]	DF	Sum of squares	Mean square	Error DF	F value	Pr > F
Trt [z]	F	4	95906	23977	98	22	<.0001*
Var [y]	F	2	1053253	526627	98	491	<.0001*
Eml [x]	F	2	145158	72579	930	76	<.0001*
Trt*eml	F	8	30673	3834.18	930	4	0.0001*
Var*eml	F	4	15805	3951.32	930	4.2	0.0024*
Trt*var	F	8	8442.88	1055.36	98	0.98	0.4527
Trt*var*eml	F	16	8159.44	509.965	930	0.5	0.9289
Year	R	1	415856	415856	6	472	<.0001*
Rep(year)	R	6	5290.35	881.726	98	0.8	0.555
Rep*trt*var(year)	R	98	105034	1071.77	930	1.1	0.1984
Residual	R	930	884627	951.212	.	.	.

[z] Trt = fertilizer rate.

[y] Var= variety.

[x] Eml= harvest time (early, middle, late).

[w] There are two types of effects. F stands for fixed effects, and R stands for random effects.

Table 2. Fertilizer rate effect on the number of nodes, fresh weight (Wt) and fresh weight/nodes (W/N).

Rate (ppm)	**2011**			**2012**		
	Nodes [z]	Wt (dwt) [y]	W/N [x]	Nodes	Wt (dwt)	W/N
50	113 c [w]	106 c	1.00 b	90 ab	90 b	1.07 b
100	142 ab	157 ab	1.22 a	104 a	117 a	1.20 ab
200	146 a	179 a	1.30 a	99 a	118 a	1.24 a
300	135 ab	165 ab	1.27 a	93 ab	115 a	1.31 a
400	124 bc	150 b	1.27 a	79 b	95 b	1.30 a
Average	132	151	1.21	93	107	1.22

Data combined all harvests and all varieties in each year.

[z] Nodes = number of nodes per pot based on 10 plants.

[y] Wt = fresh weight of all vines per pot (dwt) (1 dwt=1.6 g).

[x] W/N = the ratio of fresh weight to nodes.

[w] Comparisons were made by columns. Means with different letters are statistically different at α=0.05 using Tukey HSD.

Table 3. Variety response to number of nodes, fresh weight and fresh weight/nodes (W/N).

Variety	2011			2012		
	Nodes [z]	Wt (dwt) [y]	W/N [x]	Nodes	Wt (dwt)	W/N
Beauregard	146 b [w]	176 a	1.18 b	103 b	123 a	1.19 b
Covington	81 c	132 b	1.61 a	59 c	93 c	1.59 a
Evangeline	170 a	146 b	0.84 c	117 a	105 b	0.89 c
Average	132	151	1.21	93	107	1.22

Data combined all harvests and all fertilizer rates in each year.

[z] Nodes = number of nodes per pot based on 10 plants.

[y] Wt = fresh weight of all vines per pot (dwt) (1 dwt=1.6 g).

[x] W/N = the ratio of fresh weight to nodes.

[w] Comparisons were made by columns. Means with different letters are statistically different at α=0.05 using Tukey HSD.

Table 4. Effect of harvest time (early, middle and late seasons) on node production in 2011 and 2012.

Harvest time	**Number of nodes**	
	2011	**2012**
Early	98 b [z]	94 ab
Middle	146 a	98 a
Late	152 a	87 b
Average	132	93

Data combined all three varieties and all fertilizer rates.

[z] Comparisons were made by columns. Means with different letters are statistically different at α=0.05 using Tukey HSD.

Table 5. Interaction between harvest time and fertilizer rate on node production.

Rate (ppm)	**Number of nodes**					
	2011			**2012**		
	Early	Middle	Late	Early	Middle	Late
50	83 b [z]	134 a	121 c	95 a	102 a	73 c
100	110 a	152 a	163 ab	103 a	113 a	95 ab
200	102 ab	155 a	181 a	95 a	106 a	96 a
300	100 ab	152 a	155 abc	92 a	92 ab	95 ab
400	96 ab	137 a	140 bc	86 a	76 b	75 bc

Data combined all three varieties in each year.

[z] Comparisons were made by columns. Means with different letters are statistically different at $\alpha=0.05$ using Tukey HSD.

Table 6. Effect of fertilizer rate on the percentage of plants survived after each harvest time period (early, middle and late) in both years.

	Fertilizer rate	**Survival of plants (%)**			**Significance**
	(ppm)	Early	Middle	Late	**across harvest** [z]
2011	50	100 a [y]	100 a	100 a	NS
	100	100 a	98 a	98 a	NS
	200	100 a	97 a	97 a	NS
	300	100 a	96 ab	87 a	***
	400	98 a	87 b	72 b	**
2012	50	100 a	100 a	99 a	NS
	100	100 a	100 a	100 a	NS
	200	99 a	98 a	96 ab	NS
	300	94 a	93 a	89 b	NS
	400	80 b	59 b	50 c	***

[z] Comparisons were made across harvest time (by row). Significance was evaluated and NS, *, **, *** represented non-significant or significant at p=0.05, 0.01 and 0.001 respectively.

[y] Comparisons were made by column and within year. Means with different letters are statistically different at α=0.05 using Tukey HSD.

Table 7. Tissue nitrogen analysis for different varieties at various harvest times.

		Tissue N (%)			
		Early	Middle	Late	Average
2011	Beauregard	6.67 a [z]	5.99 ab	6.12 ab	6.26 a
	Covington	6.47 b	6.14 a	6.24 a	6.31 a
	Evangeline	6.36 b	5.73 b	5.97 b	6.02 b
2012	Beauregard	7.28 a	7.74 a	6.77 a	7.27 a
	Covington	7.08 a	7.79 a	6.82 a	7.23 a
	Evangeline	7.07 a	7.59 a	6.31 b	6.99 b

Data combined all fertilizer rates in each year.

[z] Comparisons were made by columns and within year. Means with different letters are statistically different at $\alpha=0.05$ using Tukey HSD.

Table 8. Tissue and soilless media nitrogen concentration comparisons between leaching and non-leaching treatments at different fertilizer rate treatments (200 to 400 ppm).

		Tissue N (%)			Soilless media N (ppm)		
		200	300	400	200	300	400
2011	NL[z]	6.74	6.88	7.18	110.36	163.83	275.92
	L[y]	6.27	6.65	6.88	37.56	74.68	174.58
	Contrast (NL vs L) [x]	***	*	**	***	***	**
2012	NL	7.40	7.61	7.96	281.28	404.06	532.26
	L	7.05	7.40	7.63	91.43	139.42	217.39
	Contrast (NL vs L)	***	NS	**	***	***	***

Data combined all three sampling times (early, middle and late) and all varieties in each year.

[z] NL= Not leaching.

[y] L= Leaching.

[x] Contrasts were made between NL and L treatments and labeled as *, ** and *** representing significance at P=0.1, 0.05 and 0.01, respectively.

Table 9. Nodal yield comparison between leaching and non-leaching treatments at different fertilizer rate treatments (200 to 400 ppm).

		Number of nodes		
		200	300	400
2011	NL[z]	145.95	135.46	124.36
	L[y]	143.62	150.68	148.39
	Contrast (NL vs L) [x]	NS	**	**
2012	NL	98.9	92.84	79.04
	L	110.14	101.77	97.82
	Contrast (NL vs L)	**	*	***

Data combined all harvests and all varieties in each year.

[z] NL= Not leaching.

[y] L= Leaching.

[x] Contrasts were made between NL and L treatments and labeled as NS, *, ** and *** representing no significance or significance at P=0.1, 0.05 and 0.01, respectively.

Table 10. Weight (g) of storage roots produced per pot at the end of 2011 and 2012 study.

Variety	2011		2012	
	# Storage roots [z]	Wt (g) [y]	# Storage roots	Wt (g)
Beauregard	1.8 ab	36 ab	1.1 a	22 a
Covington	2.3 a	54 a	1.5 a	38 a
Evangeline	0.5 b	10 b	0.4 a	7 a
Average	1.5	33	1.0	22

Data is displayed on a per pot basis combing all five fertilizer rate treatments for each variety.

[z] # Storage roots = number of storage roots with diameter equal to or greater than 10 mm.

[y] Wt (g) = weight (g).

Table 11. Effects of fertilizer rate on transplanting plant stand and yield.

		Fertilizer rate (ppm)					Average [y]	Sig. [x]
		50	100	200	300	400		
Plant stand %		97	96	95	96	97	95	NS
Yield [z] (lb)	#1	2.1	2.3	2.5	2.4	2.5	2.1	NS
	Canner	0.3	0.2	0.2	0.3	0.2	0.3	NS
	Jumbo	2.5	2.4	2.9	2.4	3.0	2.5	NS
	Cull	0.0	0.0	0.0	0.0	0.0	0.0	NS

Data combined all three varieties and there was no statistical difference found between treatments with respect to yield or plant stand.

[z] Yield was displayed on a per plant basis.

[y] Average of all fertilizer rates.

[x] Significance was evaluated across fertilizer rates (within row). 'NS' stands for not significant.

Table 12. Initial pH, EC, and nutrient concentration (ppm) in the Fafard 2 Mix soilless media used in 2011 and 2012.

	NH4-N	NO3-N	P	K	Ca	Mg	S	Cl	Na	Fe	EC (dS/m)	pH
2011	1 [z]	18	1.8	38	49	51	93	12	27	0.7	80	5.4
2012	13	110	1.5	12	13	13	20	12	2	1.4	212	5.2

[z] Units for all values displayed in this table are "ppm" except EC and pH.

Table 13. Average 2m solar radiation between every harvest period in 2012.

Duration	**# of harvest**	**Solar radiation (W/m^2)**
3/27-4/9	3	208 a [z]
3/6-3/26	2	163 ab
4/24-5/3	5	156 abc
6/8-6/19	9	152 bc
5/16-5/25	7	140 bc
5/26-6/7	8	137 bc
5/4-5/15	6	124 bc
4/10-4/23	4	115 c
1/31-3/5	1	110 c
Whole season average	1-9	141

Data courtesy of State Climate Office of North Carolina, Reedy Creek Field Laboratory Station, Raleigh, NC.

[z] Comparisons were made among different harvest periods. Means with different letters are statistically different at α=0.05 using Tukey HSD.

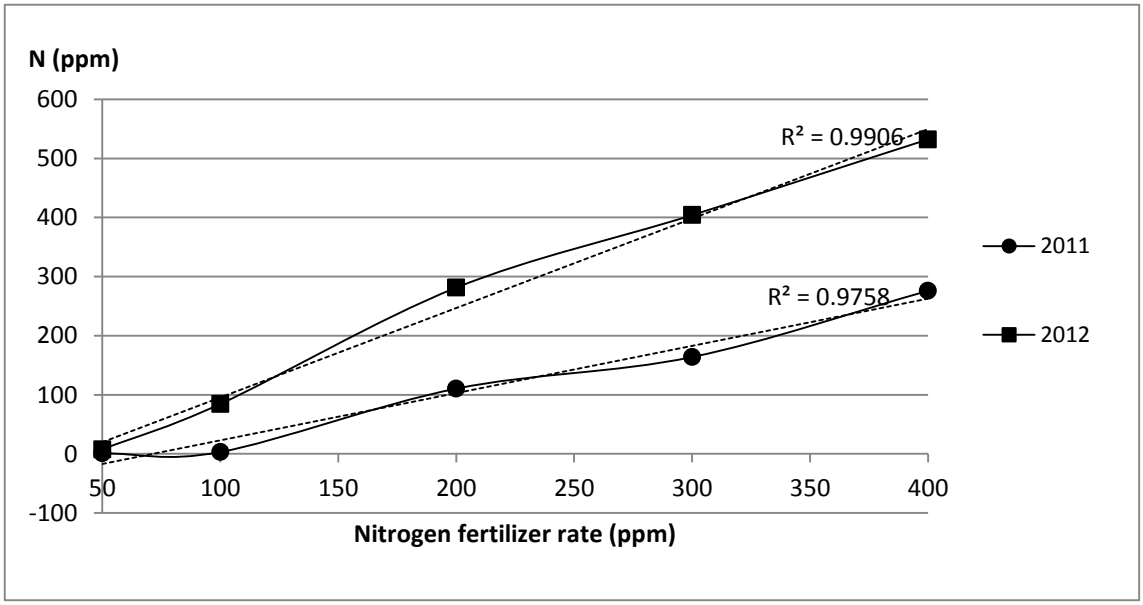


Figure 1. Linear relationship between nitrogen fertilizer rate and the accumulation of nitrogen in soilless media. Dash-lines represent the best fit of the data following a linear regression, with R square value indicated above each line. Soilless media data were collected at the end of 2011 and 2012 seasons.

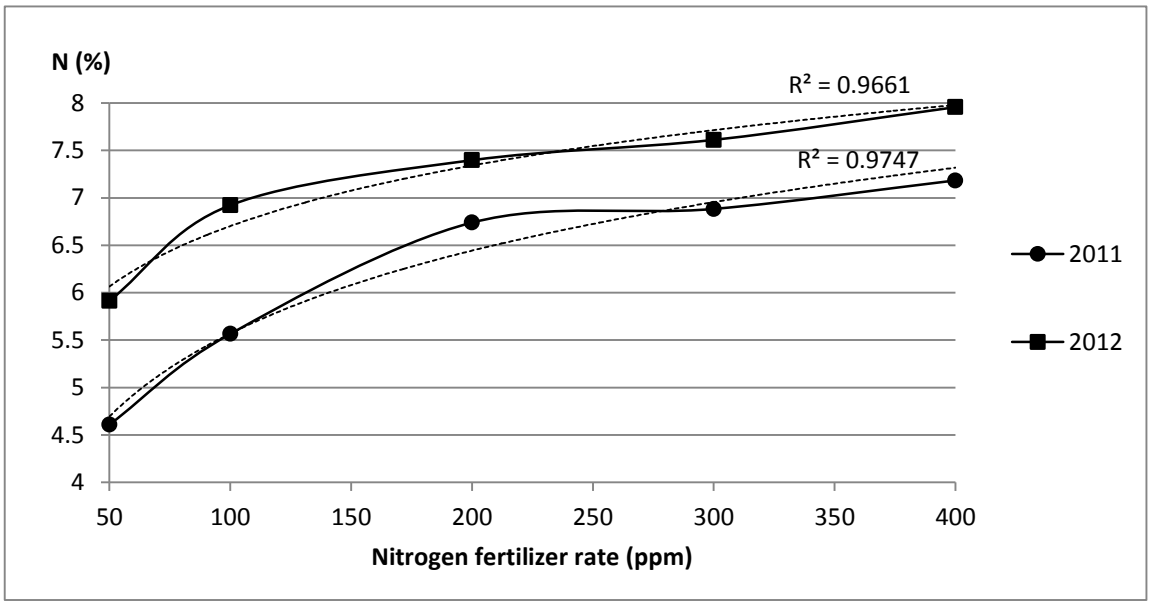


Figure 2. Logarithmic relationship between nitrogen fertilizer rate and nitrogen uptake in plant tissue. Dash-lines represent the best fit of the data following a logarithmic regression, with R square value indicated above each line. Tissue nitrogen data were obtained by averaging all three samplings (early, middle and later harvests) throughout the seasons.

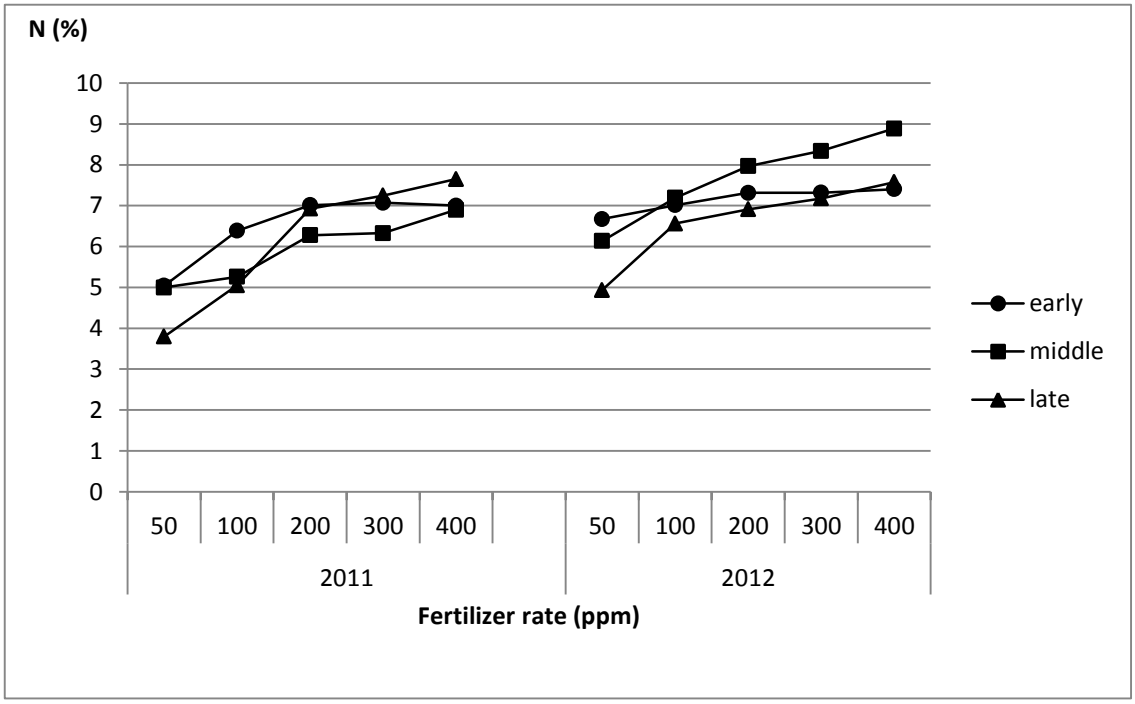


Figure 3. Tissue nitrogen concentration (%) in response to different fertilizer rates in "early", "middle" and "late" seasons of both years. Data were averaged across all three varieties.

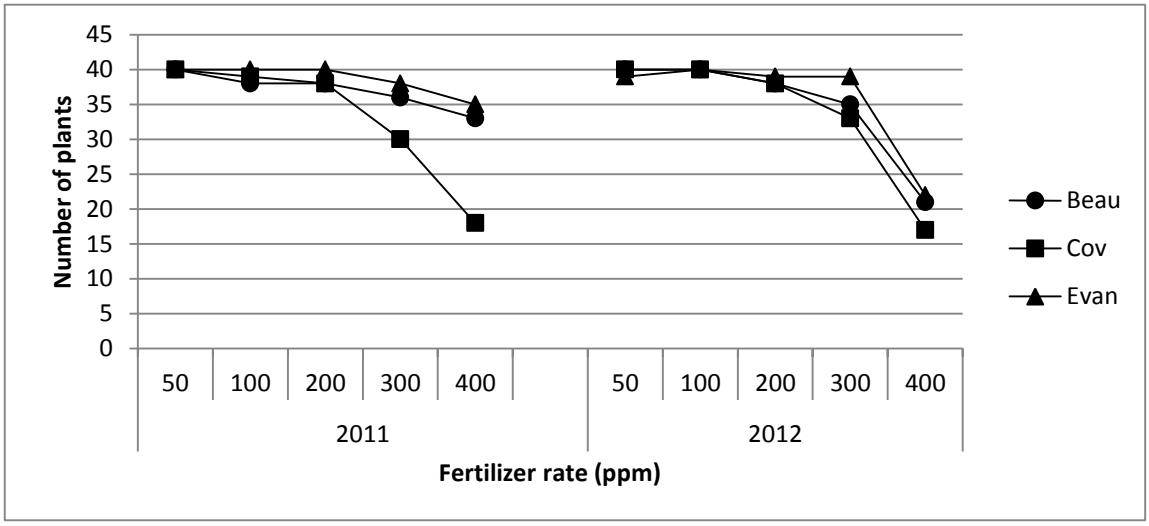


Figure 4. Effects of fertilizer rate and variety on plant mortality.

X axis = Fertilizer rate (ppm) for each year.

Y axis = Number of plants survived at each fertilizer rate for each variety at the end of season.

There are originally 40 plants for each variety by fertilizer treatment.

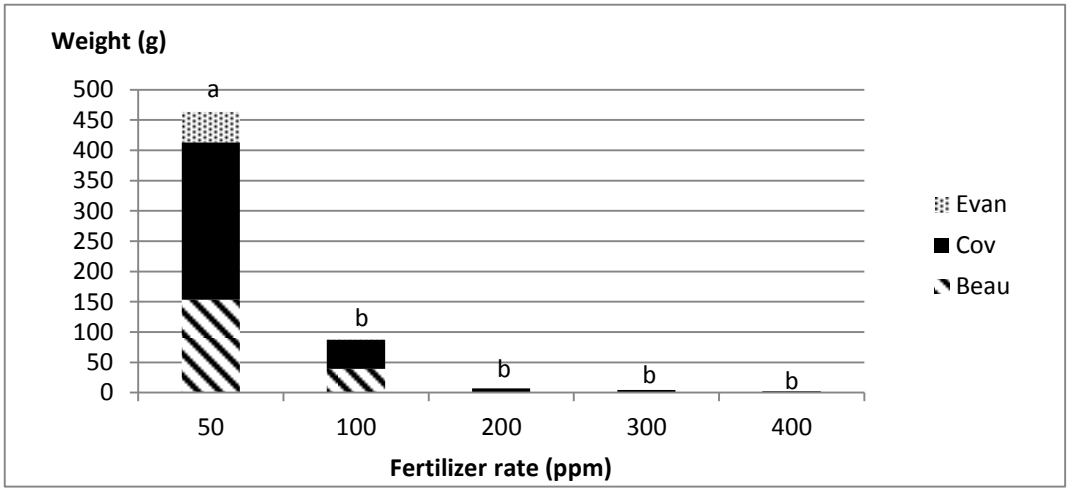


Figure 5. Weight of storage roots per pot in response to fertilizer rates. Different patterns in the bars represent different varieties. Evan= Evangeline, Cov= Covington, Beau= Beauregard. Letters on top of each bar separate the means of weight by fertilizer rate. Different letters were significant different at $\alpha = 0.05$.

Figure 6. Storage root formation under different fertilizer rates for ‘Evangeline’, ‘Beauregard’ and ‘Covington’. Photo was taken from 2011 study. Number of roots shown in each pile represents total number of roots produced per treatment combining all 4 replications (4 pots).

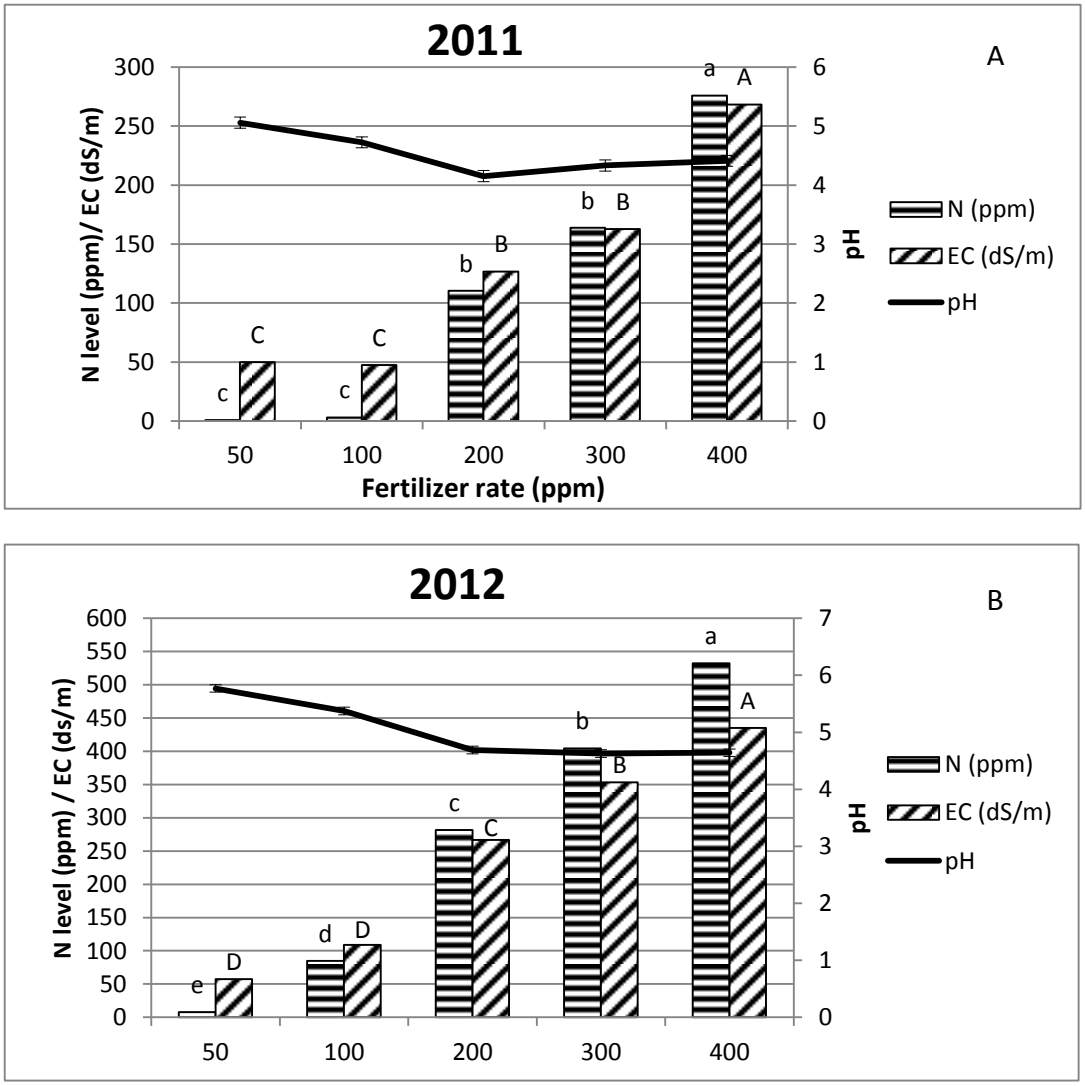


Figure 7 A and B. Relation between media nitrogen level and media EC / pH value in 2011 and 2012. Means for each parameter (N, EC, pH) were separated by Tukey HSD. Lower-case letters represent N (ppm), upper-case letters represent EC (dS/m); bars with different letters were significant different at α= 0.05. pH means were separated by error bars at α= 0.05.

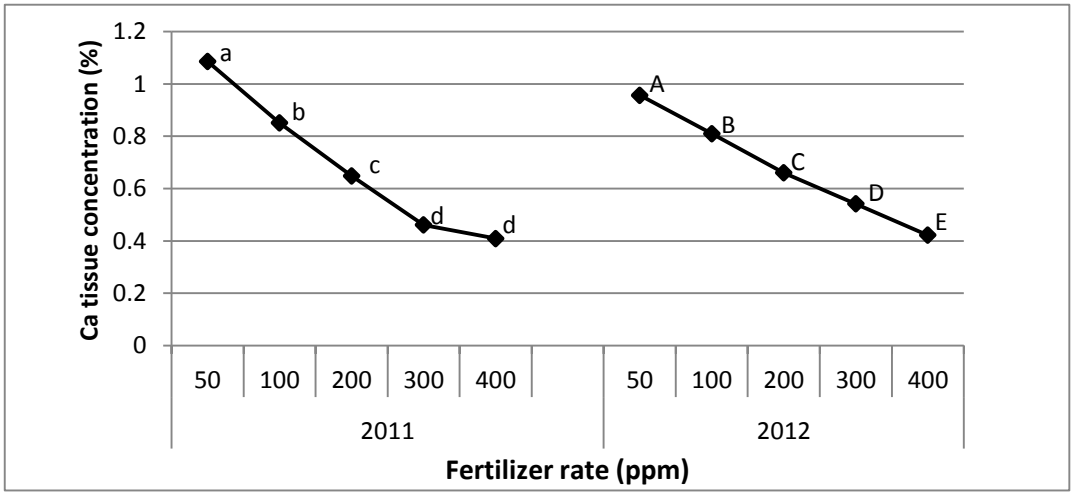


Figure 8. Relation between N fertilizer rate and Ca concentration in plant tissue. Data were obtained from the late season sampling of both 2011 and 2012. Means were separated by letters with different letters statistically different at $\alpha=0.05$.

CHAPTER II. INTERNAL NECROSIS OF 'COVINGTON' SWEETPOTATO – ITS OCCURRENCE AND SEVERITY IN THE COMMERCIAL INDUSTRY AND ITS RESPONSE TO VARIOUS CHEMISTRIES

Chen Jiang, Sylvia M. Blankenship, Michael D. Boyette, Zvezdana Pesic-VanEsbroeck, Penelope Perkins-Veazie and Jonathan R. Schultheis

A series of studies was undertaken to understand the occurrence and causes of internal necrosis (IN) in 'Covington' sweetpotato. A survey was conducted for two seasons across nearly 25 NC growers or businesses per season to investigate the occurrence of IN in 'Covington' during storage. Results indicated the problem was widely spread in facilities throughout the state but both incidence and severity were generally low. There were some exceptions in which a few businesses were impacted by a high percentage of IN with severe root symptoms. Storage temperature and relative humidity were monitored in the surveyed commercial facilities, with no relationship found between IN and storage temperature and relative humidity conditions. Internal tissue of symptomatic roots was cultured in both moisture chambers and acidified PDA medium and no consistent pathogen types were isolated that could be associated with IN. Laboratory studies which stored sweetpotato roots in air-tight barrels with 100 ppm ethylene did not find any relationship between ethylene gas in storage and IN. Effects of Prep (Ethephon (2-Chloroethyl) phosphonic acid) and other insecticides and herbicides were evaluated to detect the occurrence of IN. Only Prep induced IN. IN symptoms were not detectable at harvest; the earliest significant incidence was found 8 days after harvest, and symptoms became more prevalent and severe 30 days after harvest. Curing enhanced the incidence and severity of IN. Anatomical work was done using DAPI

(4', 6-diamidino-2-phenylindole) fluorescent dye to detect cell death in roots which had been exposed to Prep but prior to the presence of visual IN symptoms. This approach was not successful as the specific region on the root where IN occurs varied.

Key words: sweetpotato, sweet potato, internal necrosis, Prep, ethylene, storage, curing

Introduction

The sweetpotato cultivar ‘Covington’ was released by the NC State University Sweetpotato Genetic and Breeding program (Yencho et al., 2008). Since its release and because of its excellent quality, pack out and money making potential, the commercial sweetpotato industry in NC now grows over 80% of its acreage with this variety (Langdon, NC Sweet Potato Commission, personal communication). This means that at least 51,200 acres of the 64,000 acres produced in NC were ‘Covington’ (USDA-NASS, 2011); other states are also producing the variety ‘Covington’, adding to an approximated 40% of the 129,700 acres of sweetpotato production in the United States.

Although ‘Covington’ has many positive attributes regarding its production, there are some concerns. One of them is the occurrence of internal necrosis (IN) (Dittmar et al., 2010). Symptoms are not evident on the exterior of the storage roots; but appear when the cortex of the root is exposed through breakage or cutting. The symptoms usually start at the proximal end and progress towards the distal end as symptoms get worse. In most cases symptoms do not appear half way through the roots. Necrosis symptoms vary from small pencil-like black or brown spots to large areas of breakdown (Figure 1). The first incidence was reported by a grower in November/December 2006. Once discovered, ‘Covington’ “seed” from all of the Certified Seed Growers were evaluated in February 2007. One business, in which the original report of IN was made, had a high incidence of symptoms, some being severe. A few other businesses had less than 2% incidence while most businesses had roots exhibiting no symptoms. There have been several key items of note since the initial report of IN.

1) When roots having the IN were bedded, roots obtained from slips that had the

disorder did not exhibit IN symptoms in harvested roots the following growing season. This was confirmed through controlled studies on the research station and through growers who commercially bedded roots with IN symptoms (Schultheis and Thornton, 2007).

2) PREP ™ (EPA Reg. No. 264-418) is a brand of an ethephon compound product made by Bayer CropScience. It is composed of 55.4% ethephon (2-chloroethyl) phosphonic acid and 44.6% inert ingredients. Prep is currently registered and used to enhance defoliation of cotton and tobacco prior to harvest (http://www.cdms.net/LDat/ld333003.pdf). In recent years, Prep has also been evaluated as a spray application by the sweetpotato industry to reduce damage to roots at harvest by tightening the skin (Main et al., 2009; Wang et al., 2012). Some of the initial research with Prep was conducted on 'Beauregard' roots (Schultheis et al., 2000), while later experimentation has included more clones (Dittmar et al, 2010). More incidence and more severe incidence of IN symptoms were found with 'Covington' while much less incidence occurred in 'Beauregard' roots when Prep was applied. However, even when Prep was not applied, low incidence and less severe IN symptoms were still present in 'Covington'.

3) One grower in 2009 reported that 80,000 bushels of sweetpotato in storage were affected by IN. Another grower reported that IN affected 'Covington' sweetpotatoes in a "make shift" storage facility, while sweetpotatoes from the same harvest when placed in optimal storage conditions did not have incidence of IN.

4) The presence of pathogens has been examined on roots with IN symptoms. Small pieces of affected tissue were placed in Petri plates with Acidified Potato Dextrose Agar and incubated at room temperature. No pathogens were isolated besides occasionally presented

contaminants (Schultheis et al., 2009), which suggested a physiological problem.

Previous descriptions indicated that symptoms of IN are not genetically inherit, and both pre-harvest and post-harvest factors may cause the problem. One of the factors is ethephon, a compound that releases ethylene, may accelerate the development of the symptom and make the problem worse.

Ethylene plays a critical role in physiological disorders that occur among various kinds of vegetables during storage. Russet spot in lettuce (RS), bitterness in carrot root as a result of isocoumarin (MMHD, 3-methoxy-6-methoxy-8-hydroxy-3,4-dihydro-isocoumarin) accumulation, were both induced by exogenous ethylene in storage (Rood, 1956; Chalutz et al., 1969; Mercier et al., 1993); while symptoms of black spot in potato, and rind-staining in 'Navelina' orange fruit were reduced when they were exposed to ethylene (Timm et al., 1976; Lafuente and Sala, 2002).

Other literature showed that in many cases merely ethylene by itself is not enough to induce physiological disorders. Factors like the temperature, and increased or decreased levels of O_2, CO_2, and 1-MCP (Rood, 1956; Lafuente et al., 1996; de Wild et al., 1999; Blankenship and Dole, 2003) may also be involved in the induction/reduction of ethylene related physiological disorders. In the case of lettuce, the occurrence of RS was the most severe when the temperature was 7˚C (Rood, 1956). However, when the temperature was controlled at 0˚C, the ideal temperature for lettuce storage (Saltveit, 2004), no symptom was found in lettuce even if it was exposed to exogenous ethylene (Rood, 1956). In carrot roots, environments with increased O_2 and decreased CO_2 were reported to enhance the accumulation of isocoumarin, and that anaerobic treatment (4 days) greatly inhibited the

synthesis of isocoumarin greatly (Carlton et al., 1961; Lafuente et al., 1996). The application of 1-MCP prior to ethylene exposure also affected ethylene production from the produce itself and reduced the incidence of both lettuce RS and carrot root bitterness (Fan and Mattheis, 2000).

Sweetpotato is sensitive to ethylene in storage (Buescher et al., 1975; Kitinoja, 1987). One study found that sweetpotato exposed to 10 ppm ethylene during or after curing had enhanced levels of respiration and polyphenol oxidase activities, as well as decreased attributes of color and flavor (Buescher et al., 1975). Another study used a lower concentration of ethylene; it was reported that sweetpotato roots cured with 2 ppm ethylene or stored with 1 ppm ethylene had increased respiration rate compared to roots either cured or stored in ambient air conditions with negligible ethylene (Kitinoja, 1987). When sweetpotato roots were stored in an ethylene environment for a month, phenolics rating for either freshly air-cured or previously air-stored roots increased when only 0.1 ppm ethylene existed in storage; while phenolics rating for ethylene-cured roots stayed the same even with 10 ppm ethylene in storage. Further, the effect of ethylene on respiration rate was cultivar dependent and showed differences between ‘Jewel’, ‘Jersey’ and ‘Garnet’ varieties.

In addition to ethylene gas affecting sweetpotato roots, when cured ‘Beauregard’ roots were submerged in 2.6 mM ethephon for an hour, necrotic tissue, including pitting-type damage on the surfaces as well as tip rot-like symptoms on the tips was observed on sweetpotato in a month; while sweetpotato treated with 1-MCP in the same way did not show localized tissue death, even after three months (Villordon, 2012).

Hardcore is a disorder in sweetpotato that was first reported in the early 1970s. It is a

chill-induced disorder that shows greater incidence and severity in an ethylene environment than in ambient air (Daines et al., 1976; Timbie and Haard, 1977). Other factors like air pressure and CO_2 are also involved in hardcore induction. Exposing roots to low O_2 conditions and elevated levels of CO_2 reduced the hardcore while hyperbaric conditions increased the severity of hardcore.

Sweetpotato roots normally produce very low levels of ethylene (~0.1 µL/kg hr) (Cantwell and Suslow, 2001). However, the internal ethylene production can be increased in response to black rot fungus (*Ceratocystis fimbriata*) and cut injury (Imaseki et al., 1968a-c). A rapid production of ethylene from sweetpotato roots was observed within 6 hours after roots were sliced, as it stimulated cell metabolic activities, particularly peroxidase and polyphenol oxidase, enzymes involved in the biosynthesis of phenylpropanoid, mitochondrial content and chlorogenic acid. With respect to the incidence of hardcore, experiments showed smaller roots weighing less than 200g exhibited a lower incidence of hardcore than larger ones (Timbie and Haard, 1977). This was suspected due to the greater surface to volume ratio of small roots, as was facilitated by the diffusion of ethylene from the internal tissue.

The following studies were conducted to determine the prevalence of IN in NC sweetpotato storage facilities and to assess various factors that influenced the occurrence and the real time development of IN. We surveyed 25/23 sweetpotato growers or businesses annually in NC to gain a better understanding of the incidence and severity of IN, and at the same time, used scientific methods and repeated studies to find or eliminate factors that did not relate to the occurrence of IN.

Methods

1. Effects of post-harvest factors on the occurrence of Internal Necrosis

1.1. Commercial survey

A commercial survey on ‘Covington’ storage quality was conducted for two sweetpotato storage seasons. In 2010-2011 / 2011-2012 seasons, ‘Covington’ roots from 29/27 NC commercial facilities were collected, which involved 25/23 growers or businesses in each year. The businesses surveyed each season stayed mainly the same except for some minor adjustments, but the rooms that were sampled differed each year. In each facility for each year, ‘Covington’ US No.1 roots were sampled five times during storage, beginning soon after each harvest season in October and November and concluding the following June and July, with approximately six week intervals between each sampling. A box of 40 roots was obtained as a sample. Each root was sliced horizontally into approximately 5 mm sections from the stem-end. The incidence of IN was recorded and the severity was rated based on a 0 to 4 scale (Figure 2).

1.2. Storage condition tracking

Along with the survey, one Hobo sensor (U12-011, Onset, 470 MacArthur Blvd., Pocasset, MA 02559) was placed in each of the storage facilities. Temperature and relative humidity (RH) were monitored hourly, and data were recorded for each storage season from when the roots were placed in the facility until the last sample was obtained.

1.3. Pathogen isolation

In spring 2012, pathogen isolation was conducted using selected surveyed samples which were identified with high IN incidence. Two problem roots from each of those

samples were evaluated. Three small pieces of internal tissue near the infected region were cut from both roots and tissue pieces from each root were placed in an Acidified PDA (Potato Dextrose Agar) media. The remainder of each root was placed into a moisture chamber. Both moisture chambers and PDA plates were incubated at 33 ℃ for 7 days. Growth of microbes or pathogens in the moisture chamber or on the PDA medium was identified through microscopic examination.

1.4. Ethylene test

A laboratory experiment was conducted to evaluate the response of sweetpotato to IN at high levels of ethylene in storage. 'Covington' roots, which were mostly US No.1 size, were obtained from sweetpotato field research trials at NC State in which the crop was grown using standard cultural practices (Kemble et al., 2012)**.**

Whole roots were exposed to 100 ppm ethylene gas in storage for 7 days and sliced periodically afterwards to examine them for the occurrence of IN. Twenty roots were sliced and examined before the experiment started to confirm the lack of IN before any ethylene treatment was applied. The roots from the field sample were then randomly separated into two groups and put into two air-tight iron barrels with barrel 1 (B1) containing ethylene (100 ppm) and barrel 2 (B2) containing ambient air. Ethylene concentration in both barrels was measured by gas chromatography, and it was confirmed that B1 had near 100 ppm ethylene while B2 had no negligible ethylene. Roots were placed in barrels for 7 days and then were removed to ambient air storage conditions. This experiment was conducted twice using roots from both 2010 and 2011 harvest. Roots obtained from 2010 season were treated with ethylene starting 29 March 2011, after the roots had been cured and in storage for about 5

months; and these roots were evaluated in storage after 14 and 49 days of ethylene treatment. Roots from the 2011 season were treated soon after harvest and curing started 11 November 2011, and were evaluated 21, 82, and 123 days after treatment. For each sample, twenty roots were sliced from the proximal end into approximately 5 mm increments and evaluated for the occurrence and severity of IN using scale 0-4 (Figure 2).

2. Effect of pre-harvest factors on the occurrence of Internal Necrosis

2.1. Insecticide studies

Two insecticide trials were conducted in 2011 on Cunningham Research Stations, Kinston and Horticultural Crops Research Station, Clinton to determine if various chemistries caused IN. Table 1 displays all the insecticides and applications that were used. The insecticides evaluated were those that were either registered for use in sweetpotato or were possible candidates for registered use. Each study used a randomized complete block design with 4 replications. There were two harvest times; one during the growing season, the other at normal harvest time at least 100 days after planting. Plants were set in the field on 7 June in Kinston and 9 June in Clinton. Roots for the first harvest were dug by hand at 28 August in both locations (82 days after planting in Kinston and 80 days after planting in Clinton). These samples, which were mostly large canner size roots, were cut and evaluated soon after harvest. The rest of the roots were mechanically harvested on 14 September (100 days after planting) in Kinston and 21 September (105 days after planting) in Clinton. Roots were cured for 7 days after harvest and placed in storage. Using mostly US No.1 roots, a total of four samplings were taken while in storage from the Kinston or Clinton locations. The

dates for each sample were 1 November (or 19 October), 29 November (or 22 November), 17 January (or 18 January) and 13 March (or 15 March) for the Kinston (or Clinton) study. Every sample from each plot was comprised of 25 roots, and these roots were sliced and evaluated for visual symptoms based on the severity scale from 0 to 4 (Figure 2).

2.2. Herbicide study

One herbicide study was conducted in 2011 at the Horticultural Crops Research Station, Clinton, NC to determine if various weed control/growth regulator chemistries resulted in IN. Table 2 displays all the herbicides and application methods that were used. The plant growth regulator Prep was included because there was interest in it being used to tighten the sweetpotato skin (Schultheis et al., 2000; Main et al., 2009; Wang et al., 2012) and because it has been shown to cause IN (Dittmar et al., 2010). There were two Prep applications which were applied at different times at the same application rate, 1.3 pt/ Acre. These two treatments are noted in this manuscript as “Prep Early” and “Prep Late”, indicating that Prep was applied 5 weeks and 1 week before harvest, respectively. This field study used a randomized complete block design with 3 replications. There was a one row buffer between each treatment. Transplants were planted on 12 July 2011, and two Prep treatments were applied on 19 September (69 days after planting) and 21 October 2011 (101 days after planting). Roots were all mechanically harvested on 28 October 2011 (108 days after planting) and the first sampling was conducted immediately after harvest. The rest of the roots were cured for 7 days immediately after harvest, and then sampled twice during storage, either right after curing (7 days after harvest) and 30 days after harvest. Every sample from each plot was consisted of 25 roots of various sizes (due to the shortage of

samples), and these roots were sliced and evaluated for visual symptoms based on the severity scale from 0 to 4 (Figure 2). Data on IN incidence and severity were subjected to SAS 9.2 PROC GML (100 SAS Campus Dr. Cary, NC 27513).

2.3. Prep study

Based on research which linked the occurrence of IN with application of Prep (Dittmar et al., 2010) and its occurrence in our herbicide study, we followed up with two additional Prep studies in 2012. The goals of these studies were to determine the time frame in which Prep caused IN by evaluating the visual symptoms from time of Prep application (15 days prior to mechanical harvest) until 30 days after harvest, and the effects of curing versus not curing. In addition, microscopic techniques were used to characterize cell death prior to the appearance of visual symptoms. An early and later planting of 'Covington' was established 1 May and 21 May 2012. Each trial was a randomized complete block design comprised of six 6-row plots. Plants were 107 cm between rows and 30 cm within a row. There were two treatments (Prep and no Prep (control)) and 3 replications. Prep was applied 6 August for the first planting (97 days after planting) and 27 August for the second planting (98 days after planting) using 1.46 kg/ ha. Prior to Prep application of each study, 30 US No.1 roots were sampled across the entire field and cut immediately to examine the occurrence of IN. After Prep was applied, samples of 20 roots, also US No.1 size, were taken from each plot for multiple evaluation times according to the schedule in Table 3. In study 1, roots for all sampling times were dug by hand due to the wet soil condition. In study 2, hand harvest was made during the first two samplings (4 and 9 DAPA, days after Prep application) and then all roots were mechanically harvested at 15 DAPA. All samples were taken from the

center 4 rows in the field plots to avoid spray drift contamination from nearby plots. The goals of the sampling schedule were to determine: 1) if IN develops in the field and how Prep application and duration affects the development of symptoms; 2) how IN develops after roots are harvested and handled using standard postharvest procedures that includes curing at 85 F and 85% relative humidity for 7 days followed by storage at 58 F (Edmunds et al., 2008). In the first study, we did not compare the effects that curing versus not curing had on IN. This comparison was made in the second study. Twice the amount of roots were sampled from Prep treated plots; half of them were placed directly in the storage room at 58 F without getting cured. Those roots were cut and evaluated based on the same sampling schedule as the rest of samples which were cured.

Several staining techniques, including TTC (2, 3, 5-triphenyltetrazolium chloride), Evans Blue and DAPI (4', 6-diamidino-2-phenylindole), were evaluated in order to characterize cell death prior to the appearance of visual IN symptoms. Our hypothesis was that Prep would result in cell death prior to visual symptoms being observed without the aid of the microscope, and all techniques we evaluated were to detect cell death. TTC is a redox indicator, which can differentiate living and non-living tissue based on its cellular respiration. In living cells, the white compounds will enzymatically reduce and become a red compound TPF (1, 3, 5-triphenylformazan). By using TTC, we expected to differentiate living cells in a sweetpotato root which would stain red while injured or dead cells which later developed visual IN symptoms would remain white. Evans Blue is frequently used in viability assays as this azo dye can penetrate into non-viable cells. We expected this dye to move through the non-living intercellular space and stain both the intercellular space and dead cells with blue

color.

DAPI is a fluorescent stain that can bind to A-T (adenine-thymine) rich regions in DNA. It can pass through an intact cell membrane and therefore stain both live and fixed cells. Our goal was to distinguish if cells were healthy, which, when stained with DAPI, would stain the nuclei DNA and thus cause the nuclei to fluoresce under the fluorescent microscope. If the cells are stressed or dead, the nuclei would collapse and thereby not be stained by the dye and not fluoresce. Based on this hypothesis and some preliminary work, we set up a scoring system from 1 to 5 to rate different stages of cell stress (Figure 7). Three sweetpotato roots were sampled from each plot following the schedule in Table 4. Sections were taken both longitudinally and transversely from sweetpotato roots between 1.5 and 3 cm from the proximal end. Tissue sections were made by a vibratome (Leica VT1000 S Vibrating Blade Microtome, 1700 Leider Lane, Buffalo Grove, IL 60089) with consistent thickness of 170 μm. DAPI solution (0.2 mM/L) was made by dissolving pure DAPI powder (D9542, Sigma-Aldrich, 3050 Spruce St., St. Louis, MO 63103) into phosphate-buffered saline (PBS). At each sampling time, sections were made and stained with DAPI solution, and then they were immediately evaluated by the fluorescence microscope. A score from 1 to 5 was given to every root based on the stages of cell stress in both bright and fluorescent field of images. Both visual incidence and DAPI anatomic rating data were analyzed by JMP 9.0 (100 SAS Campus Dr. Cary, NC 27513) multiple comparisons Tukey HSD.

Results

1. Effect of post-harvest factors on the occurrence of IN

1.1. Commercial survey

The survey results for the incidence of IN for each commercial facility in 2010-2011 and 2011-2012 seasons are presented (Figures 3a and 3b). Percentage incidence was obtained by averaging numbers from every sampling time. The same facility number in one year did not correspond with the same storage room in the second season. The incidence of IN in 'Covington' roots varied widely among facilities in NC. There were only 2 out of 29, and 4 out of 27 facilities in 2010-2011 and 2011-2012 respectively, that did not have any IN during the entire storage season. The incidence of IN was generally low (less than 10%) in the majority of the storage facilities. In 2010-2011 and 2011-2012 respectively, there were only 2 or 1 facility that displayed incidence higher than 30%, and those facilities in two years were not from the same businesses. In the 2010-2011 survey, from the facility that had the highest IN incidence (41%), roots that were cured and stored in the same room but harvested from different fields all had IN , though the incidence percentage varied among roots harvested from different fields (Data not shown).

The severity of IN was obtained by averaging the severity scores of all roots with IN symptoms (severity score>0) (Figures 4a and 4b). In both years, most of the IN symptoms were rated as Severity 1 or 2, which meant symptoms were either negligible or obvious but confined to the stem end (less than or around 1 cm diameter) (Figure 2). Roots with these symptoms would generally be considered as marketable, as long as incidence is relatively low. There were a few facilities each year in which symptoms scored higher than 2. Those

roots were found with multiple and large areas of black marbling or necrotic tissue, and roots with these kinds of internal problems would be unmarketable.

In terms of symptom development over time in storage, observations from the five sampling times in each year did not indicate any kind of increasing or decreasing trends of either the incidence or severity of IN (Appendix B, Table 1 and 2). In some facilities, the incidence and severity was consistently high throughout the whole storage season. In some other facilities, the incidence was high in one or two sampling times but not the rest. In the rest of the facilities, which was the case in most instances, the incidence and severity was consistently low throughout the whole time in storage.

1.2. Storage condition tracking

Results showed that storage conditions in some commercial facilities were maintained very well and temperature and relative humidity (RH) varied within a very small range near recommended levels (Temperature 58 F and RH 90%, Kushman, 1975) (Appendix B, Figure 1, 2, 4). However, in some facilities the temperature and RH greatly deviated from the recommended values over time (Appendix B, Figure 3, 5). No relationship was found between storage temperature or RH conditions and the occurrence of IN. In some storage facilities, temperature exceeded 80 F for days (especially in early storage period) yet still did not show symptoms of IN; in some other facilities the RH fluctuated and some rooms were primarily maintained between 60% and 70% RH throughout the entire time, but there was no or minimal IN incidence. However, in some facilities where optimum storage temperature and RH were maintained, a high incidence of IN was detected. It appeared as though room temperature or RH in storage had little or no impact on the occurrence of IN.

1.3. Pathogen isolation

Pathogen isolation from IN roots resulted in various kinds of bacteria and fungi in either the moisture chamber or the PDA medium (Appendix B, Table 3). Some isolates were "*Fusarium*-like" but did not show consistent growth in PDA and the moisture chamber simultaneously. The types of pathogen were various from one plate to the other, and were most likely secondary contaminants. There was no relationship found between the occurrence of IN and the growth of certain types of pathogens.

1.4. Ethylene test

In both years, a small amount of IN was found in both ethylene treated roots and ethylene non-treated roots (Table 5). The incidence for non-treated roots ranged from 0 to 10% while treated roots ranged from 0 to 20%. The 20% incidence only occurred once at 82 days in 2011, however, no similar occurrence in the same treatment was found in the following sampling at 123 days. Because the 20% incidence appeared to be a random event, data from this study did not seem to indicate any relationship between ethylene gas in storage and the occurrence of IN. The small amount of symptoms observed from both ethylene treated and non-treated samples corresponded to most of the cases that occurred in the commercial facilities, which had low incidence and minimal severity.

2. Effect of pre-harvest factors on the occurrence of IN

2.1. Insecticide studies

Internal Necrosis was rarely found in roots in all insecticide treatments from both field test locations. No or minimal incidence (between 0 and 2%) occurred across all

treatments and all sampling times (Tables 6 and 7). The occurrence of IN appeared to be random with minimal incidence and severity which corresponded to what happened in most of the commercial facilities. These symptomatic roots were likely caused by factors not related to the application of any given insecticide.

2.2. Herbicide study

Among all herbicide treatments, "Prep Late" resulted in the highest IN incidence, which was significantly different from any of the other treatments (Table 8). "Prep Early" induced some IN but not as much as "Prep Late". Severity of IN for both "Prep Early" and "Prep Late" were significantly higher than other treatments. There were some occasional roots in other herbicide treatments as well as the untreated check that had IN symptoms, however, the sporadic and low occurrence was likely an artifact and not a treatment effect. Treatment "Rely" was included in the test, but its results were excluded from Table 8 as 2 out of the 3 plots were contaminated by Prep treatments next to them. The occurrence of IN in the one "Rely" plot indicated no relationship between the occurrence of IN and this herbicide.

The development of IN over time in storage was also evaluated in the herbicide study (Table 9). When a HSD multiple comparisons were made across treatments by sampling time, besides the two Prep treatments, no other treatments displayed any treatment effect across all three sampling times. Because of this, we excluded all treatments except the Prep treatments and did a further analysis on the effect of application time (early and late) and sampling time of Prep. In this analysis, no application time effect was observed (Data not shown), but the effect of sampling time was significant. Thus, the data from the two Prep treatments ("Prep Early" and "Prep Late") were pooled (Figure 5). Freshly harvested roots from all Prep treated

plots did not show any IN symptoms, while IN occurred at the following two sampling times, which were after curing (7 days after harvest), and 30 days after harvest. Incidence of IN stayed statistically the same between the 2nd and 3rd samplings, while the severity increased over sampling times.

2.3. Prep study

As mentioned in the Methods section, the goals of a subsequent Prep study and its sampling schedule were to determine: 1) how IN develops in the field and how Prep application and duration affects the development of symptoms; 2) how IN develops after roots are harvested and handled with standard postharvest procedures (Edmunds et al., 2008). During each study, 30 roots were sampled across the entire field prior to Prep application. Those roots were cut immediately after they were harvested from the field. In study 1, 1 out of 30 roots was found to have IN; in study 2, no IN was found in all sampled roots (Data not shown). After Prep was applied, roots were sampled periodically. Data showed that roots from the no Prep treatments did not have IN until 8 DAH (days after harvest) (7 days in curing, 1 day storage), and the incidence never got higher than 10% even at 30 DAH (7 days in curing, 23 days storage) (Table 10). In roots from Prep treated plots, IN incidence was minimal (3.3 %) before scheduled harvest (DAH=0) and storage (study 1), and was first seen at 9 days after Prep application (9 DAPA) in study 2. The first significant incidence of IN between the Prep treated and the no Prep treatments in both studies was first measured at sampling 6, which was 8 DAH, where 27% and 25% of IN was found in studies 1 and 2, respectively. The incidence of IN in study 1 increased significantly over time and reached 67% at 30 DAH; while in study 2, though the incidence stayed statistically the same as 6

DAH, 40% of IN was still shown at both 15 and 30 DAH.

The effect of curing versus not curing on Prep treated roots was evaluated in study 2. Results showed that at 8 DAH, roots treated with Prep which had not been cured (PNC) had significantly less incidence and severity than roots treated with Prep that had been cured (PC) (Figures 6a and 6b). At most of the sampling times (0 to 30 DAH), the incidence and severity of IN in PNC roots was statistically the same with roots that had not been treated with Prep but were normally cured (NPC). There were a few exceptions when one treatment registered higher incidence and severity of IN than another treatment.

Anatomical methods were evaluated to understand the changes at the cellular level of sweetpotato tissue when Prep was applied and determine if detection of the impending visual IN symptoms could be realized earlier using microscopic tools. Our goal was to find a quick technique to detect early cell death in sweetpotato roots in response to the application of Prep.

When using TTC, the dye did not move through the tissue. When a 5 mm thick tissue section was made and soaked in TTC solution, the solution could not penetrate through the cells and stain them thoroughly and uniformly.

Evans Blue is supposed to penetrate into non-viable cells. We expected to have this dye go through the non-viable intercellular space and thus stain the dead cells in the tissue. This did not succeed either as the dye only went through the dead cells but not the non-viable intercellular space. This meant when sweetpotato tissue was soaked in the staining solution, if the surface of the section was dead, the dye could move through the surface and reach the internal tissue. However, if the surface was healthy, there was no channel for the dye to move into the tissue, even if dead tissue existed below the cut surface.

The third stain, DAPI was expected to stain the nuclei DNA in living cells and thus cause the nuclei to fluoresce. If the cells are stressed or dead, nuclei would collapse and thereby not be stained by the dye and not fluoresce. Data were obtained by scoring the stages of cell stress. Determination of definitive cell images that could be easily scored was not obtained. In addition, cell conditions were likely greatly inconsistent from one root to the other or among different sections of the given root. Due to the above reasons, a significant difference between the Prep and no Prep treatment could not be consistently distinguished; the only times this difference was seen was at 9 DAPA (days after Prep application) in study 1 and 23 DAPA in study 2, where Prep treatment had significant higher cell damage scores than the no Prep treatment (Table 11). In addition to these, differences of cell damage scores could be seen in different sampling times but no consistent trends of increasing or decreasing scores were seen. For these reasons, no relationship was found between the application of Prep as well as its duration and the stage of cell stress obtained by scoring tissue images.

As described above, all three techniques we evaluated proved to be a failure in achieving this goal.

Discussion

The commercial survey results revealed that most of sweetpotato storage facilities in NC contained roots with some level of IN. The incidence and severity were generally low in most storage facilities, usually less than 10%, and the majority of the roots were marketable (Figures 3a, 3b, 4a and 4b). However, the few businesses that experienced high incidence of IN (above 30%) with severe or intense symptoms were likely not marketable and detrimental

to their profit margin. The first samples in both years were collected within a month after curing (time varied from one grower to the other); after the first month no consistent pattern was associated with the increasing or decreasing of IN incidence and severity over time in storage (Appendix B, Table 1 and 2). This indicated that the initiation of the symptom and its physiological changes may occur before storage, such as during pre-harvest practices, at harvest, during curing or shortly after curing. However, in 2012, it was observed that roots obtained from different fields and stored in the same facility all had high incidence and severity of IN symptoms within a month of storage, indicating that curing conditions associated with the facility may play an important role in the occurrence of IN (Schultheis, personal communication).

Previous studies showed that even a small amount of ethylene in either curing or storage can affect sweetpotato root respiration rate and phenolic compounds (Buescher et al., 1975; Kitinoja, 1987). However, data from our laboratory study did not show the relationship between ethylene gas in storage and the development of IN (Table 5). Compared with previous studies, we used a much higher ethylene concentration (100 ppm instead of 10 ppm used by Buescher and 1 ppm by Kitinoja), which should be sufficient to induce IN if ethylene gas was related to this incidence in storage. The weakness of our study was that we did not treat sweetpotato roots with ethylene during curing. To come to a more confident conclusion, future studies should be continued on investigating the effect of ethylene in curing and the occurrence of IN.

Produce quality can be affected by storage CO_2 and O_2 concentration (Kader, 2002). Sweetpotato cured at 2 or 4% O_2 or 60% CO_2 + 21 or 8% O_2 stimulated decay after curing

(Delate and Brecht, 1989). Once cured and then exposed to 2 or 4% O_2 + 40% CO_2 or 4% O_2 + 60% CO_2, postharvest quality was not affected. In spite of these findings, high CO_2 and low O_2 conditions as described in the above study are unlikely to happen in a properly operated modern sweetpotato storage facility (Edmunds et al., 2008); nevertheless, data from the commercial survey showed that high IN incidence was still found in some recently built and well ventilated facilities (Data not shown).

Application of pesticide can cause injury to sweetpotato roots. For example, glyphosate resulted in external cracks to sweetpotato roots (Meyers, 2009) and post-planting application of halosulfuron could cause root injury too. The external symptoms for halosulfuron injury were described as a blackened area with blistering on the root surface while the internal injury consisted of small red-brown spots inside the roots (Dittmar et al., 2012). In our study, no other commonly used herbicides or insecticides, other than Prep, were found to have an impact on IN in 'Covington' (Tables 6, 7, 8). These results confirmed the findings of another study, where Prep caused IN on several sweetpotato varieties, including 'Covington', "Beauregard' and 'Carolina Ruby', but varieties varied in their IN expression (Dittmar et al., 2010).

Even when Prep was applied, no or very minimal incidence (3.3%) of IN was found in freshly harvested roots. Previous assumptions included that the time period between Prep application and root harvest might affect the occurrence of this symptom. However, in the herbicide study, no IN was found in roots cut at 0 DAH, regardless of whether treatment was from an early or late Prep application (Figure 5), in which applications were a month apart. Correspondingly, in the Prep study, very minimal IN was found at 0 DAH, no matter if the

roots were harvested 5 days, 10 days or 15 days after Prep was applied (Table 10). The earliest significant IN was seen in the Prep study at 6 DAH when roots were still in curing, and a similar incidence was observed in the herbicide study soon after curing. Based on the above findings, no matter when Prep was applied, significant incidence of IN was visually apparent only after roots were harvested, and cured and stored for at least 6 days.

Ethephon, the active ingredient of Prep, is used in many crops as a preharvest application to accelerate defoliation (Tiernan, 1983). It can also affect postharvest quality and lead to fast deterioration (Gerasopoulos and Stavroulakis, 1999). In sweetpotato production, foliar application of ethephon prior to harvest causes defoliation on sweetpotato plants (Arancibia et al., 2011a). Defoliation (or leaf senescence) in plants normally happens either as a genetically programmed process during plant development, or as a response to external environmental stresses (Buchanan-Wollaston, 1997). This period involves a highly regulated series of events such as the massive remobilization of nutrients, cessation of photosynthesis, degradation of cell structure etc., which eventually lead to cell death (Lim et al., 2007). Sweetpotato Tip Rot incidence has been reported in 'Beauregard' and exhibits different symptoms from IN, however, like IN, sweetpotato tip rot incidence can be greatly enhanced by spraying ethephon during the preharvest period (Arancibia et al., 2011a). It is suspected that the tip rot is caused by ethephon-induced stresses that dispose the root to pathogen infections. Interestingly, this incidence could be reduced when standard curing practice was used, which was opposite to the case of IN (Figures 6a and 6b).

Biochemical changes have been found in storage roots when sweetpotato plants or roots encountered ethylene. Plant phenolics play roles in pigment formation, lignification as

well as resistance to pathogens and insects (Lattanzio, 2006). Studies have found that total phenolics in storage roots increased when sweetpotato plants were sprayed with ethephon (Arancibia et al., 2011b) or when roots were stored in an ethylene environment (Kitinoja, 1987). Polyphenol oxidase (PPO) and peroxidase (POD) are the enzymes involved in the process of browning, which happens when the enzyme and substrate are mixed and the cell structure is destroyed (Jang and Moon, 2011). PPO activities were enhanced when storage roots were exposed to ethylene in storage (Buescher et al., 1975) while POD activities increased when storage roots were sliced and rapid ethylene was produced endogenously (Imaseki, 1970). In addition, foliar ethephon application prior to harvest also caused more browning in the flesh of baked sweetpotatoes, while their texture, color and flavor were ranked lower than those from non-ethephon treated plants (Sheibani et al., 2011). To date, no study has been done on the physiological and biochemical changes in sweetpotato roots as a result of IN, but in cassava (*Manihot esculenta* Crantz), studies on the postharvest physiological deterioration (PPD) have pointed to an increase of reactive oxygen species (ROS) as one of the earliest recognized steps in PPD development (Zidenga, 2012). It is suspected that this ROS increase may come from hydrolysis from the cyanogenic alkaloid linamarin to cyanide (Getzin and Fellman, 2011). This process can occur when roots are cut off from the stem and harvested, which may interrupt the respiration in mitochondria and thus lead to PPD. Sweetpotato is harvested in a similar way as cassava, and 'Covington' roots tend not to separate from the vines as easily as another less susceptible variety 'Beauregard' (Dittmar et al., 2010; Schultheis, personal communication). Future studies on the physiological and biochemical changes of the sweetpotato plant and roots at harvest as

well as when IN occurs can be valuable in understanding the development of this disorder.

It should be emphasized that we have learned that Prep (or ethephon) exacerbates the IN; however, the crops we examined in commercial storage facilities were not treated with Prep. This indicated there are other causes of IN that we cannot account for. Prep caused leaf senescence which seemed to trigger a plant stress response and thus induced IN. In this regards, other preharvest or at harvest environmental stresses may also trigger this response and thereby cause IN in cases where Prep is not used. Evidences of preharvest or at harvest disorders associated with environmental factors have been described in other root (tuber) crops. Internal Blackspot (IBS) and Internal Heat Necrosis (IHN) are two examples in Irish potato (*Solanum tuberosum* L.). IBS forms as a result of mechanical injury during harvest (Timm et al., 1976); but its occurrence is strongly associated with soil-plant nutrition (Mulder, 1955; Jacob, 1959; Mondy et al., 1967), firmness of tubers (Sawyer and Collins, 1960) and the handling temperature (Ophuis et al., 1958). IHN is a production problem that does not relate to postharvest handlings; high temperature, low soil moisture and poor soil nutrients are all factors that affect the incidence and severity of this disorder (Yencho et al., 2008).

We have one hypothesis that might explain what occurs with the foliar application of Prep. Prep may induce programmed cell death (PCD) in plants and thus result in IN. Prep is a plant growth regulator that can trigger the plant to start its defense system to fight with any potential environmental stress. As part of the defense, the plant may deliberately sacrifice some of its cells in order to protect the whole system, which is called PCD. The most common form of PCD is the phenomenon of hypersensitive reaction (HR), where cell death

happens as a normal immune response around a diseased area and thus stops the disease from invading. PCD has also been seen during the formation of aerenchyma cells as a response to ethylene or hypoxia in maize roots (Arunika et al., 2001). It has also been documented that cells characterized as having PCD usually show increased levels of ROS (Gadjev et al., 2008) and phenolic compounds (Beckman, 2000), which have both been identified in sweetpotato roots that were treated with ethylene (Buescher et al., 1975; Kitinoja, 1987; Arancibia et al., 2011 b). In addition, a later stage of PCD defense can result in periderm formation (Beckman, 2000), which we found around the necrosis area of sweetpotato roots with IN (Appendix B, Figure 7). Based on the above literature and our laboratory observation, we hypothesize that the occurrence of IN during Prep application may be a PCD process that comes from the defense of plant itself.

Summary

This research demonstrated that the disorder internal necrosis in ‘Covington’ variety is widely spread among NC commercial facilities but the overall incidence and severity is relatively low. Temperature, relative humidity and the existence of ethylene gas in storage do not seem to induce this symptom. However, other postharvest factors could not be excluded and further studies are needed to focus on the environmental factors during the curing process. Besides Prep, an ethephon compound, no other currently used pesticides have been found to induce IN. The time period between foliar Prep application and root harvest did not seem to be critical in inducing IN. In our studies, significant IN incidence was found 8 DAH and increased to 40 to 66.7 % at 30 DAH. Curing can enhance the incidence and severity of IN. Future studies could focus on the biochemical and physiological changes in sweetpotato roots as a response to unfavorable preharvest and curing environment.

References

Arancibia, R.A., J.L. Main, and X. Wang. 2011a. Sweetpotato Tip Rot Disorder is enhanced by preharvest application of ethephon and reduced by curing. HortScience 46(9):S253 (Abstr.).

Arancibia, R.A., J.L. Main, and X. Wang. 2011b. Pre-harvest applications of ethephon increase phenolics and skinning tolerance in sweetpotato. HortScience 46(9): S297 (Abstr.).

Arunika, H.L. and A.N. Gunawardena, D.M. Pearce, M.B. Jackson, C.R. Hawes, D.E. Evans. 2001. Characterization of programmed cell death during aerenchyma formation induced by ethylene or hypoxia in roots of maize (*Zea mays* L.). Planta. 212(2):205-14.

Beckman, C.H. 2000. Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defense responses in plants? Physiol. & Mol. Plant Pathol. 57(3):101-110.

Blankenship, S.M. and J.M. Dole. 2003. 1-Methylcyclopropene: a review. Postharvest Biol. Technol. 28(1):1-25.

Buchanan-Wollaston, V. 1997. The molecular biology of leaf senescence. J. Expt. Bot. 48(2):181-199.

Buescher, R.W., W.A. Sistrunk, and P.L. Brady. 1975. Effects of ethylene on metabolic and quality attributes in sweetpotato roots. J. Food. Sci. 40(5):1018-1020.

Cantwell, M. and T. Suslow. 2001. Sweet potato: recommendations for maintaining

postharvest quality. Dept. Plant Sci., UC-Davis, Davis, CA. http://postharvest.ucdavis.edu/pfvegetable/SweetPotato/

Carlton, B.C., C.E. Peterson, and N.E. Tolbert. 1961. Effects of ethylene & oxygen on production of a bitter compound by carrot roots. Plant Physiol. 36(5):550-552.

Chalutz, E., J.E. Devay, and E.C. Maxie. 1969. Ethylene-induced isocoumarin formation in carrot root tissue. Plant Physiol. 44(2):235-241.

Daines, R.H., D.F. Hammond, N.F. Haard, and M.J. Ceponis. 1976. Hardcore development in sweet potatoes: a response to chilling and its remission as influenced by cultivar, curing temperatures and time and duration of chilling. Phytopathol. 66:582-587.

de Wild, H.P.J., E.J. Woltering, and H.W. Peppelenbos. 1999. Carbon dioxide and 1-MCP inhibit ethylene production and respiration of pear fruit by different mechanisms. Jour. Exp. Bot. 50(335):837-844.

Delate, K.M. and J.K. Brecht. 1989. Quality of tropical sweetpotatoes to controlled atmosphere treatments for postharvest insect control. J. Amer. Soc. Hort. Sci. 114(6):963-968.

Dittmar, P.J., D.W. Monks, K.M. Jennings, and J.R. Schultheis. 2012. Effects of halosulfuron POST on sweetpotato yield and storage root quality. Weed Technol. In-Press.

Dittmar, P.J., K.M. Jennings, D.W. Monks, and J.R. Schultheis. 2010. Determining the effect of ethylene on internal black marbling expression in sweetpotato. HortScience 45(4):488-489 (Abstr.).

Edmunds, B., M. Boyette, C. Clark, D. Ferrin, T. Smith, and G. Holmes. 2008. Postharvest handling of sweetpotatoes. North Carolina Cooperative Extension Service. p. 12. Raleigh, NC.

Fan, X. and J.P. Mattheis. 2000. Reduction of ethylene-induced physiological disorders of carrots and iceberg lettuce by 1-methylcyclopropene. HortScience 35(7):1312-1314.

Gadjev, I., J.M. Stone, and T.S. Gechev. 2008. Programmed cell death in plants: new insights into redox regulation and the role of hydrogen peroxide. Intl. Rev. Cell & Mol. Biol. 270:87-144.

Gerasopoulos, D. and G.Stavroulakis. 1999. Temperature and ethephon effects on storability of 'Sceptar' red raspberries. Mediterranean Post-harvest Network 2nd Workshop, Chania, Greece. 42:50-56.

Getzin, E. and J.K. Fellman. 2011. Confocal imaging studies of postharvest physiological deterioration in cassava (*Manihot esculenta* crantz) root tissue. ISHS Acta Hort. 945: IV Intl. Conf. Postharvest Unlimited 2011.

Imaseki, H. 1970. Induction of peroxidase activity by ethylene in sweet potato. Plant Physiol. 46(1):172-174.

Imaseki, H., I. Uritani, and M.A. Stahmann. 1968a. Production of ethylene by injured sweet potato root tissue. Plant Cell Physiol. 9(4):757-768.

Imaseki, H., M. Uchiyama and I. Uritani. 1968c. Effect of ethylene on inductive increase in Metabolic Activities in Sliced Sweet Potato Roots. Agri. Biol. Chem. 32(3):387-389.

Imaseki, H., T. Teranishi and I. Uritani. 1968b. Production of ethylene by sweet potato roots infected by black rot fungus. Plant Cell Physiol. 9(4):769-781.

Jacob, W.C. 1959. Studies on internal black spot of potatoes. p. 368. Cornell Uni. Agri. Expt. Station, New York State College of Agriculture, Ithaca, NY.

Jang, J.H. and K.D. Moon. 2011. Inhibition of polyphenol oxidase and peroxidase activities on fresh-cut apple by simultaneous treatment of ultrasound and ascorbic acid. Food Chemistry 124(2): 444-449.

Kader, A.A. (ed.). 2002. Postharvest technology of horticultural crops. Univ. California, Agri. Natl. Resources, Publication 3311.

Kemble, J.M., K. Ivors, F.J. Louws, K.M. Jennings, and J.F. Walgenbach (eds.). 2012. Southeastern U.S. 2012 vegetable crop handbook. p. 96-97. du Pont.

Kitinoja, L.A. 1987. Effects of low concentrations of ethylene on sweetpotatoes (*Ipomoea batatas* (L.)(Lam) during curing and storage. Acta Hort. 201:37-42.

Kushman, L.J. 1975. Effect of injury and relative humidity during curing on weight and volume loss of sweet potatoes during curing and storage. HortScience 10:275-277.

Lafuente, M.T. and J.M. Sala. 2002. Abscisic acid levels and the influence of ethylene, humidity and storage temperature on the incidence of postharvest rindstaning of 'Navelina' orange (*Citrussinensis* L. Osbeck) fruit. Postharvest Biol. Technol. 25(1):49-57.

Lafuente, M.T., G. López-Gálvez, M. Cantwell, and S.F. Yang. 1996. Factors influencing ethylene-induced isocoumarin formation and increased respiration in carrots. Jour. Amer. Soc. Hort. Sci. 121:537-542.

Lattanzio, V., M.T. Veronica, and A. Cardinali. 2006. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. p. 23-67. In: Imperato, F. (ed). Phytochemistry: Advances in Reseach 2006. Research Signpost, Kerala, India.

Lim, P.O., H.G. Nam, and H.J. Kim. 2007. Leaf senescence. Annu. Rev. Plant Biol. 58: 115-136.

Main, J.L., M.W. Shankle, and T.F. Garrett. 2009. A comparison of ethephon and conventional vine removal systems for Mississippi sweetpotato. HortScience 44(3):574-574 (Abstr.).

Mercier, J., J. Arul, and C. Julien. 1993. Effect of UV-C on phytoalexin accumulation and resistance to *Botrytis cinerea* in stored carrots. J. Phytopathol. 139:17-25.

Meyers, S.L. 2009. Interference and control of Palmer Amaranth (*Amaranthus palmeri*) in sweetpotato. NC State Univ., MS Diss..

Mondy, N.I., E.O. Mobley, and S.B. Gedde-Dahl. 1967. Influence of potassium fertilization on enzymatic activity, phenolic content, and discoloration of potatoes. J. Food Sci. 32(4):378-381.

Mulder, E.G. 1955. Effect of mineral nutrition of potato plants on respiration of the tubers. Acta Botanica Neerlandica 4(3):429-451.

Ophuis, B.G., J.C. Hesen, and E. Kroesbergen. 1958. The influence of temperature during handling on the occurrence of blue discoloration inside potato tubers. Eur. Potato J. 1(3):48-65.

Rood, P. 1956. Relation of ethylene and post-harvest temperature to brown spot of lettuce. Proc. Amer. Soc. Hort. Sci. 68:296-303.

Saltveit, M.E. 2004. Lettuce. In: K. Gross. The commercial storage of fruits, vegetables, and florist and nursery stocks. Agriculture Agri. Handbook Number 66. U.S. Dept. Agr., Agr. Research Service.

Sawyer, R.L. and G.H. Collins. 1960. Black spot of potatoes. Amer. Potato J. 37:115-126.

Schultheis, J.R. and A.C. Thornton. 2007. Determining the expression or lack of expression of internal marbling in Covington roots via vegetative propagation during the growing season and in storage; refinement of nitrogen application rate and timing to optimize yields and root sizing of Covington sweetpotato. North Carolina sweetpotato research and extension reports 2007. NC State Univ., Raleigh, NC.

Schultheis, J.R., S.M. Blankenship, D.W. Monks, and M.D. Boyette. 2000. Preharvest methods to reduce skinning in 'Beauregard' sweetpotato. HortScience 35(3):444 (Abstr.).

Schultheis, J.R., Z. Pesic-VanEsbroeck, K.M. Jennings, P.J. Dittmar, and A.C. Thornton. 2009. Effects of environmental stress and pathogens on the Internal Mottling and End Rots of sweetpotato in new commercial varieties ('Hatteras' and 'Covington'), and established commercial varieties ('Beauregard' and 'Carolina Rudy'). North Carolina sweetpotato research and extension reports 2009. NC State Uni., Raleigh, NC.

Sheibani, E., R. Arancibia**,** T. Kim, J. Main, and J.L. Silva. 2011 Preharvest ethylene and postharvest curing effects on baked sweet potato (*Ipomoea batatas* L. Lam) quality. IFT Annul. Mtg., New Orleans, LA.

Tiernan, P.I. 1983. The use of chemical aids in pre-harvest defoliation of *Phaseolus vulgaris* L. and their effect on crop yield. Acta Hort. 137:395-400.

Timbie, M. and N.F. Haard. 1977. Involvement of ethylene in hardcore syndrome of sweet-potato roots. J. Food Sci. 42(2):491-493.

Timm, H., M. Yamaguchi, D.L. Hughes, and M.L. Weaver. 1976. Influence of ethylene on black spot of potato-tubers. Am. Potato J. 53(2):49-56.

Villordon, A.Q. 2012. Differential response of 'Beauregard' sweetpotato storage roots to ethephon, 1-Methylcyclopropene and water submergence treatments. ASHS Natl. Conf. 2012, Miami, FL.

Wang, X., R.A. Arancibia, J.L. Main, and M. Shankle. 2012. Pre-harvest application of ethephon enhances skin lignification and increases the force required to peel sweetpotato storage roots. ASHS-HortTalks, Miami, FL (Abstr.).

Yencho, G.C., K.V. Pecota, J.R. Schultheis, Z. Pesic-Vanesbroeck, G.J. Holmes, B.E. Little, A.C. Thornton, and V. Truong. 2008. 'Covington' sweetpotato. HortScience 43(6):1911-1914.

Yencho, G.C., P.H. McCord, K.G. Haynes, and S.B. Sterrett. 2008. Internal Heat Necrosis of potato - a review. Amer. J. Potato Res. 85(1):69-76.

Zidenga, T., E. Leyva-Guerrero, H. Moon, D. Siritunga, and R.T. Sayre. 2012. Extending cassava root shelf life via reduction of reactive oxygen species production. Plant Physiol. 159(4):1396-1407.

[USDA-NASS] U.S. Dept. Agr.- Natl. Agr. Stat. Service. 2011. 2011 Census of Agri. Washington D.C.: U.S. Dept, Agr.

Table 1. 2011 insecticide treatments in two locations (Insecticide studies).

Location	Treatment	Treatment description	Application code [z]
Kinston	1	Lorsban 0.5 gal/Acre	A
	2	Brigade 19.2 fl oz/Acre	A
	3	Mocap 6.9 fl oz/1000 Row-FT	B
	4	Coragen 7 fl oz/Acre	C
	5	Belay 12 fl oz/Acre	A
	6	Admire Pro 10.5 fl oz/Acre	A
	7	Brigade 19.2 fl oz/Acre	B
	8	Brigade 19.2 fl oz/Acre	D
	9	Untreated check	
Clinton	1	Lorsban 64 fl oz /Acre	A
		Brigade 19.2 fl oz /Acre	D
	2	Mocap 60.8 fl oz /Acre	A
	3	Belay 12 fl oz /Acre	A
	4	Coragen 7 fl oz /Acre	A
	5	Admire Pro 10.5 fl oz /Acre	A
	6	Untreated Check	

[z] Application code: A= broadcast pre plant incoporated soil application; B = at plant in-furrow application; C = at plant soil drench; D= broadcast post plant incorporated soil application.

Table 2. 2011 herbicide treatments (Herbicide study).

Treatment	Treatment Description	Application code [z]
1	Prep early application	C
2	Prep late application	D
3	Rely	A
4	Valor 3	A
5	Command	B
6	Paraquat	A
7	Dual Magnum 0.75 pt/A	B
8	Dual Magnum 1.25 pt/A	B
9	Glyphosate (Roundup)	A
10	Devrinol	B
11	Check (nontreated)	

[z] Application code: A= broadcast pre plant (1 day before planting) application; B=broadcast post plant (1 day after planting) application; C= broadcast application on 19 September, 2011 (69 days after planting); D= broadcast application on 21 October, 2011 (101 days after planting).

Table 3. Visual symptoms sampling schedule based on Prep application and days roots being cured/ stored (Prep study).

Sampling #	DAPA [z]	Days cured	Days stored	DBH (-) or DAH (+) [y]
1	4	0	0	-11
2	9	0	0	-6
3	15	0	0	0
4	18	3	0	+3
5	21	6	0	+6
6	23	7	1	+8
7	30	7	8	+15
8	35	7	13	+20
9	45	7	23	+30

[z] DAPA = days after Prep application.

[y] DBH= days before harvest; DAH= days after harvest.

Table 4. Anatomic sampling schedule based on Prep application and days roots being cured/ stored (Prep study).

Sampling #	DAPA [z]	Days cured	Days stored	DBH (-) or DAH (+) [y]
1	4	0	0	-11
2	9	0	0	-6
3	15	0	0	0
4	18	3	0	+3
5	21	6	0	+6
6	23	7	1	+8

[z] DAPA = days after Prep application.

[y] DBH= days before harvest; DAH= days after harvest.

Table 5. Relation between the existence of ethylene (C_2H_4) gas and IN (Ethylene study).

Sample Source	**Sampling Times**	**Days after C_2H_4 treatment**	**C_2H_4 rate (ppm)**	**Incidence of IN [z]**	**Percentage IN %**	**Sev. [y]**
2010	1	0	-	0	0	0
	2	14	100	0	0	0
	2	14	0	1	5	2
	3	49	100	0	0	0
	3	49	0	2	10	1
2011	1	0	-	0	0	0
	2	21	100	0	0	0
	2	21	0	0	0	0
	3	82	100	4	20	1
	3	82	0	0	0	0
	4	123	100	0	0	0
	4	123	0	1	5	1

[z] Number of roots with IN symptoms; total roots per sampling time was 20.

[y] Severity of IN, referring to Figure 2.

Table 6. Incidence of IN in response to different insecticides at different sampling times, Clinton, NC, 2011 (Insecticide studies).

	IN incidence (%)					
Insecticide treatment	**9/6 (mid-harvest)**	**10/19**	**11/22**	**1/18**	**3/15**	**Avg.**
Admire Pro 10.5 fl oz /Acre A [z]	0	0	1	0	2	0.6
Belay 12 fl oz /Acre A	0	0	1	0	1	0.4
Coragen 7 fl oz /Acre A	0	0	0	0	0	0
Lorsban 64 fl oz /Acre A; Brigade 19.2 fl oz /Acre B	0	0	0	0	0	0
Mocap 60.8 fl oz /Acre A	0	0	1	0	1	0.4
Untreated Check	1	0	1	0	0	0.4
Significance [y]	NS	NS	NS	NS	NS	NS

[z] Capital letters at the end of each treatments indicated the application codes (Table1.)

[y] NS= not significant at α=0.05.

Table 7. Incidence of IN in response to different insecticides at different sampling times, Kinston, NC, 2011 (Insecticide studies).

	IN incidence (%)					
Insecticide treatment	**9/1 (mid-harvest)**	**11/1**	**11/29**	**1/17**	**3/13**	**Avg.**
Lorsban 0.5 gal/Acre A [z]	0	0	0	0	0	0
Brigade 19.2 fl oz/Acre A	0	0	0	2	0	0.4
Mocap 6.9 fl oz/1000 Row-FT B	0	2	0	0	2	0.8
Coragen 7 fl oz/Acre C	0	0	0	0	0	0
Belay 12 fl oz/Acre A	0	1	0	0	0	0.2
Admire Pro 10.5 fl oz/Acre A	1	1	0	0	0	0.4
Brigade 19.2 fl oz/Acre B	1	0	0	0	0	0.2
Brigade 19.2 fl oz/Acre D	0	1	1	0	2	0.8
Untreated check	0	0	1	0	0	0.2
Significance [y]	NS	NS	NS	NS	NS	NS

[z] Capital letters at the end of each treatments indicated the application codes (Table1.)

[y] NS= not significant at α=0.05.

Table 8. Incidence and severity of IN in response to different herbicides treatments across time (Herbicide Study).

Treatment	Incidence	Severity
Prep late	21.4 a [z]	1.3 a
Prep early	9.3 b	0.9 a
Command	0.6 b	0.3 b
Devrinol	0.4 b	0.1 b
Check (nontreated)	0.4 b	0.1 b
Valor 3	0 b	0 b
Dual Magnum 0.75 pt/A	0 b	0 b
Roundup	0 b	0 b
Dual Magnum 1.25 pt/A	0 b	0 b
Paraquat	0 b	0 b

Data generated over three sampling times (at harvest, after curing and 30 days after harvest).

[z] Comparisons were made by columns. Means with different letters are significantly different at α=0.05 using Tukey HSD.

Table 9. Incidence and severity of IN in response to different herbicides treatments in each sampling time (Herbicide Study).

	At harvest		After curing		30 days	
	Inc. %	**Severity**	**Inc. %**	**Severity**	**Inc. %**	**Severity**
Check (nontreated)	1 a [z]	0.3 a	0 b	0 b	0 b	0 b
Command	0 a	0 a	0 b	0 b	2 b	0.5 b
Devrinol	1 a	0.3 a	0 b	0 b	0 b	0 b
Dual Magnum 0.75 pt/Acre	0 a	0 a	0 b	0 b	0 b	0 b
Dual Magnum 1.25 pt/Acre	0 a	0 a	0 b	0 b	0 b	0 b
Paraquat	0 a	0 a	0 b	0 b	0 b	0 b
Prep early	0 a	0 a	13 ab	0.9 ab	15 b	1.7 a
Prep late	0 a	0 a	29 a	1.5 a	35 a	2.4 a
Roundup	0 a	0 a	0 b	0 b	0 b	0 b
Valor 3	0 a	0 a	0 b	0 b	0 b	0 b

[z] Comparisons were made by columns. Means with different letters are significantly different at α=0.05 using Tukey HSD.

Table 10. Relationship between Prep application and the percentage (%) and development of IN (Prep Study).

					Study 1		Study 2	
Sampl -ing #	**DAPA [z]**	**Days being cured**	**Days being stored**	**DBH (-) or DAH (+) [y]**	**No Prep**	**Prep**	**No Prep**	**Prep**
1	4	0	0	-11	0.0 a [x]	0.0 e	0.0 a	0.0 b
2	9	0	0	-6	0.0 a	0.0 e	0.0 a	1.7 b
3	15	0	0	0	0.0 a	3.3 de	0.0 a	1.7 b
4	18	3	0	+3	0.0 a	1.7 e	0.0 a	1.7 b
5	21	6	0	+6	0.0 a	18.3 cde	0.0 a	10.0 ab
6	23	7	1	+8	1.7 a	26.7 bcd	1.7 a	25.0 ab
7	30	7	8	+15	0.0 a	28.3 bc	8.3 a	40.0 a
8	35	7	13	+20	0.0 a	48.3 ab	5.0 a	26.7 ab
9	45	7	23	+30	3.3 a	66.7 a	10.0 a	40.0 a

[z] DAPA= days after Prep application.

[y] DBH= days before harvest; DAH= days after harvest.

[x] Comparisons were made by columns. Means with different letters are significantly different at α=0.05 using Tukey HSD.

Table 11. Anatomic image evaluation on the stages of cell stress in response to Prep application and its duration, scoring scale is from 1 to 5 [z] (Prep Study).

	DAPA [y]	No Prep	Prep	Contrast [w] (No Prep Vs Prep)
Study 1	4	2.2 b [x]	2.7 b	NS
	9	3.0 ab	3.3 ab	*
	15	2.5 b	3.2 ab	NS
	18	2.8 b	2.9 ab	NS
	21	3.7 a	3.7 a	NS
	23	2.8 b	2.7 b	NS
	Overall	2.8	3.1	
Study 2	4	3.3 a	3.5 ab	NS
	9	3.1 a	3.2 b	NS
	15	3.9 a	3.4 ab	NS
	18	3.4 a	3.9 ab	NS
	21	3.7 a	3.2 b	NS
	23	3.2 a	4.0 a	**
	Overall	3.4	3.5	

[z] Scale refers to Figure 7.

[y] DAPA= days after Prep application.

[x] Comparisons were made by columns and by study. Means with different letters are significantly different at α=0.05 using Tukey HSD.

[w] Contrasts were made between Prep and No Prep treatments (within rows) and significance was labeled as NS, * and **representing significant or not significant at P= 0.05 and 0.01 respectively.

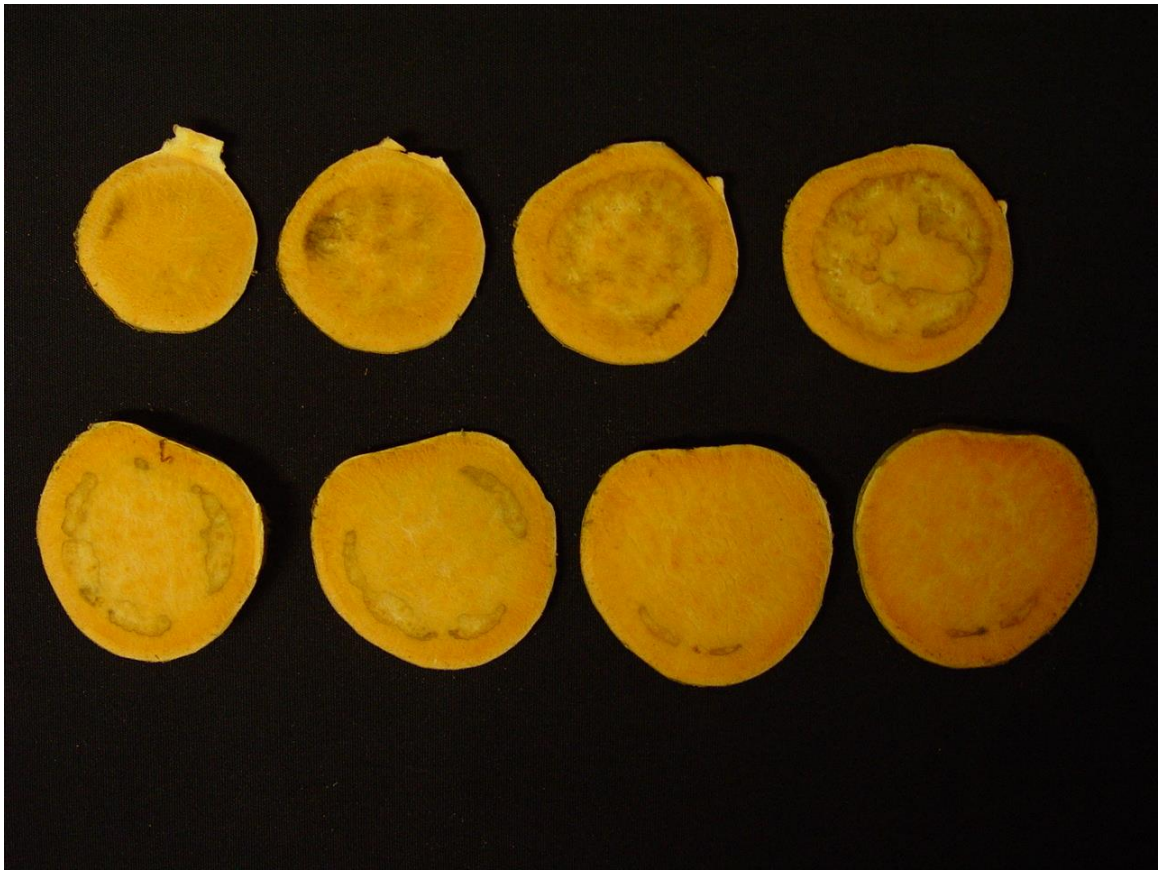


Figure 1. IN symptom from one root showing progressively worse necrosis from the stem end in the top row, and becoming less evident in the middle half of the root as shown in the bottom row.

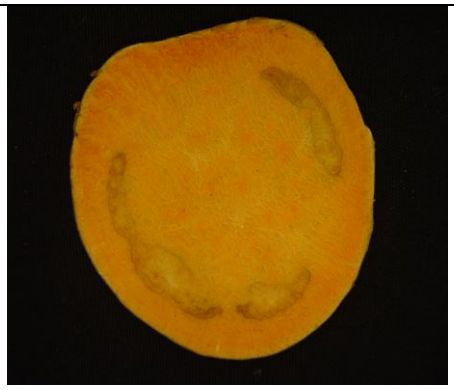
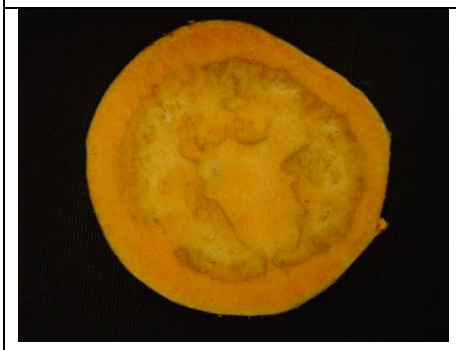
 0 = no symptom.	 1 = negligible but visible symptoms.
 2 = obvious symptoms but mainly confined to stem end.	 3 = extensive symptoms that either go deeper into the root or have multiple and larger diffuse areas of the root affected and could render roots unmarketable.
 4 = extreme symptoms that go at least 1/3 of the way into the root from the stem end and render the root unmarketable.	

Figure 2. Rating scale of IN, from 0 to 4.

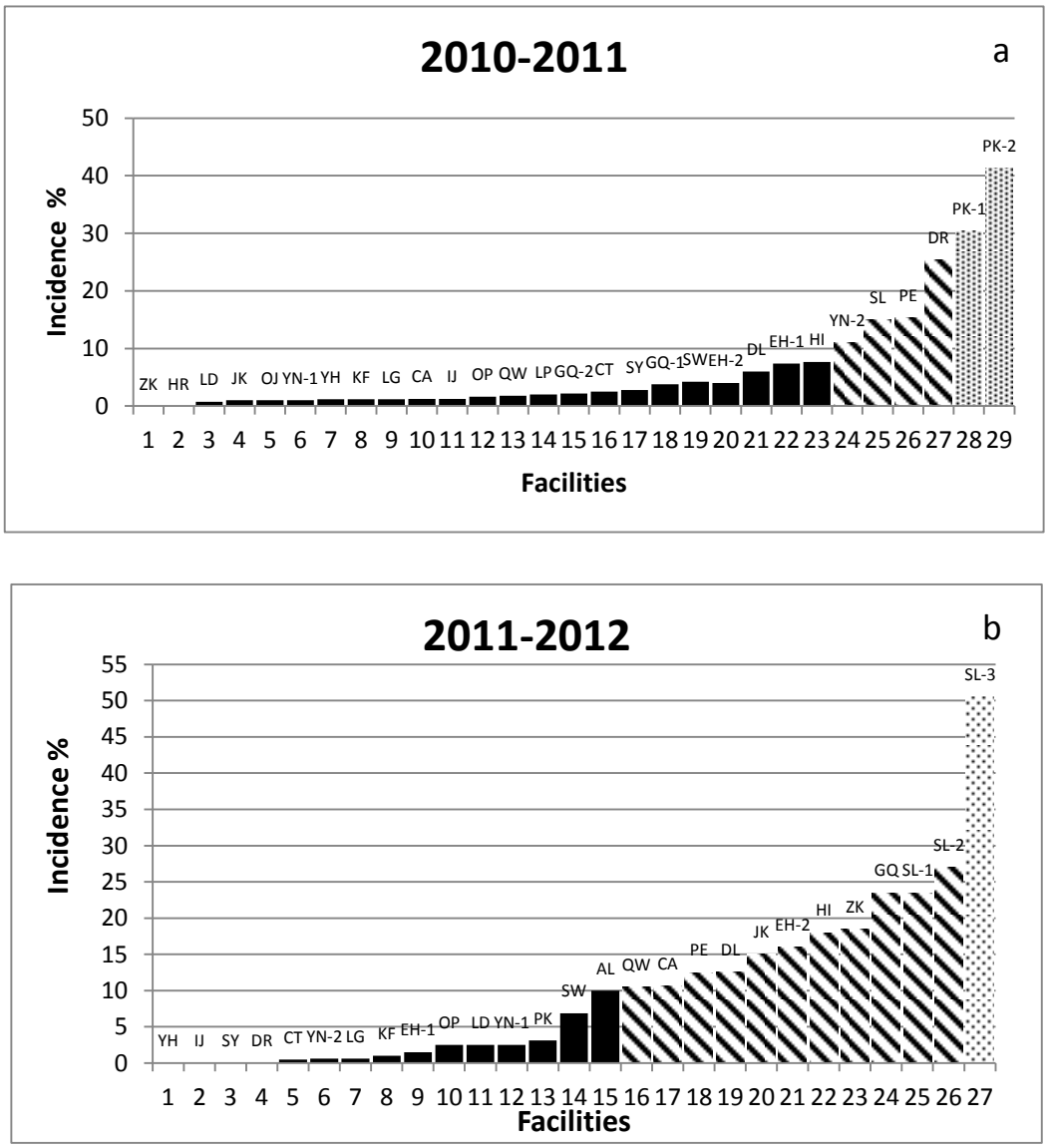


Figure 3a and 3b. Average incidence of IN for each facility in 2010-2011 and 2011-2012 surveys. Each number indicates a facility. A random two-letter code was assigned for each business/grower, for those businesses where more than one facility was sampled, a number was followed by the code. The businesses/growers shared the same code in two years were the same businesses/grower.

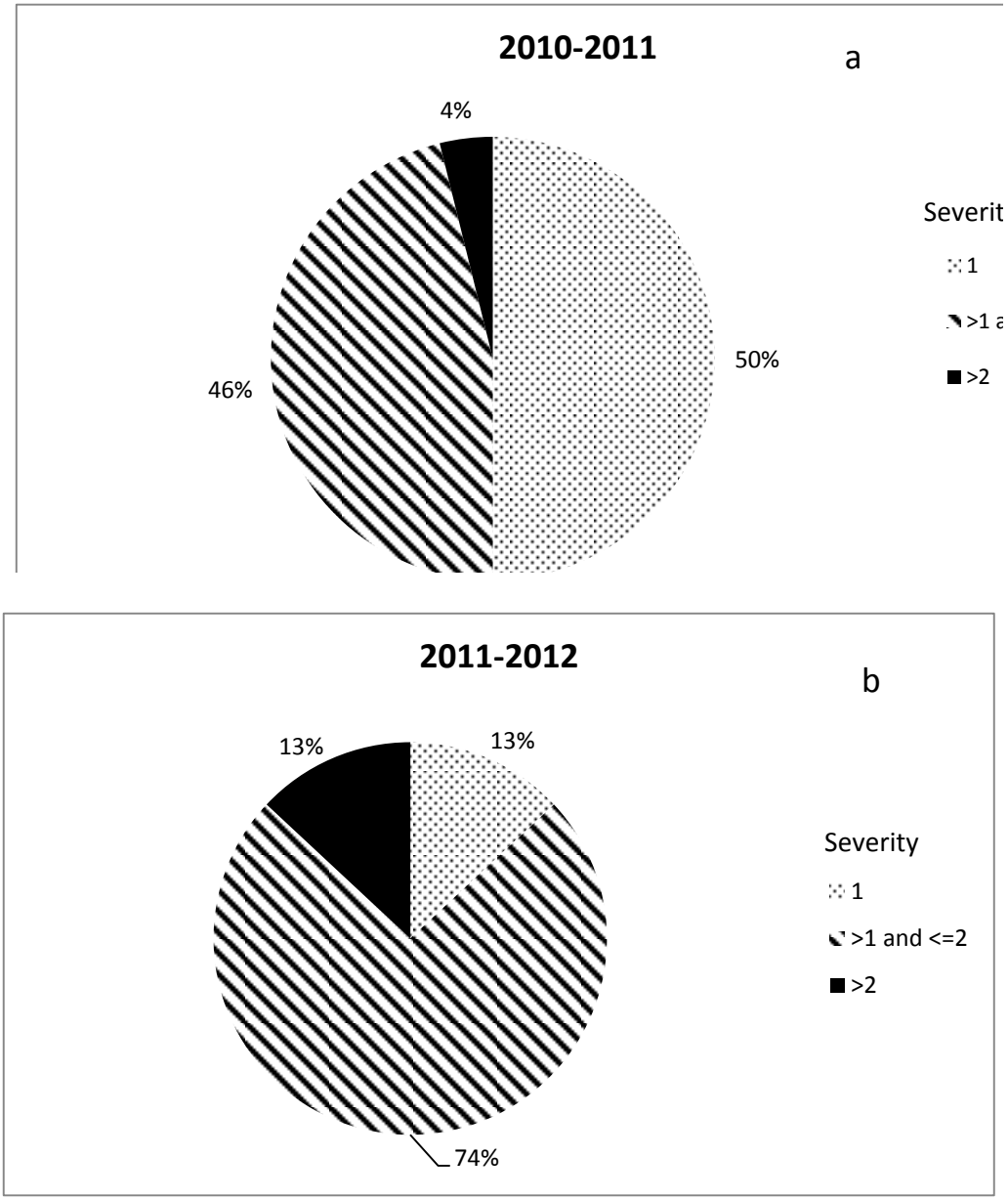


Figure 4a and 4b. Average percentage occurrence of various severity scores of all roots with IN in 2010-2011 and 2011-2012 commercial surveys.

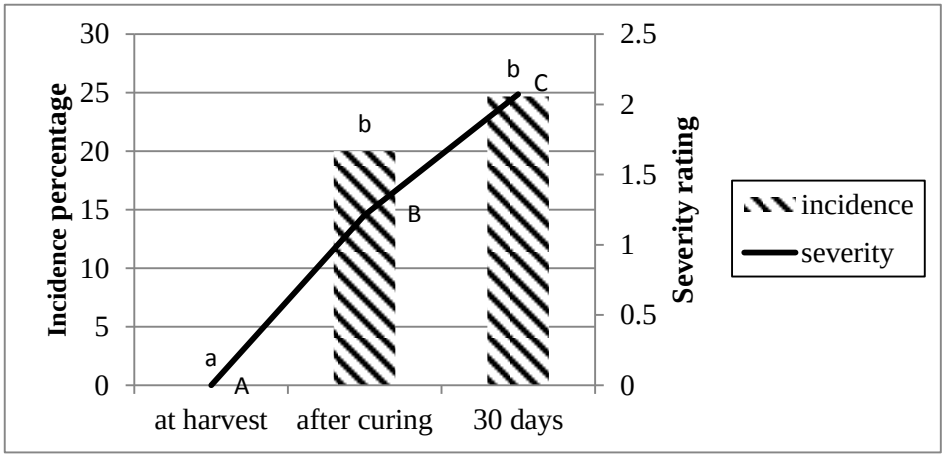
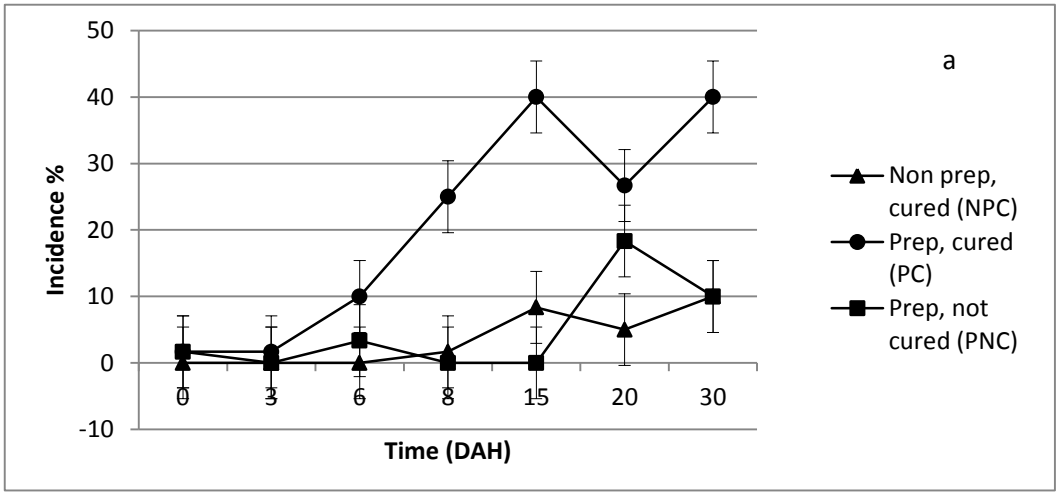


Figure 5. Effects of Prep treatments and storage time on the incidence and severity of IN, Herbicides study.

"After curing" also meant 7 days after harvest. Data were pooled between "Prep Early" and "Prep Late" treatments as treatment effect was not significant. Means were separated by Tukey HSD. Different letters are significantly different at α=0.05. Upper-case letters represent the severity, and lower-case letters represent the incidence.



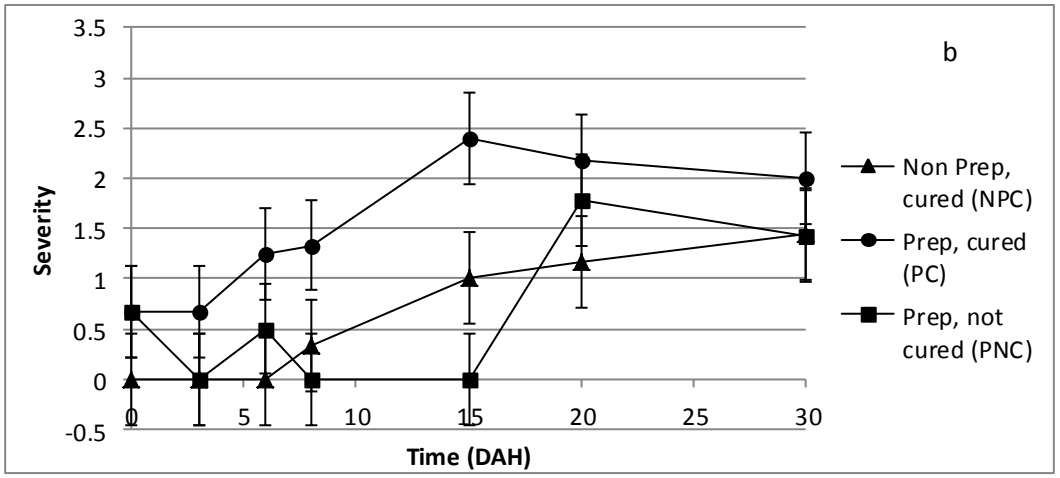


Figure 6a and 6b. Effects of curing and Prep on the incidence (a) and severity (b) of IN over time.

DAH= days after harvest. Means were separated by Tukey HSD. Data points can be compared either within treatments (lines) or within time (DAH). Data points with error bars not overlapped are significantly different at α=0.05.

Figure 7. Different stages of cells under stress induced by Prep application.

For each stage, two pictures were taken, one was under bright field (a) and the other was under fluorescence field (b).

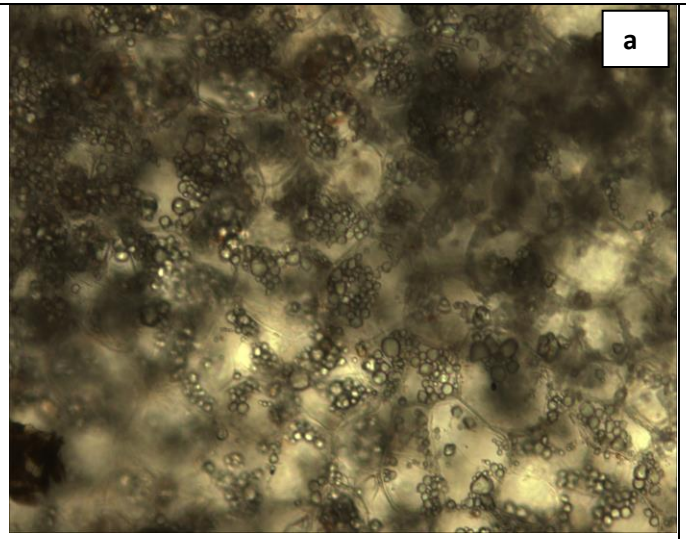
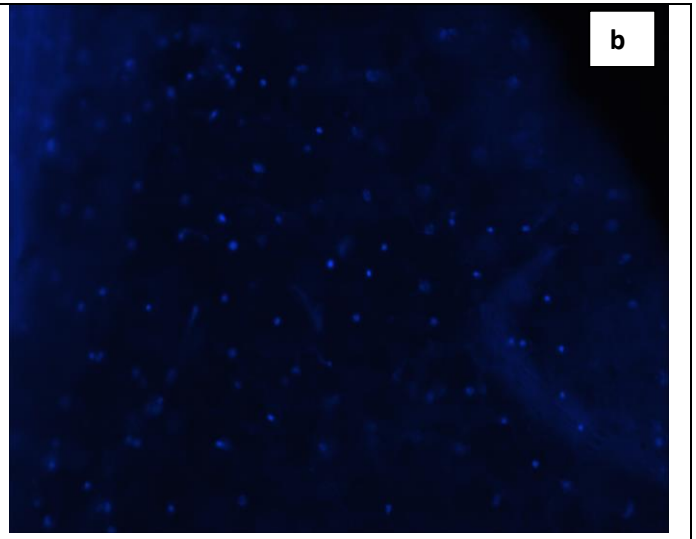
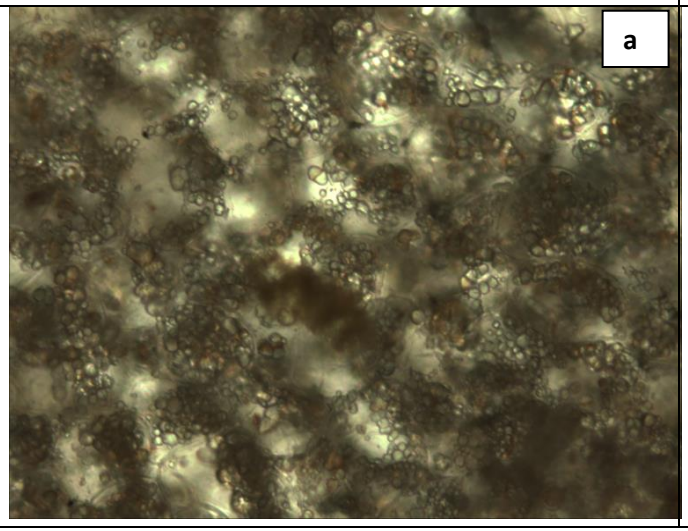
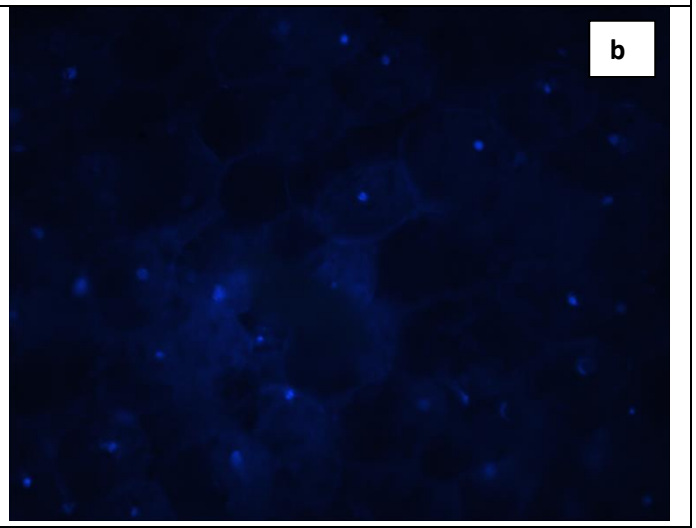
Stage 1. Cells in bright field appear healthy, clear and full of starch. Fluoresent image displayes bright and distinguished nuclei with abundant amount; they are labled by DAPI, a fluorescent dye that combines DNA. No other parts of the cell can be clearly seen in the field beside the nuclei.

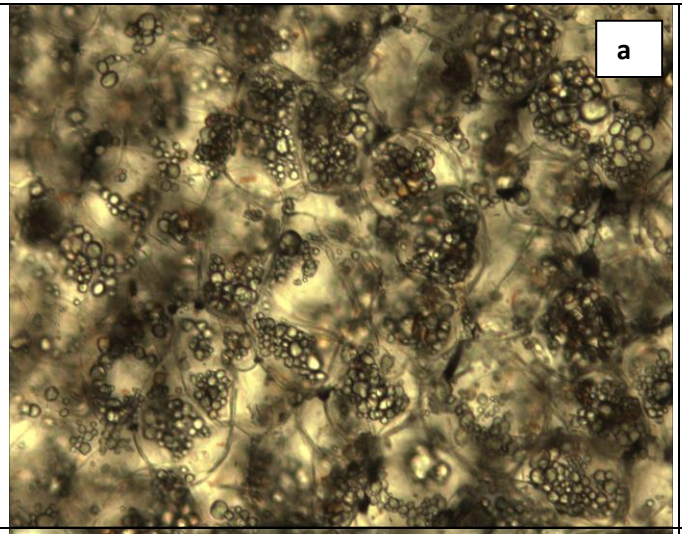
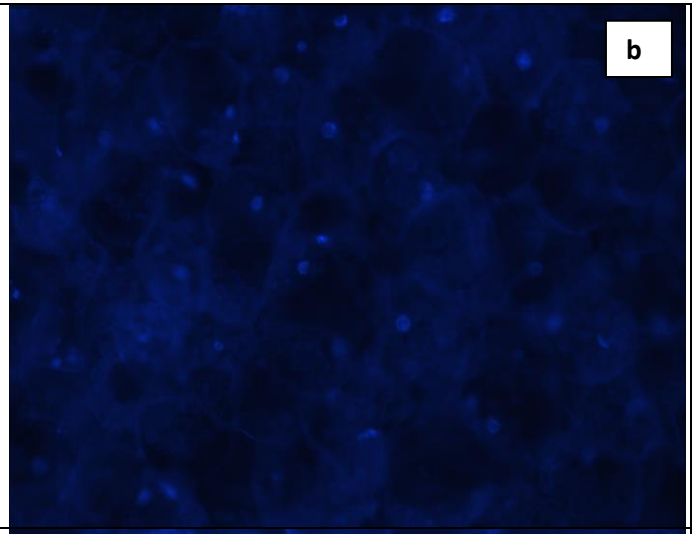
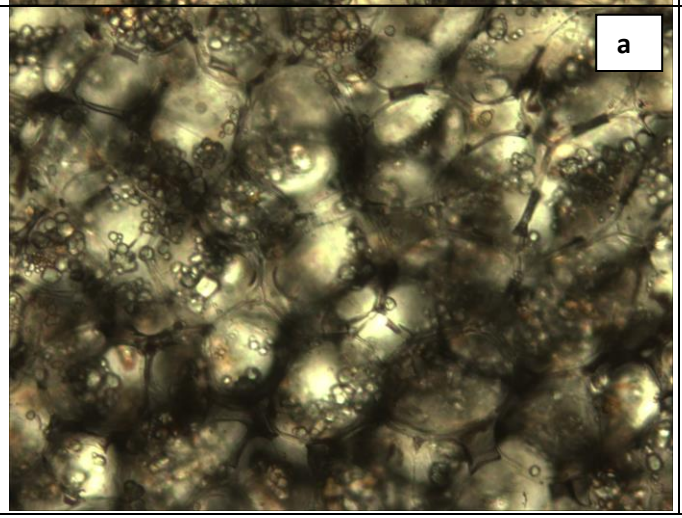
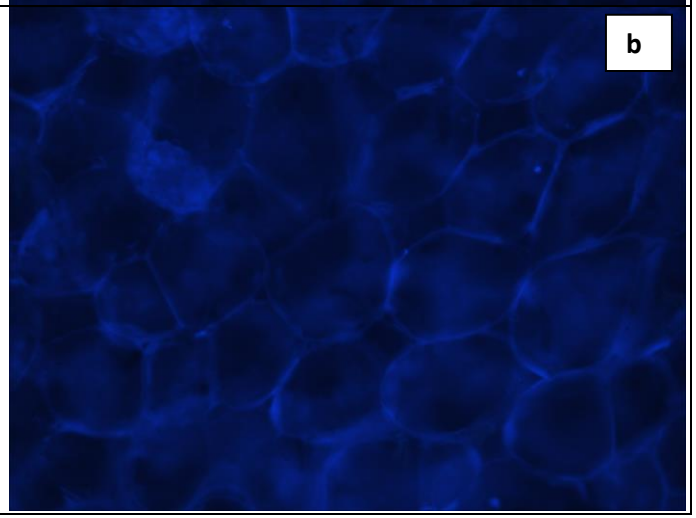
Stage 2. Cells in bright field appear as healthy as stage 1, which are full of starch. Fluoresent image displayes fluorescent nuclei that are bright and distinguished, and nuclei are less than on stage 1. Some other parts of cells (such as plastid and cell wall) show fluorescence too, which seems to be the autofluorescence from cell material itself instead of from DAPI, as DAPI only labels the DNA structure.

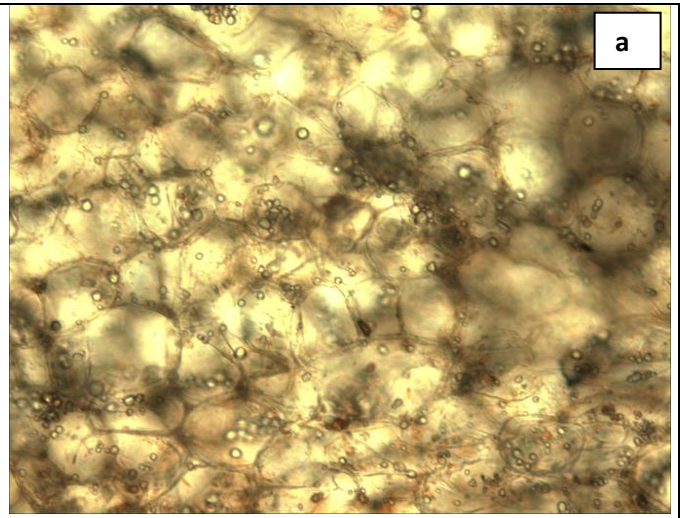
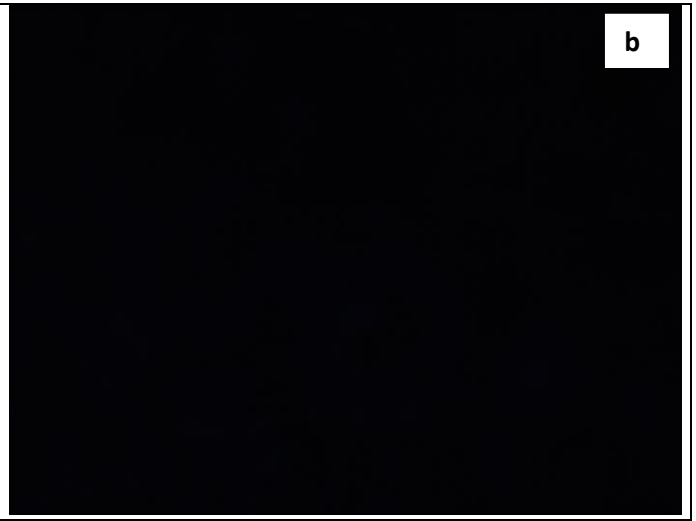
Stage 3. Cells in bright field still appear healthy. Fluoresent image displayes nuclei but they look blurry, which may indicate the break down of nuclei. Abundant and clear autofluorescence from other cell parts (plastid and cell wall) can be seen, which indicate the cells seem to be under stress.

Stage 4. Cells in bright field have lost some of the starch; darker areas along cell walls are suspected to be an artificial effect of air within the intercellular space. Fluorescent image rarely displays any fluorescent nuclei, only autofluorescent plastids and cell walls can be seen.

Stage 5. Cells in bright field are depleted of starch and cell structure are getting collapsed. Nothing can be seen in the fluoresent image.

Stage 1		
Stage 2		

Stage 3		
Stage 4		

Stage 5		

APPENDICES

Appendix A. Chapter I

Table 1. Type 3 Analysis of Variance for node production

Source	Expected Mean Square	Error Term
Trt [z]	Var(Residual) + 9 Var(rep*trt*variety*year) + Q(trt,trt*variety,trt*eml,trt*variety*eml)	MS(rep*trt*variety*year)
Var [y]	Var(Residual) + 9 Var(rep*trt*variety*year) + Q(variety,trt*variety,variety*eml,trt*variety*eml)	MS(rep*trt*variety*year)
Trt*var	Var(Residual) + 9 Var(rep*trt*variety*year) + Q(trt*variety,trt*variety*eml)	MS(rep*trt*variety*year)
Eml [x]	Var(Residual) + Q(eml,trt*eml,variety*eml,trt*variety*eml)	MS(Residual)
Trt*eml	Var(Residual) + Q(trt*eml,trt*variety*eml)	MS(Residual)
Var*eml	Var(Residual) + Q(variety*eml,trt*variety*eml)	MS(Residual)
Trt*var*eml	Var(Residual) + Q(trt*variety*eml)	MS(Residual)
Year	Var(Residual) + 9 Var(rep*trt*variety*year) + 135 Var(rep*year) + 540 Var(year)	MS(rep*year)
Rep(year)	Var(Residual) + 9 Var(rep*trt*variety*year) + 135 Var(rep*year)	MS(rep*trt*variety*year)
Rep*trt*var (year)	Var(Residual) + 9 Var(rep*trt*variety*year)	MS(Residual)
Residual	Var(Residual)	.

[z] Trt- fertilizer rate

[y] Var- variety

[x] Eml- harvest time

Table 2. **2011** Soilless media nutrient analysis (parts per million except for EC and pH)

	N Rate (ppm)	NO3-N	NH4-N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Na	Cl	EC (ms/m)	pH
Beau	50	0.3	0.4	4.7	1.8	7.1	5.5	51.5	1.4	0.0	0.0	0.1	0.0	80.4	11.9	46.8	5.1
	100	0.6	0.3	11.9	3.8	6.0	4.2	44.7	2.4	0.0	0.1	0.1	0.0	76.5	8.2	43.0	4.5
	200	38.0	23.6	37.8	47.5	12.0	8.3	44.8	5.9	0.2	0.1	0.2	0.2	95.4	30.9	95.8	4.2
	300	83.4	42.1	45.5	132.3	12.0	9.0	46.3	9.7	0.1	0.2	0.3	0.2	111.7	41.9	124.3	4.3
	400	196.0	107.0	88.1	274.8	21.5	20.9	47.0	10.1	0.5	0.4	0.5	0.5	100.5	36.4	281.8	4.3
	200 Leach	19.1	4.4	29.9	19.0	12.2	8.9	65.6	4.4	0.1	0.1	0.2	0.1	127.2	15.5	81.5	4.6
	300 Leach	32.7	11.7	39.4	33.9	10.4	6.5	32.2	4.3	0.1	0.1	0.1	0.1	90.1	19.5	76.0	4.2
	400 Leach	89.3	51.9	55.8	96.3	15.6	10.4	33.8	7.2	0.3	0.1	0.2	0.2	98.1	30.5	143.5	4.1
Cov	50	0.6	0.6	5.4	3.8	8.4	6.3	54.2	1.4	0.0	0.0	0.1	0.0	85.0	12.0	49.8	5.1
	100	3.8	0.6	14.2	8.7	5.2	3.6	40.0	2.4	0.0	0.1	0.1	0.0	78.1	10.8	44.3	5.0
	200	74.5	76.5	42.2	105.8	13.5	9.2	44.1	6.4	0.2	0.1	0.1	0.1	89.7	31.2	134.3	4.2
	300	145.7	71.5	66.3	232.5	19.8	17.8	47.2	5.3	0.3	0.2	0.3	0.2	86.8	32.2	220.5	4.5
	400	164.0	85.7	86.2	278.3	13.6	12.4	33.0	5.1	0.4	0.3	0.4	0.4	60.7	22.7	260.0	4.6
	200 Leach	29.8	11.2	27.6	34.7	8.8	6.1	43.7	3.2	0.1	0.1	0.1	0.0	95.3	15.0	76.7	4.7
	300 Leach	68.9	28.9	36.0	101.5	12.5	8.6	33.7	4.4	0.2	0.1	0.1	0.1	80.7	27.3	116.8	4.1
	400 Leach	129.0	67.3	65.0	180.0	15.8	11.2	36.5	6.8	0.3	0.2	0.2	0.2	94.4	28.4	188.3	4.2
Evan	50	0.2	0.3	6.5	3.2	10.3	7.9	50.9	1.2	0.0	0.0	0.1	0.0	86.5	25.6	53.0	5.0
	100	3.6	0.5	19.6	8.9	9.7	6.1	47.9	2.6	0.0	0.1	0.1	0.0	92.3	21.5	55.3	4.7
	200	83.2	35.2	31.5	80.7	28.9	16.9	53.4	7.0	0.3	0.1	0.1	0.2	123.6	52.3	150.0	4.1
	300	97.6	51.0	27.5	127.0	11.5	8.0	27.4	4.8	0.2	0.1	0.2	0.2	67.6	29.8	143.8	4.2
	400	183.5	91.4	77.5	261.5	17.7	15.2	36.4	6.7	0.4	0.3	0.4	0.5	72.8	27.1	263.0	4.3
	200 Leach	41.2	7.9	29.4	30.9	16.1	11.4	48.3	2.9	0.1	0.1	0.1	0.1	122.9	31.8	94.3	4.7
	300 Leach	58.7	23.1	35.1	66.0	19.9	11.5	42.6	4.7	0.2	0.1	0.1	0.1	103.1	41.3	113.8	4.1
	400 Leach	122.5	63.2	37.2	139.0	15.1	9.2	29.0	6.7	0.3	0.1	0.2	24.3	78.2	68.1	132.5	4.1

Table 3. **2012** Soilless media nutrient analysis (parts per million except for EC and pH)

	N Rate (ppm)	NO3-N	NH4-N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Na	Cl	EC (ms/m)	pH
Beau	50	5.9	3.0	12.6	5.5	7.5	5.6	65.9	0.7	0.0	0.1	0.1	0.1	105.8	9.2	57.3	5.8
	100	30.2	2.1	34.8	29.1	11.1	7.9	45.3	2.2	0.1	0.2	0.2	0.2	125.0	16.6	79.0	5.2
	200	171.0	58.6	57.9	230.6	34.8	30.3	28.6	6.3	0.4	0.4	0.3	0.3	109.7	37.3	209.8	4.7
	300	340.5	98.5	109.8	478.7	47.1	41.6	30.8	10.4	0.7	1.2	0.7	0.5	132.9	40.9	385.8	4.7
	400	332.6	136.6	124.2	476.8	27.8	25.1	22.5	5.8	0.5	0.6	0.4	0.6	72.0	23.6	386.8	4.7
	200 Leach	35.3	3.6	23.7	43.7	9.5	6.8	23.1	1.7	0.1	0.1	0.1	0.1	83.9	21.8	64.3	5.1
	300 Leach	66.7	9.0	33.8	106.3	14.5	10.8	23.8	3.8	0.2	0.3	0.2	0.2	89.6	26.4	101.5	4.6
	400 Leach	119.7	55.4	56.3	190.6	15.2	11.8	21.4	4.2	0.3	0.1	0.1	0.3	76.5	26.3	158.3	4.6
Cov	50	4.5	1.4	11.8	12.5	7.2	4.9	60.8	0.8	0.0	0.1	0.1	0.0	102.2	9.4	55.5	5.7
	100	58.3	9.7	45.3	89.3	14.9	11.2	72.4	3.1	0.1	0.7	0.4	0.1	163.1	24.6	127.3	5.7
	200	219.9	65.4	97.5	339.4	45.6	39.0	56.3	7.8	0.6	1.2	0.8	0.3	134.2	35.1	283.0	4.8
	300	308.7	92.2	124.3	468.7	46.3	38.0	34.6	7.1	0.9	0.8	0.5	0.5	105.3	28.0	360.0	4.6
	400	409.4	158.2	155.8	591.1	45.5	39.1	30.5	6.6	0.9	0.8	0.4	0.7	88.3	28.9	466.8	4.7
	200 Leach	56.7	16.3	29.0	108.0	11.5	8.6	30.5	1.5	0.1	0.1	0.1	0.1	86.0	22.0	89.0	5.2
	300 Leach	94.8	9.1	45.1	173.3	18.6	13.7	34.8	3.7	0.2	0.2	0.2	0.1	98.9	24.0	135.5	4.8
	400 Leach	184.5	71.1	90.6	311.3	23.0	17.6	35.6	5.5	0.5	0.4	0.3	0.3	99.0	26.7	235.8	4.8
Evan	50	5.8	2.5	12.5	8.5	10.1	6.8	58.8	0.6	0.0	0.1	0.0	0.1	104.3	26.3	58.5	5.7
	100	69.7	1.2	31.9	52.1	28.9	21.3	51.7	1.2	0.1	0.2	0.1	0.2	159.0	41.3	119.5	5.2
	200	264.6	64.5	61.1	352.1	59.6	46.0	45.1	8.1	0.7	0.6	0.4	0.4	137.9	53.6	307.3	4.6
	300	281.9	90.5	64.6	391.2	34.2	27.0	22.0	5.5	0.6	0.3	0.2	0.5	81.4	30.4	313.5	4.5
	400	410.4	149.7	124.7	551.0	51.0	42.4	24.8	6.0	1.0	0.5	0.3	0.7	84.2	29.5	451.8	4.6
	200 Leach	71.1	22.6	22.1	98.8	23.7	16.3	30.9	2.2	0.2	0.1	0.1	0.2	100.2	46.8	107.5	4.9
	300 Leach	124.8	9.1	25.2	176.1	30.5	20.7	25.7	4.0	0.4	0.2	0.1	0.2	108.0	42.6	152.3	4.5
	400 Leach	159.9	61.6	37.7	232.6	19.3	13.8	18.5	3.1	0.4	0.1	0.1	0.3	75.0	27.3	189.0	4.5

Table 4. **2011** tissue sample nutrient analysis in the **early** season.

Early 2011	**N rate (ppm)**	**N %**	**P %**	**K %**	**Ca %**	**Mg %**	**S %**	**Fe (ppm)**	**Mn (ppm)**	**Zn (ppm)**	**Cu (ppm)**	**B (ppm)**	**Na %**
Beau													
	50	5.2	0.6	4.1	0.89	0.72	0.42	73	195	47	7	39	0.03
	100	6.4	0.8	4.4	0.89	0.72	0.42	72	153	52	8	46	0.03
	200	7.3	1.4	5.2	1.08	0.84	0.42	71	213	57	16	52	0.03
	300	7.2	1.4	5.0	0.88	0.66	0.42	80	165	52	13	49	0.03
	400	7.2	1.6	5.3	0.93	0.69	0.39	72	164	51	14	48	0.03
	200 Leach	6.8	1.1	4.5	1.00	0.78	0.39	74	159	51	9	46	0.03
	300 Leach	7.0	1.6	5.2	1.08	0.78	0.4	89	177	56	11	49	0.03
	400 Leach	7.0	1.5	5.1	0.98	0.74	0.37	75	137	45	9	45	0.02
Cov													
	50	5.1	0.5	3.8	0.7	0.64	0.39	69	111	42	7	32	0.06
	100	6.3	0.8	4.4	0.77	0.69	0.4	161	106	46	5	37	0.05
	200	6.9	1.2	5.0	0.9	0.74	0.37	71	130	44	8	43	0.06
	300	7.2	1.3	4.5	0.75	0.62	0.41	67	129	52	11	47	0.04
	400	6.9	1.1	4.3	0.71	0.6	0.36	62	108	47	10	45	0.04
	200 Leach	6.8	1.2	4.6	0.88	0.77	0.39	86	116	41	6	39	0.06
	300 Leach	7.1	1.3	5.1	0.85	0.71	0.36	62	94	45	7	39	0.05
	400 Leach	7.1	1.2	4.7	0.81	0.68	0.39	72	95	47	9	40	0.05
Evan													
	50	4.8	0.5	3.9	0.78	0.69	0.37	82	163	44	9	30	0.03
	100	6.4	0.8	4.1	0.64	0.66	0.36	59	115	43	6	32	0.03
	200	6.9	1.4	4.6	0.73	0.72	0.4	69	151	58	10	37	0.03
	300	6.8	1.5	4.4	0.65	0.63	0.39	73	136	55	11	38	0.02
	400	6.8	1.8	4.9	0.79	0.73	0.41	81	154	64	17	45	0.03
	200 Leach	7.0	1.2	4.3	0.75	0.71	0.41	93	134	56	9	37	0.03
	300 Leach	6.9	1.7	4.8	0.84	0.79	0.4	81	149	55	11	40	0.03
	400 Leach	6.7	1.6	4.8	0.84	0.76	0.36	79	124	79	13	42	0.04

Table 5. **2011** tissue sample nutrient analysis in the **middle** season.

	N rate (ppm)	N %	P %	K %	Ca %	Mg %	S %	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)	Na %
Beau													
	50	5.2	0.6	3.8	0.8	0.67	0.49	90	92	53	12	57	0.03
	100	4.9	0.7	3.9	0.97	0.77	0.46	90	133	38	15	54	0.05
	200	6.2	1.0	5.3	0.83	0.63	0.4	131	152	43	19	60	0.05
	300	6.5	1.1	5.1	0.78	0.57	0.42	91	171	50	23	70	0.06
	400	7.1	1.4	5.2	0.72	0.43	0.44	116	214	56	25	79	0.03
	200 Leach	5.8	0.7	3.8	0.96	0.73	0.49	93	135	47	14	58	0.04
	300 Leach	6.0	0.9	5.0	0.86	0.64	0.43	92	158	45	19	62	0.05
	400 Leach	6.2	1.1	5.6	0.78	0.56	0.4	167	194	50	24	64	0.04
Cov													
	50	5.1	0.6	3.9	0.67	0.58	0.44	77	65	49	12	39	0.08
	100	5.6	0.7	3.8	0.65	0.52	0.43	97	92	45	13	44	0.15
	200	6.4	1.0	4.5	0.63	0.48	0.4	106	114	55	19	55	0.09
	300	6.4	1.0	4.6	0.62	0.46	0.38	109	120	50	20	57	0.08
	400	7.1	1.2	4.8	0.49	0.39	0.42	108	150	56	23	58	0.09
	200 Leach	5.4	0.7	3.8	0.7	0.58	0.46	95	94	49	12	44	0.13
	300 Leach	6.2	0.9	4.6	0.71	0.57	0.45	116	113	57	18	54	0.11
	400 Leach	6.8	1.0	4.9	0.58	0.45	0.4	101	133	60	20	59	0.08
Evan													
	50	4.7	0.6	4.0	0.67	0.64	0.41	94	100	40	11	40	0.02
	100	5.3	0.8	4.4	0.81	0.73	0.44	81	144	45	18	45	0.03
	200	6.2	1.0	4.7	0.71	0.63	0.4	184	178	51	21	46	0.03
	300	6.1	1.3	5.0	0.69	0.66	0.38	317	263	54	22	49	0.03
	400	6.5	1.5	4.7	0.63	0.52	0.39	238	310	55	25	53	0.02
	200 Leach	5.3	0.7	4.4	0.83	0.72	0.46	88	138	44	16	47	0.03
	300 Leach	6.2	1.0	5.4	0.73	0.63	0.41	130	159	50	23	48	0.03
	400 Leach	6.5	1.1	5.3	0.63	0.57	0.39	235	238	57	24	47	0.02

Table 6. **2011** tissue sample nutrient analysis in the **late** season.

	N rate (ppm)	N %	P %	K %	Ca %	Mg %	S %	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)	Na %
Beau													
	50	3.6	0.3	2.6	1.13	0.76	0.29	95	211	33	15	50	0.04
	100	5.2	0.4	2.7	0.89	0.64	0.39	118	152	42	19	50	0.10
	200	7.0	1.1	4.3	0.56	0.49	0.45	110	159	55	21	54	0.14
	300	7.1	1.2	4.9	0.51	0.45	0.45	180	200	54	26	62	0.06
	400	7.6	1.5	4.8	0.36	0.36	0.41	116	246	59	30	63	0.04
	200 Leach	6.5	0.7	3.2	0.85	0.63	0.48	99	144	50	19	55	0.14
	300 Leach	6.9	0.8	4.2	0.69	0.56	0.51	138	185	51	22	63	0.09
	400 Leach	7.3	1.0	4.4	0.55	0.46	0.43	113	185	59	22	68	0.10
	50	4.3	0.4	2.8	0.99	0.73	0.37	100	156	37	15	46	0.18
	100	5.0	0.4	3.1	0.83	0.67	0.42	109	207	42	19	50	0.04
	200	6.8	1.0	4.2	0.68	0.58	0.46	147	176	55	23	61	0.08
	300	7.3	1.1	4.8	0.43	0.41	0.43	141	190	54	27	52	0.03
	400	7.5	1.3	4.8	0.44	0.4	0.43	267	350	60	28	72	0.03
	200 Leach	6.5	0.7	3.4	0.79	0.62	0.47	124	135	46	18	54	0.14
	300 Leach	6.7	0.8	4.2	0.67	0.53	0.47	122	175	58	22	60	0.12
	400 Leach	7.1	1.0	4.5	0.58	0.5	0.45	161	239	56	24	62	0.05
Evan													
	50	3.5	0.3	2.8	1.14	0.78	0.32	92	212	33	14	53	0.05
	100	5.0	0.5	3.1	0.84	0.6	0.43	128	148	48	19	49	0.21
	200	7.0	0.9	4.1	0.71	0.54	0.46	144	165	58	22	72	0.15
	300	7.3	1.2	4.4	0.45	0.38	0.41	153	176	55	28	61	0.10
	400	8.0	1.2	4.9	0.45	0.35	0.45	150	213	62	27	89	0.05
	200 Leach	6.3	0.6	3.4	0.84	0.64	0.43	117	177	48	19	48	0.06
	300 Leach	6.7	0.8	4.3	0.59	0.52	0.46	116	166	56	22	55	0.07
	400 Leach	7.2	1.1	4.4	0.51	0.44	0.42	135	191	65	23	60	0.09

Table 7. **2012** tissue sample nutrient analysis in the **early** season.

	N rate (ppm)	N %	P %	K %	Ca %	Mg %	S %	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)	Na %
Beau	50	6.8	0.9	4.9	0.77	0.66	0.48	100	18	5	1	49	0.02
	100	7.4	1.3	5.3	0.79	0.68	0.50	93	20	6	1	54	0.03
	200	7.4	1.5	5.2	0.75	0.61	0.49	96	18	5	2	55	0.03
	300	7.6	1.5	5.3	0.71	0.58	0.48	91	22	5	2	55	0.03
	400	7.3	1.6	5.3	0.77	0.58	0.46	92	21	5	2	66	0.03
	200 Leach	7.2	1.3	5.9	0.76	0.64	0.53	74	19	6	2	55	0.03
	300 Leach	7.5	1.4	5.8	0.77	0.61	0.50	86	21	6	2	56	0.03
	400 Leach	7.5	1.6	5.8	0.73	0.59	0.52	81	23	5	2	61	0.03
Cov													
	50	6.7	0.8	4.9	0.74	0.68	0.45	76	12	5	1	38	0.04
	100	6.4	0.9	4.5	0.62	0.60	0.44	80	12	5	1	39	0.04
	200	7.3	1.2	4.8	0.60	0.57	0.44	88	15	5	2	42	0.04
	300	7.4	1.1	4.8	0.59	0.52	0.41	85	13	5	2	43	0.04
	400	7.6	1.2	4.6	0.60	0.52	0.43	76	15	5	2	53	0.03
	200 Leach	7.3	1.0	4.9	0.53	0.52	0.45	74	10	6	1	35	0.04
	300 Leach	7.4	1.1	5.4	0.59	0.53	0.46	72	14	6	2	37	0.04
	400 Leach	7.7	1.2	5.2	0.59	0.54	0.45	69	13	5	2	42	0.04
Evan													
	50	6.6	0.9	4.8	0.65	0.69	0.48	79	19	6	1	34	0.03
	100	7.3	1.2	5.1	0.59	0.63	0.44	71	15	6	1	33	0.03
	200	7.3	1.6	5.1	0.64	0.65	0.43	74	19	6	2	38	0.02
	300	6.9	1.7	4.9	0.67	0.63	0.44	86	19	6	2	40	0.02
	400	7.3	1.8	4.8	0.70	0.66	0.44	87	22	5	2	47	0.03
	200 Leach	7.1	1.3	5.0	0.61	0.65	0.47	62	18	6	2	34	0.03
	300 Leach	7.2	1.4	5.1	0.56	0.56	0.47	64	17	8	2	33	0.03
	400 Leach	7.2	1.3	4.6	0.52	0.51	0.44	63	15	5	2	32	0.02

Table 8. **2012** tissue sample nutrient analysis in the **middle** season.

	N rate (ppm)	N %	P %	K %	Ca %	Mg %	S %	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)	Na %
Beau	50	6.1	0.6	3.6	1.18	0.90	0.52	94	21	4	2	74	0.04
	100	7.2	0.8	3.9	0.90	0.69	0.46	107	14	4	2	67	0.04
	200	8.0	1.2	5.6	0.77	0.49	0.45	105	24	5	3	82	0.02
	300	8.6	1.3	5.3	0.62	0.42	0.48	93	28	6	3	97	0.03
	400	8.8	1.3	5.0	0.52	0.38	0.44	97	26	6	3	100	0.03
	200 Leach	7.4	0.8	4.4	0.85	0.64	0.44	97	15	5	2	66	0.04
	300 Leach	8.0	1.1	5.2	0.72	0.46	0.43	89	20	5	3	78	0.03
	400 Leach	8.4	1.0	5.5	0.55	0.38	0.45	90	22	5	3	72	0.03
Cov													
	50	6.0	0.6	3.6	0.78	0.68	0.52	94	13	5	1	57	0.05
	100	7.1	0.8	4.1	0.65	0.58	0.46	81	7	5	2	57	0.09
	200	8.2	0.9	4.3	0.58	0.46	0.44	100	12	6	4	67	0.05
	300	8.4	0.9	4.6	0.45	0.40	0.42	96	14	7	4	87	0.05
	400	9.2	1.1	4.9	0.44	0.38	0.43	122	17	7	3	116	0.05
	200 Leach	7.5	0.8	4.3	0.57	0.50	0.40	92	8	5	2	55	0.09
	300 Leach	7.7	0.9	4.4	0.55	0.44	0.42	93	13	6	3	76	0.05
	400 Leach	8.0	0.9	4.0	0.46	0.38	0.43	97	14	6	3	72	0.04
Evan													
	50	6.3	0.8	4.1	1.07	0.90	0.54	98	24	5	2	64	0.03
	100	7.4	0.9	4.4	0.80	0.68	0.45	93	13	5	2	51	0.03
	200	7.7	1.6	5.3	0.74	0.57	0.49	95	26	6	4	71	0.02
	300	8.0	1.8	5.2	0.61	0.49	0.48	106	33	7	4	69	0.03
	400	8.7	1.7	5.0	0.47	0.40	0.47	94	28	6	4	78	0.02
	200 Leach	7.2	0.9	4.9	0.74	0.64	0.43	100	14	5	3	51	0.03
	300 Leach	7.8	1.4	5.0	0.66	0.49	0.45	86	22	6	3	61	0.03
	400 Leach	7.9	1.3	5.1	0.50	0.40	0.47	75	22	6	3	59	0.03

Table 9. **2012** tissue sample nutrient analysis in the **late** season.

	N rate (ppm)	N %	P %	K %	Ca %	Mg %	S %	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)	Na %
Beau													
	50	4.9	0.5	2.8	1.02	0.77	0.49	82	127	33	13	53	0.03
	100	6.6	0.8	3.7	0.95	0.66	0.51	106	129	46	22	65	0.05
	200	7.2	1.2	4.7	0.71	0.45	0.52	121	195	55	29	79	0.03
	300	7.6	1.4	5.0	0.61	0.37	0.47	144	191	63	35	101	0.04
	400	7.7	1.4	4.8	0.46	0.33	0.46	131	224	68	30	99	0.02
	200 Leach	6.5	0.8	3.6	0.72	0.52	0.56	112	136	52	25	70	0.04
	300 Leach	7.1	1.0	5.0	0.68	0.53	0.50	138	188	57	29	78	0.05
	400 Leach	7.5	1.2	4.6	0.69	0.41	0.48	125	224	58	28	82	0.03
Cov													
	50	5.2	0.6	3.1	0.91	0.70	0.53	100	148	43	14	50	0.09
	100	6.6	0.8	3.7	0.69	0.56	0.48	120	98	52	22	53	0.14
	200	7.2	1.1	4.4	0.59	0.46	0.49	161	146	64	37	79	0.15
	300	7.3	1.1	4.7	0.49	0.35	0.47	151	171	69	33	91	0.08
	400	7.8	1.3	4.7	0.37	0.31	0.42	153	153	63	29	##	0.09
	200 Leach	6.7	0.9	4.0	0.66	0.53	0.57	132	132	63	29	68	0.12
	300 Leach	7.0	1.1	4.3	0.70	0.51	0.51	166	180	62	30	80	0.12
	400 Leach	7.4	1.1	4.5	0.49	0.37	0.45	156	163	68	29	78	0.08
Evan													
	50	4.7	0.5	3.2	0.94	0.73	0.42	83	148	36	14	46	0.01
	100	6.5	0.8	4.1	0.79	0.62	0.43	86	121	44	23	51	0.03
	200	6.4	1.3	4.3	0.68	0.47	0.47	123	200	60	31	71	0.03
	300	6.7	1.8	4.4	0.53	0.41	0.47	141	291	83	26	73	0.02
	400	7.3	1.9	4.6	0.44	0.36	0.45	146	264	71	31	87	0.02
	200 Leach	6.5	0.9	4.2	0.71	0.55	0.51	107	156	50	27	58	0.03
	300 Leach	7.0	1.2	4.7	0.60	0.51	0.46	135	216	57	26	66	0.03
	400 Leach	7.2	1.7	4.7	0.58	0.42	0.45	147	255	60	26	71	0.03

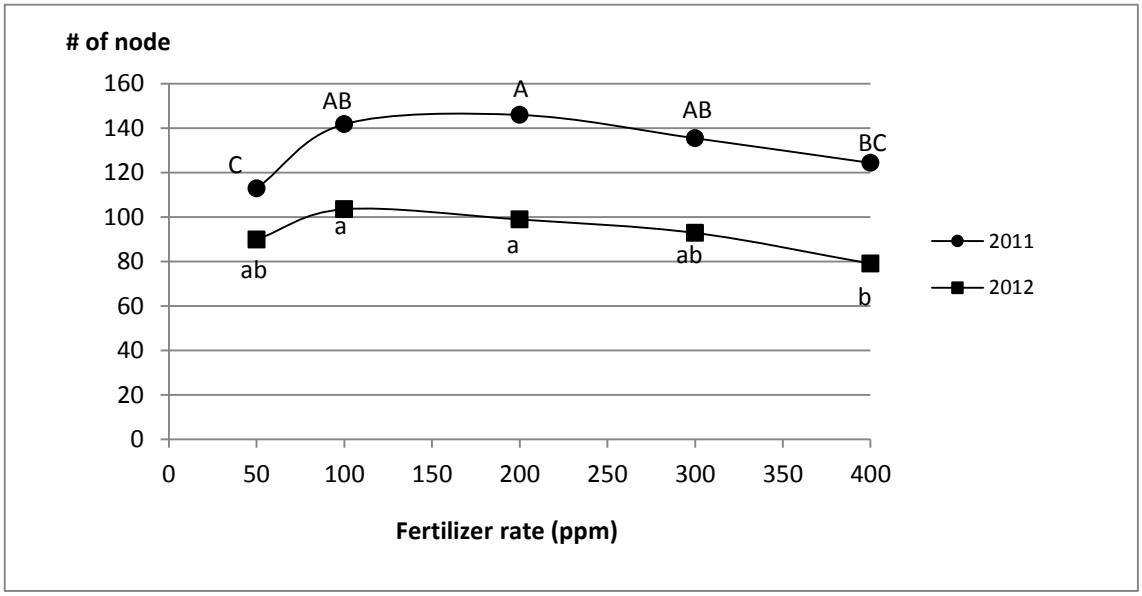


Figure 1. Effect of fertilizer rate (trt) on plant node production. Data combined all harvest times in each year and all three varieties. Data combined all harvest times in each year and all fertilizer rates. Tukey HSD comparisons were made within year, and different letters indicated significance at $\alpha=0.05$. Upper case letters represented 2011 and lower case letters represented 2012.

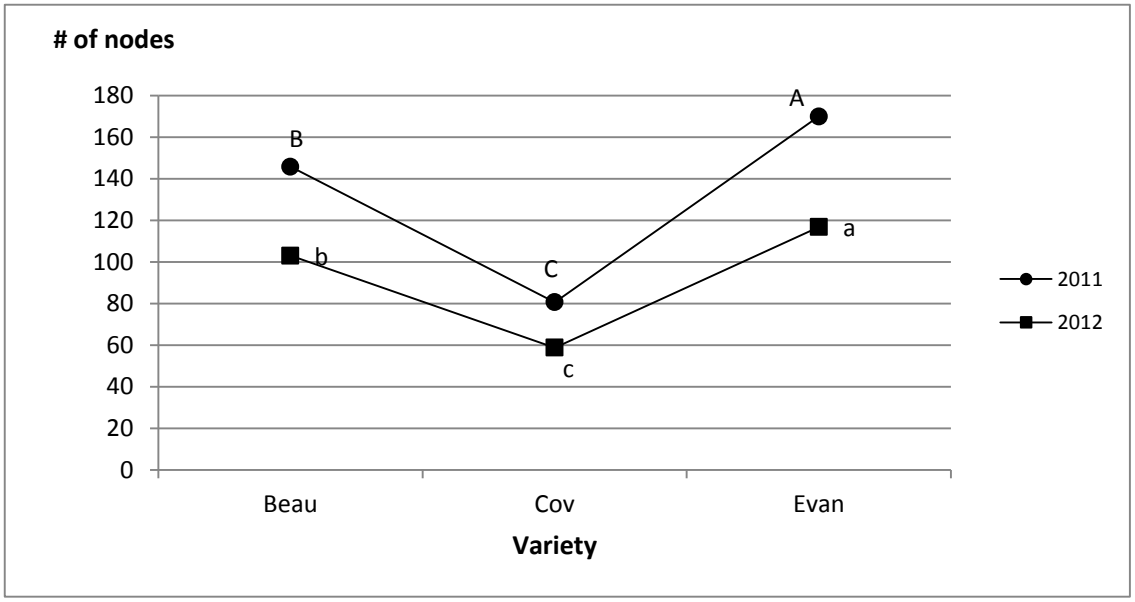


Figure 2. Effect of variety on plant node production in 2011 and 2012. Data combined all harvest times in each year and all fertilizer rates. Tukey HSD comparisons were made within year, and different letters indicated significance at $\alpha=0.05$. Upper case letters represented 2011 and lower case letters represented 2012.

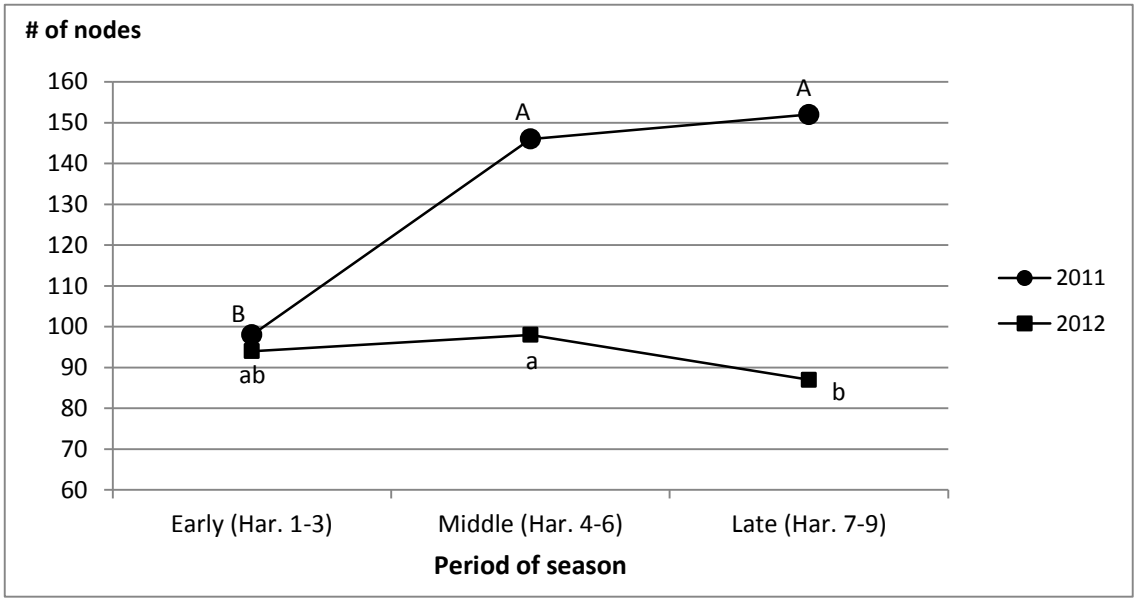


Figure 3. Effect of harvest time (eml) on node production. Data combined all fertilizer rates and all varieties. Tukey comparisons were made within year, and different letters indicated significance at α=0.05. Upper case letters represented 2011 and lower case letters represented 2012.

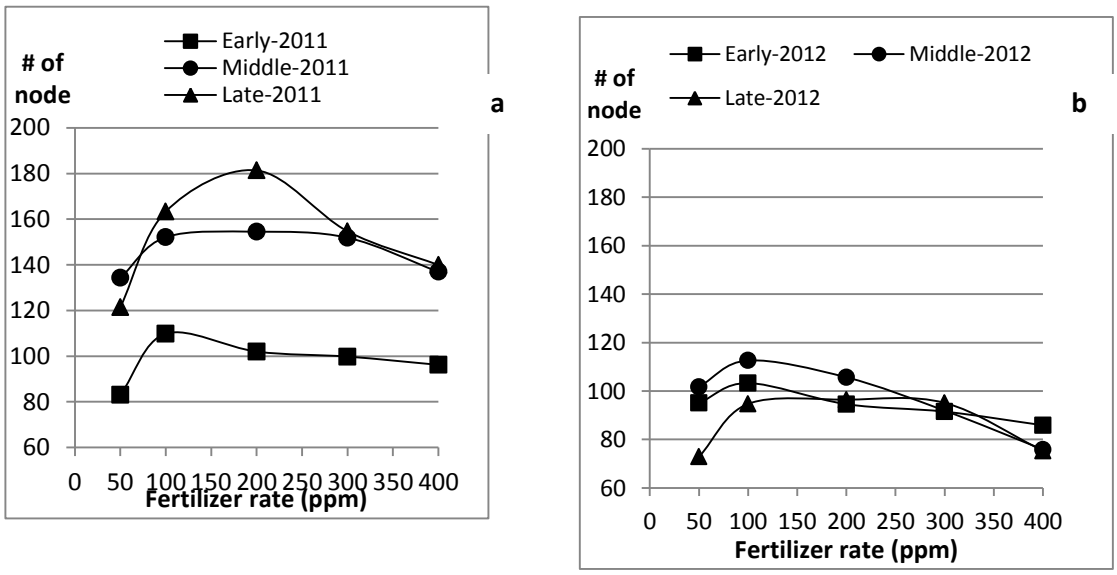


Figure 4a and 4b. Interaction effect of harvest time (eml) and fertilizer rate (trt) in each year. Data combined all three varieties.

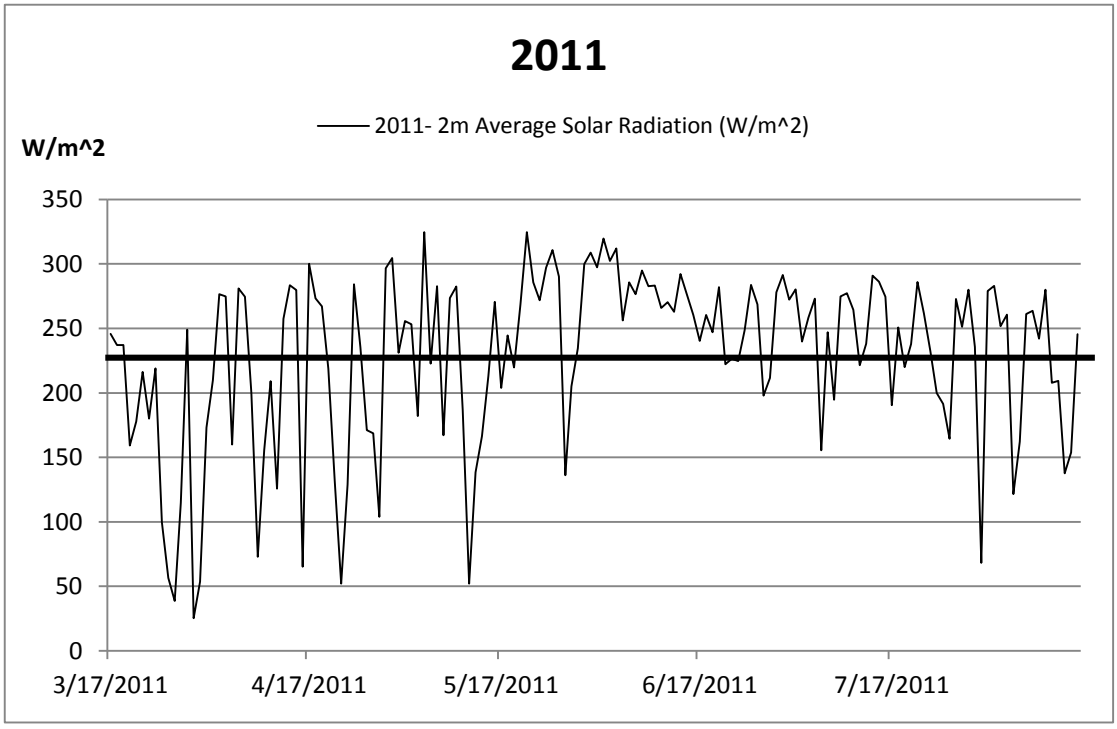


Figure 5. Average 2m solar radiation for every day during 2011 season. The solid line across the whole time period represented the average solar radiation across the whole season, which was equal to 228 W/m^2. Data courtesy of State Climate Office of North Carolina, Reedy Creek Field Laboratory Station, Raleigh, NC.

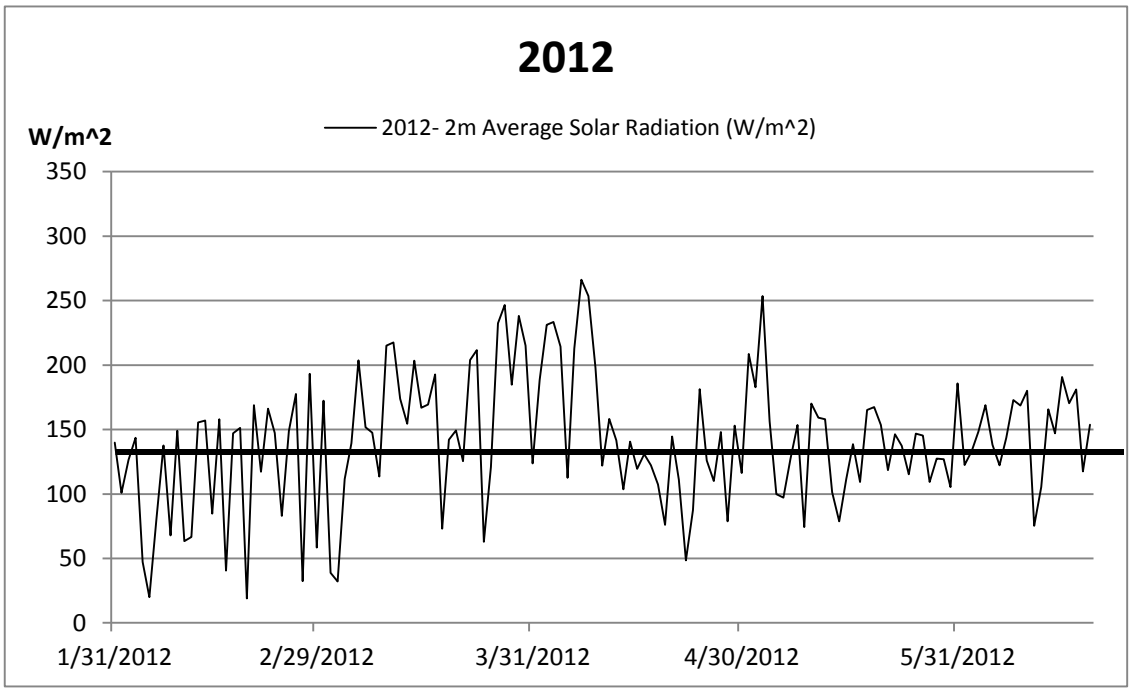


Figure 6. Average 2m solar radiation for every day during 2012 season. The solid line across the whole time period represented the average solar radiation across the whole season, which was equal to 141 W/m^2. Data courtesy of State Climate Office of North Carolina, Reedy Creek Field Laboratory Station, Raleigh, NC.

Appendix B. Chapter II

Table1. Results of 2010-2011 Survey on internal necrosis among NC sweetpotato commercial facilities.

	Sampling Time [z]									
Num.	1		2		3		4		5	
	Inc. %	Severity [y]	Inc. %	Severity	Inc. %	Severity	Inc. %	Severity	Inc. %	Severity
1	9	0.5	8	1.0	3	2.0	0	0.0	3	2.0
2	14	0.3	0	0.0	5	1.0	3	2.0	15	1.0
3	0	0.0	3	1.0	5	1.0	0	0.0	0	0.0
4	30	2.0	15	2.0	10	1.8	15	2.5	5	1.0
5	0	0.0	3	1.0	8	1.0	0	0.0	3	1.0
6	10	-	6	1.0	0	0.0	3	1.0	0	0.0
7	5	-	0	0.0	0	0.0	0	0.0	5	1.5
8	2	-	11	1.5	5	1.5	3	2.0	0	0.0
9	3	-	3	1.0	3	1.0	0	0.0	0	0.0
10	0	0.0	3	1.0	3	1.0	0	0.0	0	0.0
11	3	-	0	0.0	0	0.0	0	0.0	3	1.0
12	8	-	0	0.0	3	1.0	0	0.0	0	0.0
13	3	1.0	8	2.3	50	2.1	13	1.2	3	2.0
14	3	-	3	1.0	0	0.0	0	0.0	0	0.0
15	0	0.0	0	0.0	5	1.0	0	0.0	-	-
16	0	0.0	5	1.0	0	0.0	5	1.5	-	-
17	10	-	5	2.0	3	1.0	8	2.0	-	-
18	3	1.0	0	0.0	0	0.0	0	0.0	-	-
19	5	-	0	0.0	0	0.0	0	0.0	-	-
20	0	0.0	0	0.0	-	-	-	-	0	0.0
21	10	-	5	1.5	-	-	-	-	8	1.3
22	3	1.0	0	0.0	0	0.0	-	-	-	-
23	0	0.0	3	1.0	0	0.0	-	-	-	-
24	23	2.0	40	2.5	28	1.9	-	-	-	-
25	19	2.0	75	1.9	30	1.5	-	-	-	-
26	15	0.6	15	1.8	3	1.0	-	-	-	-
27	3	0.5	0	0.0	0	0.0	-	-	-	-
28	28	-	33	1.9	15	1.3	-	-	-	-
29	0	0.0	0	0.0	0	0.0	-	-	-	-

[z] Sample time 1 is during the period of 11/18/10 - 01/05/11, sample time 2 is during the period of 02/14/11 - 03/04/11, sample time 3 is during the period of 03/25/11 - 04/08/11, sample time 4 is during the period of 05/18/11 - 05/26/11, sample time 5 is during the period of 06/27/11 – 07/18/11.

[y] Severity is ranged from 1 to 4 as shown in the Figure 2 Chapter 2.

Table 2. Results of 2011-2012 survey on internal necrosis among NC sweetpotato commercial facilities

	Sampling time [z]									
	1		2		3		4		5	
Num.	Inc. %	Severity	Inc. %	Severity	Inc. %	Severity	Inc. %	Severity	Inc. %	Severity
1	2.5	1	0	0	2.5	2	0	0	0	0
2	12.5	1.2	20	1.3	10.3	1.5	22.5	2	15	2
3	0	0	2.5	3	0	0	2.5	1	2.5	1
4	0	0	17.5	1.7	17.5	1.1	15	1.5	12.5	1.2
5	47.5	1.6	0	0	2.5	1	0	0	2.5	1
6	0	0	47.5	1.7	2.7	1	0	0	-	-
7	0	0	0	0	0	0	0	0	0	0
8	5	2.5	45	2.1	22.5	1.2	25	1.5	20	2
9	0	0	0	0	5	1	2.5	1	5	1.5
10	0	0	0	0	0	0	0	0	2.5	1
11	0	0	0	0	0	0	0	0	0	0
12	62.5	2.4	55	2.4	20	1.4	52.5	2. 7	62.5	2.3
13	0	0	32.5	2	22.5	1	52.5	1.6	27.5	1.6
14	12.5	1.8	2.5	1	60	2.2	25	2	17.5	1.9
15	0	0	7.5	1.3	17.5	1.3	17.5	1.1	-	-
16	2.5	1	5	1.5	-	-	0	0	-	-
17	0	0	0	0	0	0	2.5	2	-	-
18	12.5	1.2	5	1.5	7.5	1	2.5	1	-	-
19	0	0	0	0	0	0	0	0	-	-
20	2.5	3	5	1.5	22.5	1.4	30	1.2	-	-
21	0	0	0	0	5	1	5	1	-	-
22		-	0	0	0	0	0	0	0	0
23	-	-	0	0	0	0	5	1	7.5	1.3
24	-	-	5	1.5	38.9	2	10	1	20	1.4
25	-	-	0	0	28.9	1.8	13.2	1.6	30	2.1
26	0	0	0	0	0	0	-	-	2.5	1
27	0	0	5	2	25	2.1	-	-	-	-

[z] Sampling time 1 is during the period of 10/14/11 – 11/14/11, sampling time 2 is during the period of 11/21/11 - 11/28/11, sampling time 3 is during the period of 02/07/12 - 02/10/12, sampling time 4 is during the period of 03/19/12 - 04/04/12, sampling time 5 is during the period of 05/10/12 – 06/12/12.

Table 3. Results of pathogen isolation from selected survey samples.

Sample ID	IN Incidence (IN roots/ total roots)	Moisture Chamber		PDA acid	
		Isolation	Result	Isolation	Result
1	21/40	2	1 bacterium	6	2 bacteria, 3 fungi
2	21/40	2	1 fungus	6	5 bacteria
3	10/40	2	1 fungus	6	3 fungi
4	12/40	2	1 fungus	6	0
5	9/40	2	1 fungus	6	1 bacteria, 2 fungi
6	10/40	2	0	6	4 fungi, 2 bacteria

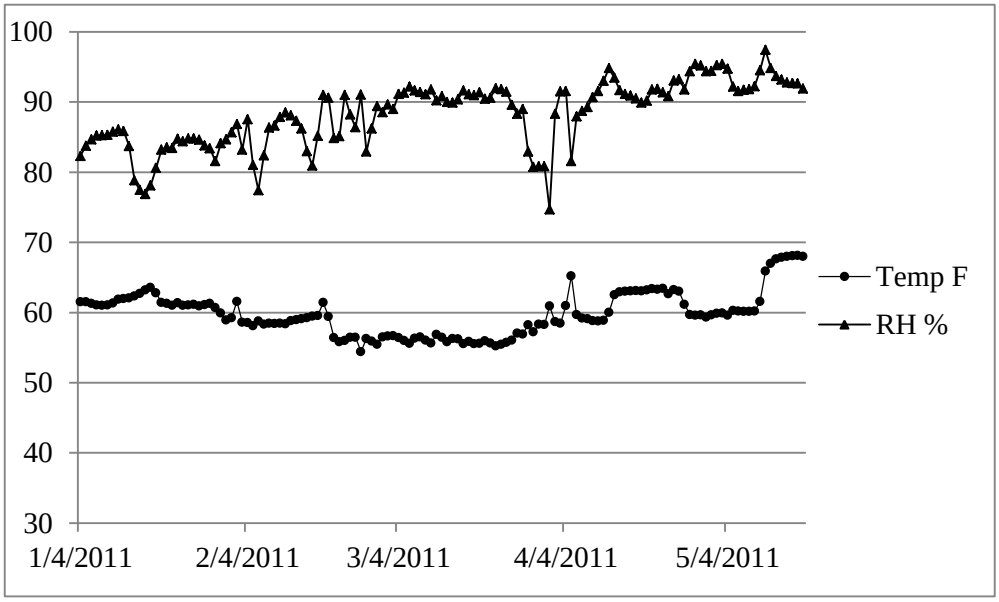


Figure 1. Storage conditions (Temperature and relative humidity) and its relation to the occurrence of IN. This facility had an average of 41% of IN across all sampling times during 2010-2011 storage.

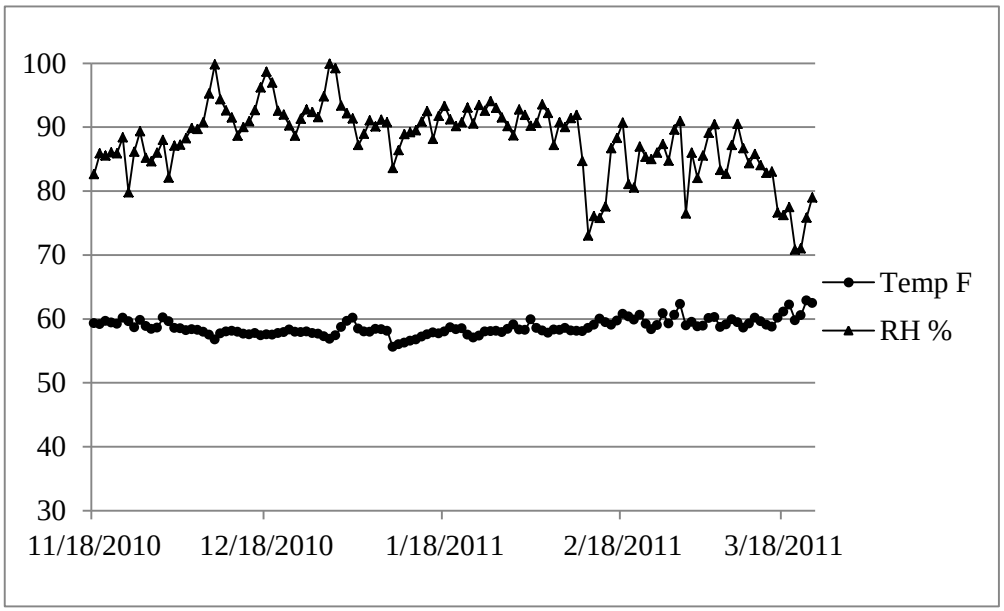


Figure 2. Storage conditions (Temperature and relative humidity) and its relation to the occurrence of IN. This facility had an average of 11% of IN across all sampling times during 2010-2011 storage.

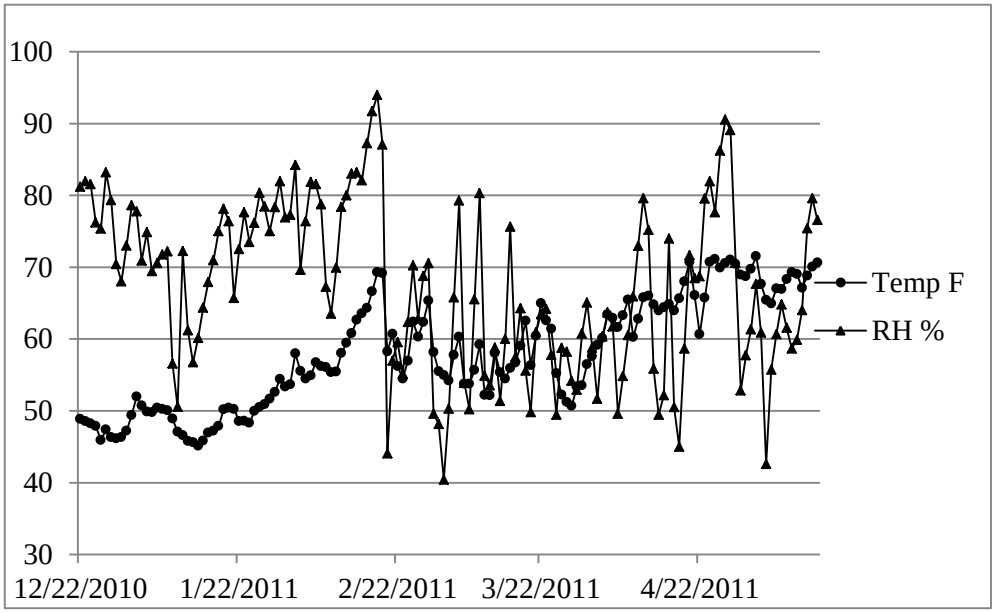


Figure 3. Storage conditions (Temperature and relative humidity) and its relation to the occurrence of IN. This facility had an average of 6.5% of IN across all sampling times during 2010-2011storage.

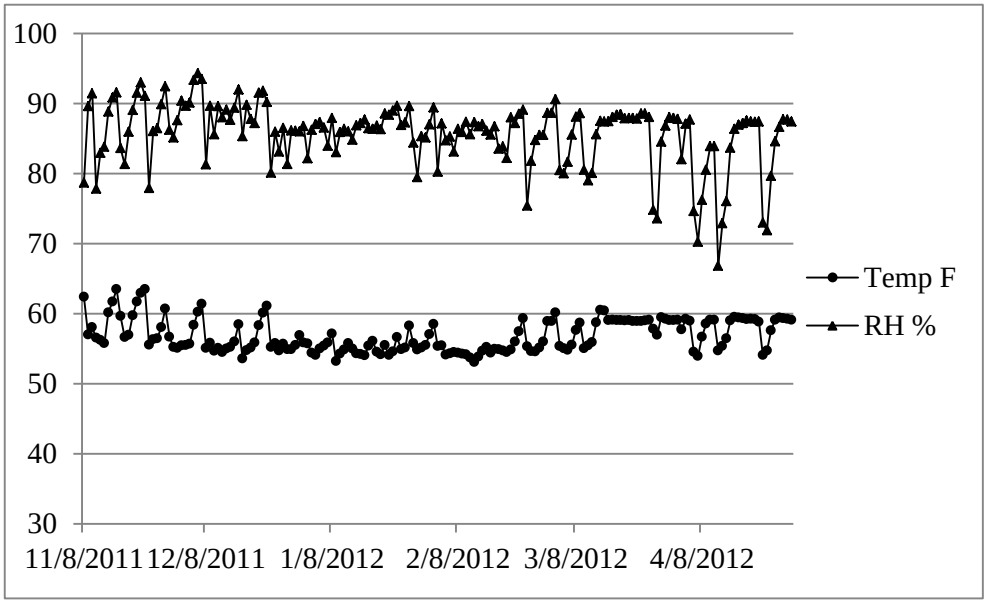


Figure 4. Storage conditions (Temperature and relative humidity) and its relation to the occurrence of IN. This facility had an average of 50.5% of IN across all sampling times during 2011-2012 storage.

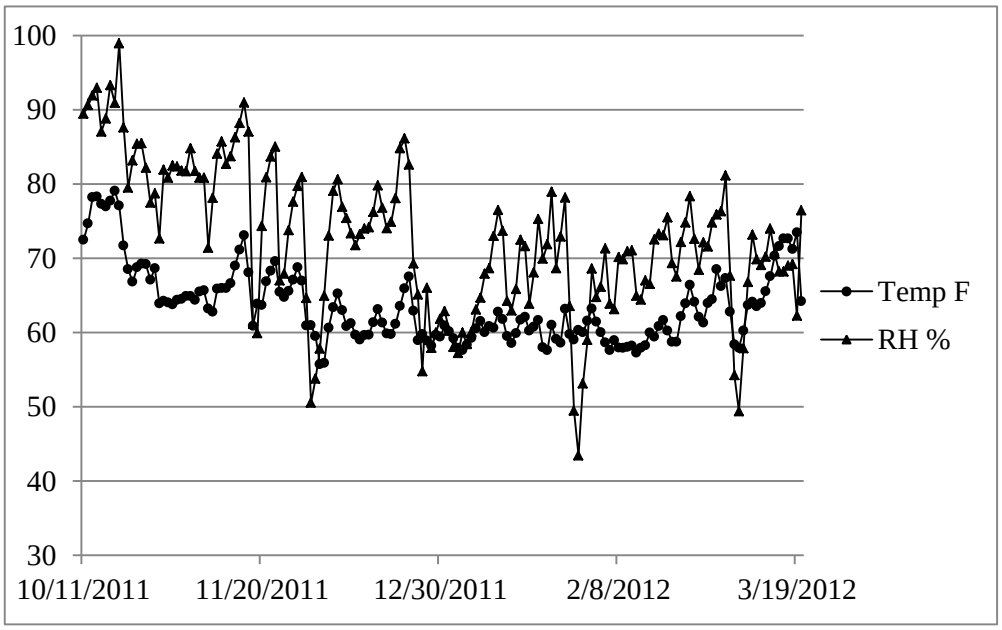


Figure 5. Storage conditions (Temperature and relative humidity) and its relation to the occurrence of IN. This facility had an average of 2.5% of IN across all sampling times during 2011-2012 storage.

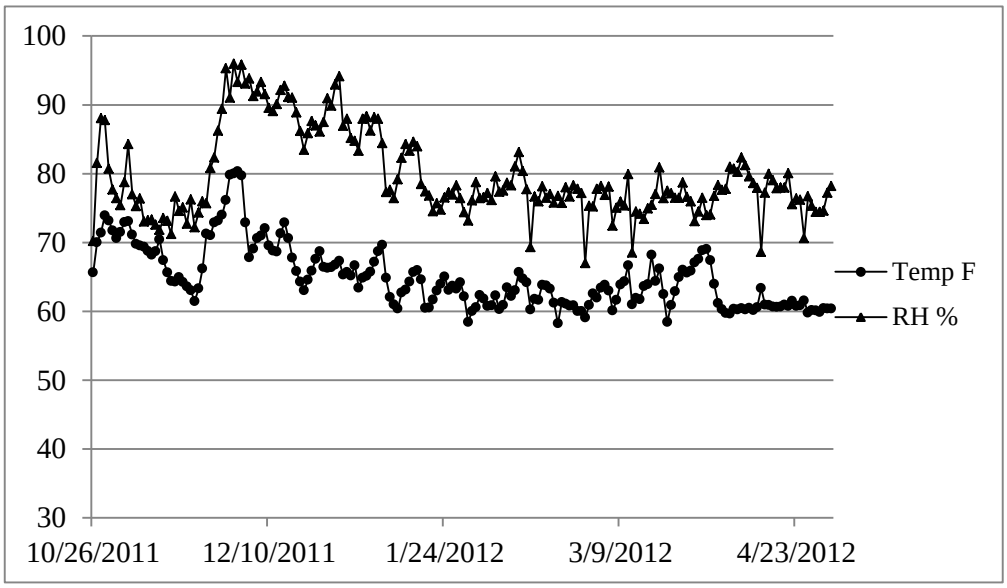


Figure 6. Storage conditions (Temperature and relative humidity) and its relation to the occurrence of IN. This facility had an average of 2.5% of IN across all sampling times during 2011-2012 storage.

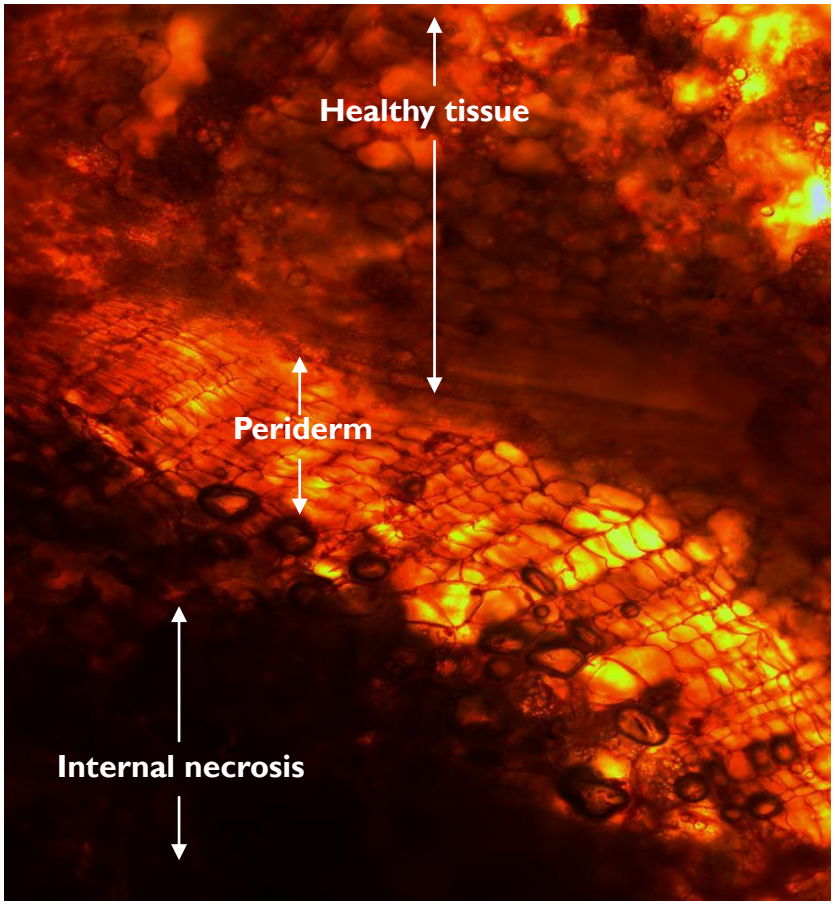


Figure 7. Sweetpotato internal tissue with IN and periderm (4X, stained with Sudan).