Postharvest Research Report 2008-2009

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Research Areas

I. Pressure bruise
a. Pressure bruise susceptibility in potato cultivars
b. Effect of watering after vine kill on pressure bruise susceptibility
c. Gene expression during pressure bruise incidence in tubers
II. Disease detection
a. Early detection of diseases in storages
III. Powdery scab population dynamics and disease spread in specialty and russet cultivars

1. Studying the phenomenon of pressure bruise susceptibility in potato cultivars

Pressure bruise is a primary concern for all market classes of potato. Pressure bruise limits the storage duration of potato and reduces grade the longer potatoes are stored. Pressure bruise can result in quality losses of 20 to 30% in long term storage potatoes, yet little is known about factors influencing this condition. Pressure bruise is primarily caused by the weight of the pile but many physiological and genetic factors play a role in affecting the structure of tubers on the bottom portion of the bin. Some cultivars are more prone to this condition. In addition to that there are a number of pre-harvest and post-harvest factors that can make this condition worse.

Pressure bruise is a complex process that involves physical damage to cells on the surface of the tuber and discoloration of internal tissues. These flattened areas of the potato exhibit crushed periderm and underlying cells when viewed with a microscope (Lulai et al., 1996). Turgor pressure is the counteractive force within the cells of potato tubers that helps to prevent the development of pressure bruise. Cells create turgor pressure by increasing water intake into the cell, thereby increasing outward force on the cell membranes and walls. Plants regulate turgor pressure during growth by increasing the osmotic potential within cells. Turgor pressure allows cell elongation leading to shoot and root expansion during plant growth. For example, turgor pressure in roots creates a penetration force of up to 1 MPa (145 psi). How that force corresponds to resistance to pressure bruise is unknown. Correspondingly, how turgor pressure, hydration level, and water potential of tuber tissue relate to resistance to pressure bruise is not known either. Tissues beneath pressure flattened areas turned black to gray within 5 days after removal from storage. The black coloration results from increased levels of melanin, a characteristic commonly associated with black-spot bruise. The delay in bruise formation has been hypothesized to be related to limited oxygen availability under the pressure-flattened areas.

Objective:

Study the correlation between early tuber weight loss and susceptibility of tubers to pressure bruise during long-term storage

Procedure:

Tubers were collected after harvest from the piler during bin loading in 10 different commercial storages. 15 tubers per bag were weighed and labeled. They were kept at 6 different locations on top of the pile in the commercial storage bins along with electronic sensors for humidity and temperature.

Pulp temperature and specific gravity of the tubers were also measured. Subsequently, every month each bag was weighed and once in every 3 months specific gravity of these tubers samples was also recorded. During the bin emptying, 180 tubers were collected randomly and pressure bruises per tuber were recorded. We also collected data from storage managers on the extent pressure bruise in those bins based on USDA inspector's reports and other data. Data collected from storages that were empty of tubers after 6 months of storage was analyzed.

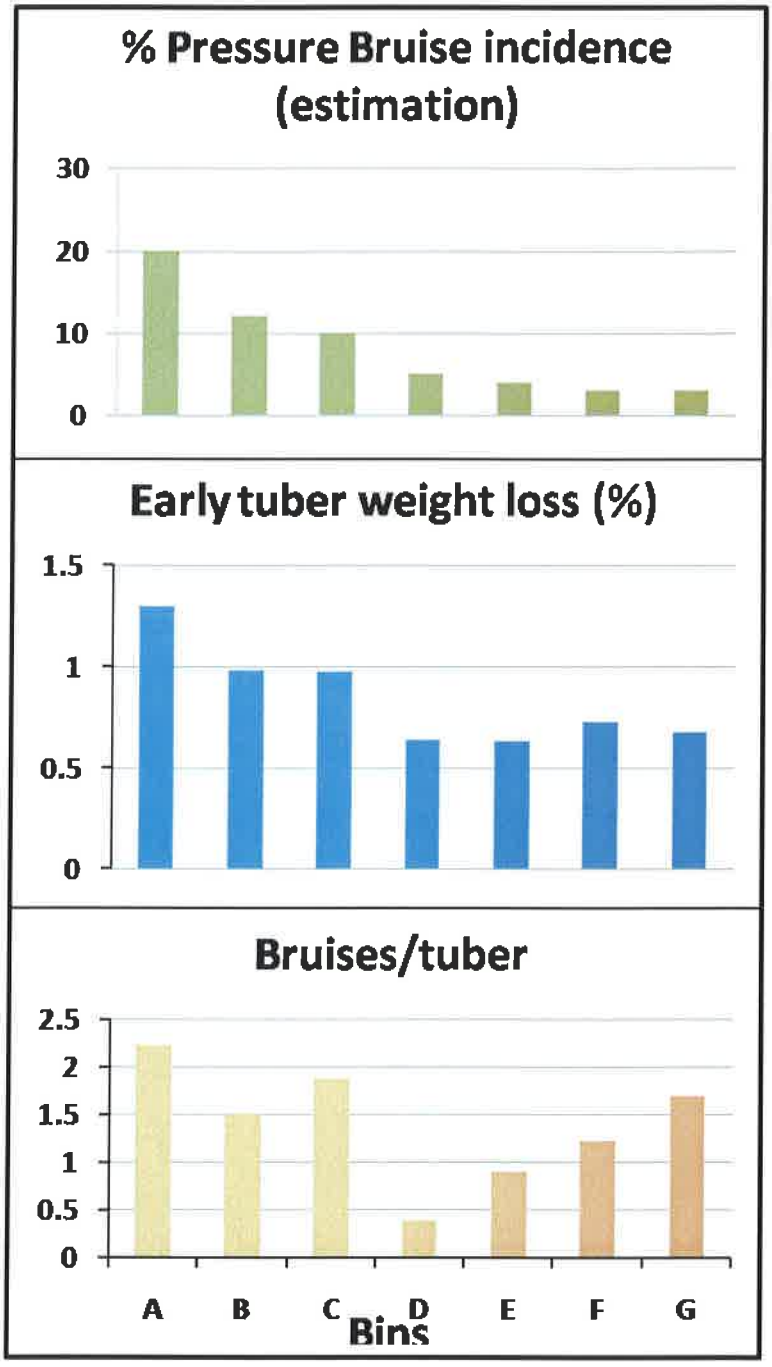

Figure 1

Conclusions:

Tuber susceptibility to pressure pre and postharvest bruise can be controlled by number of factors. In this experiment we tested whether early tuber weight loss can offer us an indication of pressure bruise incidence in long-term storage. Based on our study this year on 7 different commercial storage bins, we found a good correlation between early tuber weight loss and pressure bruise incidence. Early tuber weight loss (ETL) is calculated based on weight loss that occurred during the first three months of storage. Differences in ETL between the bins ranged from 0.6 to 1.3. These subtle differences in ETL gave an indication of pressure bruise susceptibility. In three commercial storage bins where there was around one or more than one percent ETL, we

found 10% or more than 10% pressure bruise incidence (Figure 1). The other four bins had only 3 to 5% pressure bruise incidence with a recorded 0.6 to 0.75 ETL. Tubers collected from A, B, C and G bins also showed 2 to 1.5 bruises per tuber which coincided with our ETL data. Our results suggest that there is no correlation between pulp temperature and pressure bruise susceptibility in the temperature range that we studied (54 to 65°F) (data not presented). Results based on one year's study indicate that early tuber weight loss (ETL) can be used as predictive tool for susceptibility of the tubers to pressure bruise.

2. The effect of watering after vine kill on pressure bruise susceptibility and the role of DMN on tuber weight loss during storage

During potato crop growth, turgor pressure within tuber tissues is influenced by environmental conditions. For example, as soils become drier the water potential of tuber tissue decreases as transpiration exceeds the rate of water uptake. However, we do not know how well plants or tubers are able to regulate water potential after vine kill or harvest. Tubers have little if any ability to absorb water after harvest. Likewise, we know the ability of tubers to resist pressure bruise is dependent on minimal water loss and subsequent effects on cell turgor pressure. Yet little data is available on quantifying tuber water relations in the field and after harvest. More information is needed on how these factors influence tuber quality such as shrink, weight loss and pressure bruise susceptibility for better storage management.

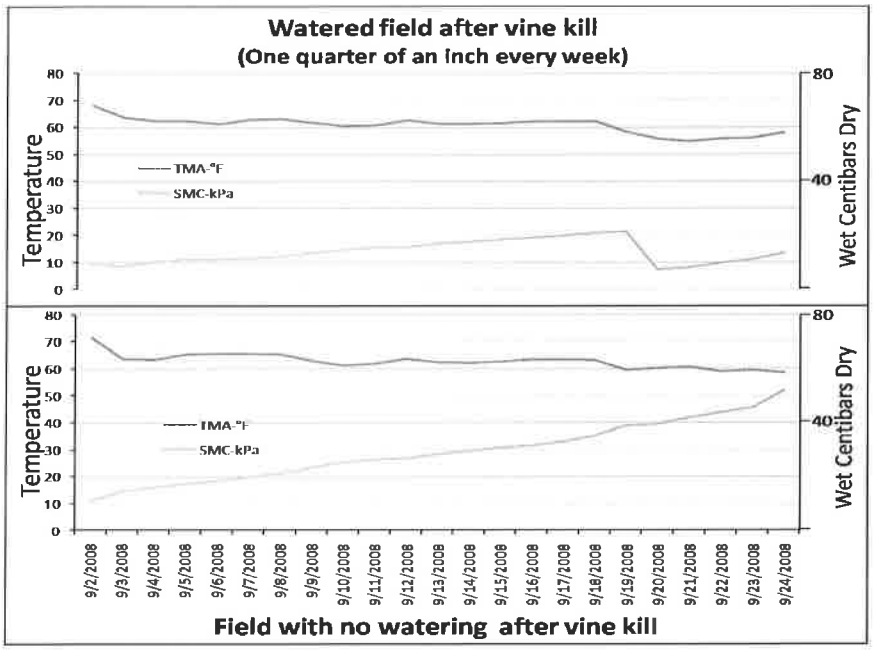

Figure 2

Ethyl substituted naphthalenes are naturally occurring in potato tubers and contribute to flavor in baked potatoes (Buttery et al 1970 and Coleman et al 1981). These compounds showed sprout suppressant activity on a short-term basis of approximately 30 days. These compounds are used in combination with CIPC to suppress sprouting and also to reduce pressure bruise incidence in long term potato storages.

Objective:

To quantify the effect of watering after vine kill on tuber weight loss in storage.

Procedure:

Potato tubers were harvested after three to four weeks from vine kill. In sandy soils especially, temperatures will go up considerably and may result in dehydration of tubers. We planted both Canela Russet and Centennial Russet at the SLV Research Center and followed recommended practices for irrigation and fertilizer. After vine kill, one half of the field received ¼" of water every week until tubers were harvested. The other half of the field received no watering after vine kill. We monitored soil moisture using moisture sensors placed at four places in the field. The average soil moisture data after vine kill for the two sides of the field is presented in figure 2. In the watered field soil moisture was maintained between 10 to 20 kPa until harvest compared to in the control field where soil dryness levels increased significantly over a 3 week period.

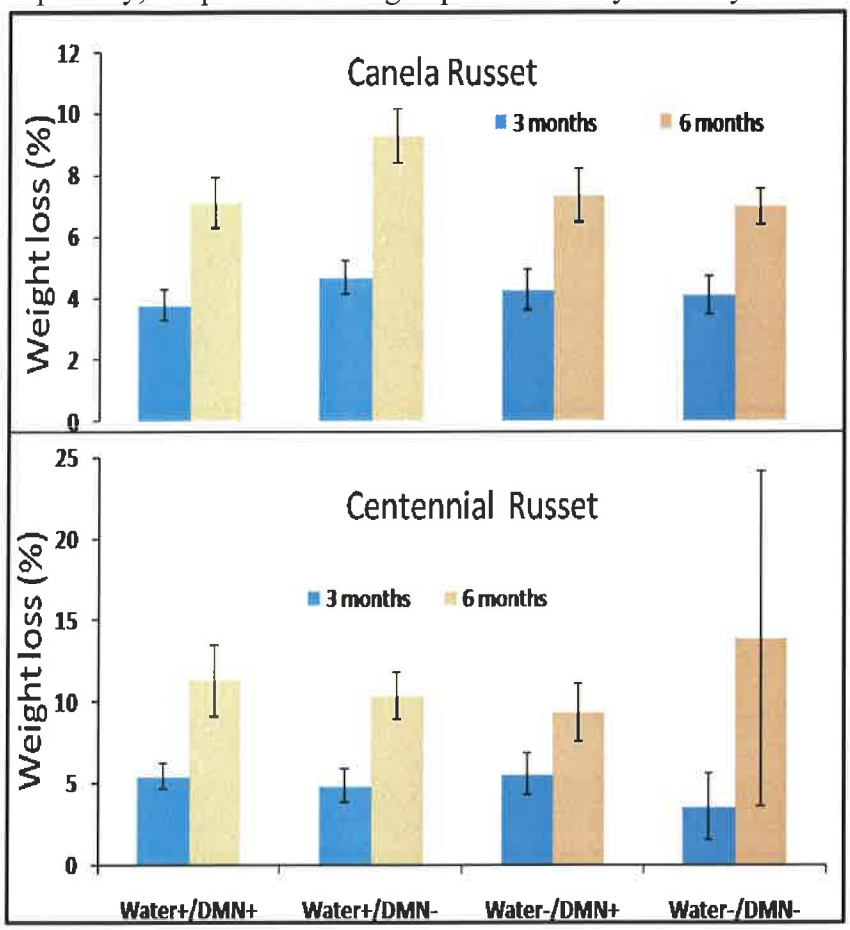

Figure 3

After harvest, tubers were treated with a 20 ppm concentration of 1, 4-DMN for 24 hours. Tubers were weighed after three and six months to assess the effect of watering after vine kill and DMN treatments on tuber weight loss.

Conclusions:

There was a significant difference in terms of dryness between fields that were watered and not watered after vine kill. The SLV Research Center has a sandy loam soils. The drying event was severe in the case of more sandy soils and had the potential to significantly dehydrate tubers when day temperatures were high. Tuber weight loss was more in Centennial Russet than Canela Russet. In general, Canela Russet tubers harvested from the watered field and treated with DMN had less weight loss when compared to other treatments. There was no effect of moisture in the case of case of Centennial Russet.

3. Comparing the gene expression during pressure bruise tubers

Plant cell wall is composed of polysaccharides, proteins, phenolic compounds, and other materials (Varner and Lin, 1989). The plant cell wall plays a determinative role in establishing the size and shape of the plant cell. For the elongation or maturation event, however, the plant cell needs to selectively modify its cell wall. The agents for cell wall modification in the plant cell include various cell wall components, such as expansins, extensins endoglucanases, xyloglucan endotransglycosylases, and hydroxyl radicals. These proteins provide flexibility to the cell wall.

Role of expansins and extensins

Expansin is a family of proteins that catalyze long-term expansion of cell walls and has been considered a principal protein that affects cell expansion in plants. Expansins are unusual proteins discovered by virtue of their ability to mediate cell wall extension in plants.

Extensin, another structural cell wall protein, can form covalent bonds with other extensin proteins through the amino acid tyrosine. In extensin, the tyrosines are evenly spaced and when they bond with tyrosine on another extensin molecule, they can wrap around other cell wall constituents "knitting" the wall together. The amount of extensin changes with development. Cells that have thick, hard walls are often rich in extensin (i.e., sclerids and fibers). The amount of extensin produced is dependent on mechanical wounding, and/or infection and these responses are mediated by plant hormones.

The cell wall integrity signaling pathway is controlled by the Rho1 factor. Rho1 is a central signaling molecule responsible for orchestrating changes to the cell wall periodically

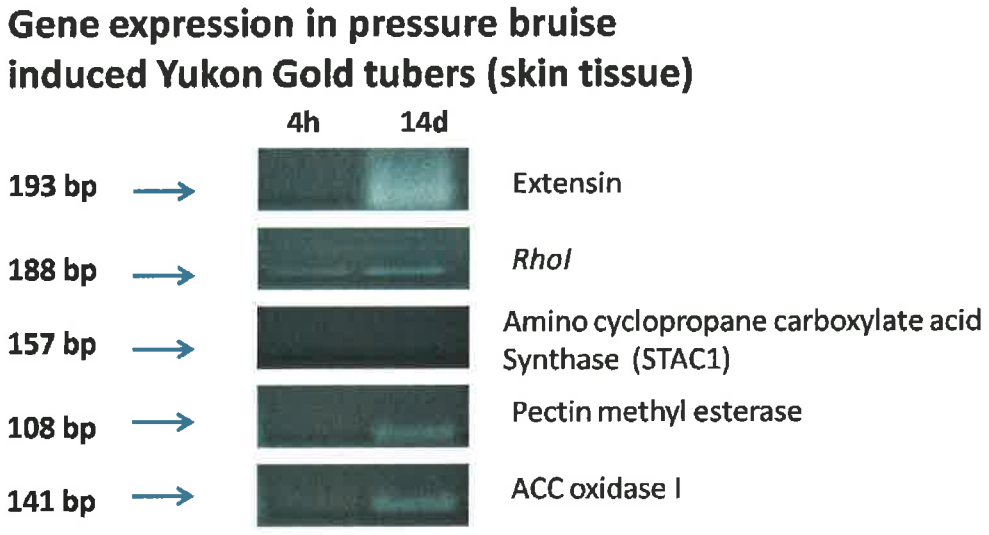

Figure-4

through the cell cycle and in response to various forms of cell wall stress such as pressure, temperature and other external stimuli. This signaling pathway acts through direct control of wall biosynthetic enzymes, transcriptional regulation of cell wall-related genes, and polarization of the pectin cytoskeleton.

Objective:

To study pressure bruise susceptibility at the molecular level and to test whether genetic markers can be used to predict pressure bruise susceptibility.

Procedure:

Pressure bruise was induced using clamps at precise locations on the tuber under laboratory conditions. We harvested cortex tissue from the tuber and extracted RNA using phenol chloroform method. We tested expression of different genes that regulate cell shape and size to see if they play any roles in pressure bruise using gene specific PCR primers.

Conclusions:

This is an initial screening to test whether some of these genes have a role in incidence of pressure bruise or cultivar susceptibility. We found differential gene expression between 4 hours and 14 day tissue samples (figure-4). Further studies are in progress to confirm these results and testing many cultivars under similar conditions.

4. Disease detection in storages

Huge losses are incurred by the potato industry due to diseases in the potato storage houses. These diseases are caused by both bacterial and fungal pathogens. The most devastating are soft rot (*Erwinia cartovora* ssp *carotovora*), black leg (*Erwinia cartovora* ssp *atroseptica*), dry rot (*Fusarium sambucinum*), ring rot (*Clavibacter michiganensis* ssp. *sepedonicus*), late blight (*Phytophthora infestans*), pink rot (*Phytophthora erythroseptica*), and leak (*Pythium ultimum*). Potentially, all the harvest operations involve some amount of bruising and wounding of the tubers which provides avenues for pathogen infection. Pathogens thrive in the storage with high humidity and other favorable conditions. Currently, visual inspection, bad odors and temperature sensors are the only means of detection for disease. Existing temperature and other sensors can detect problems only after symptoms appear and damage has been taken place.

Figure- 5

5A. Air sample collection from potato storage
5B. Incubated petri dishes with fungal pathogens collected form storages

Objectives:

To test the feasibility of using air filters to detect pathogens in potato storage

Target Organism	Disease	Primer sequence (5'→ 3')
Phytophthora infestans	Late blight	CATTACATTGCTCACATGGCTTTC ATCACGCGGGGACAAATG
Pythium ultimum	Leak	GCAGATTGTCCCGGATATTAAC CACAATAACCCGAGAATCAAAG
Phytophthora erythroseptica	Pink rot	GATGAAACTAAGCGCCTTCTC CGACAATAGTCTTCAAGGTGGAT
Erwinia carotovora	Soft rot	ACCGCCAGCACATCGTC CGGAAGGCCATCAGGAA
Fusarium sambucinum	Dry rot	CCTCTCCGTCCATCAACTGG AGGTAGTTCAAGTCGCCGTAAG
Spongospora subterranea	Powdery Scab	CCTGGGTGCGATTGTCTGTT CACGCCAATGGTTAGAGAGACG
Helminthosporium solani	Silver scurf	GTTTCAGCGGCCGCAAGGCT CAGGGCTTCAAGAAGCGCA

Figure-6

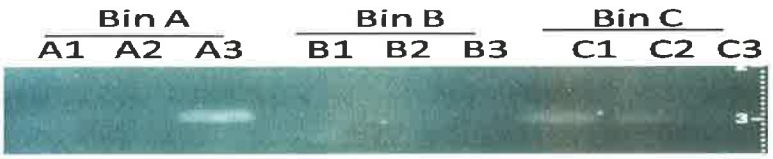

Figure - 7

Procedure:

The air samples were taken from the negative pressure duet from three commercial potato storages. The air was filtered through a 37-mm-diameter, 0.2-µm pore polycarbonate track etch membrane (Poretics Corp, Livermore, CA). The membrane filter holder was a 37-mm disposable styrene acrylonitrile two-piece assembly that holds the membrane filter. The filter holder was directly connected to a battery-operated pump (Escort Sampling; Hazco Services Inc; Dayton, OH) to filter the air at the rate of 2.0 L/min. The membrane filter was recovered by removal from the membrane holder using sterile technique under an aseptic hood and sterile solution was added to collect all spores. The solution was spread on Petri dishes containing an agar media. The plates were incubated at room temperature overnight and observed for any fungal growth. Single colonies from the agar plate were picked using a sterile pipette tip and washed with Tris buffer in an eppendorof tube. This buffer was used to test the presence of various potato pathogens using specific primers for different pathogen as described in figure-6 in a PCR reaction. Amplified DNA specific to pathogens will be visualized in an agarose gel stained with ethidium bromide (figure7).

Conclusions:

We could successfully detect common diseases that are encountered in a typical potato storage. This is a pilot study mainly looking at the sensitivity and usefulness of this technology. This can be pursued further if there is sufficient interest and when necessary funding is available.

5. Studying the powdery scab resistance mechanism in russet cultivars

Powdery scab disease caused by *Spongospora subterranea f. sp. subterranea* is one of the major concerns for potato producers in potato production regions in North America. This is a soil borne pathogen that infects root hairs, stolon epidermal cells, lenticels, eyes and wounds of developing tubers. Infected tubers and roots may have white, gall-like growths, which in turn develop into brown powdery scab lesions on tubers as they mature (Harrison et al. 1997). Powdery scab symptoms cause significant economic losses in both fresh and seed markets. Depending on the severity of symptoms, tubers could become non-marketable or the grade may be lowered in fresh, processing and seed markets. Even in processing, industry costs incurred for peeling is higher. Seed lots infected with powdery scab may or may not pass inspection depending on the regulations of the certifying agency and the degree of infection. Infected tubers

are also more susceptible to dehydration, Fusarium dry rot, bacterial soft rot and other pathogens during storage.

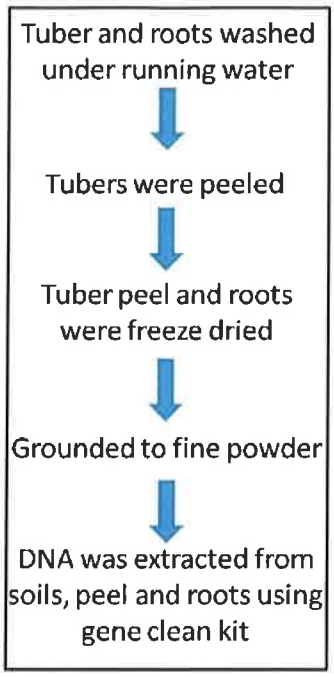

Figure 8

Christ (1993) observed that potato cultivars with smooth or light skin and red skin are more susceptible where as russet-skinned varieties are less prone to powdery scab, although root galls are common. There are reports on partial russeting in some cultivars such as Rio Grande Russet. Partial russeting and irregular skin setting can lead to disease, loss of water and prone to skin bruise, affecting tuber quality (Lulai EC, 2002). A better understanding of the russetting mechanisms will help us to develop new cultural tools for better skin set in new cultivars and to enhance the native capacity of tubers for skin set. Skin set is an important biological process in tubers that protects against biotic and abiotic stresses, and has a great impact on the potato quality.

Objectives:

1. Testing root galling susceptibility between cultivars
2. Testing russet tuber peel for pathogen presence
3. Testing whether a russet tuber from an infected field will spread inoculum as a seed.

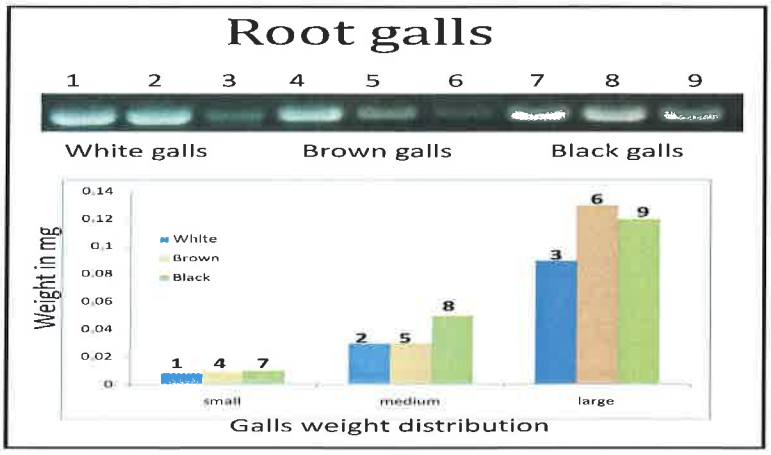

Figure-9

Procedure:

Four cultivars: Mesa Russet, Rio Grande Russet, Cherry Red (DT 6063-1R) and Rio Colorado each with different levels of susceptibility, were planted in a powdery scab infested field. Tubers, roots and the adjacent soil were collected at the end of the season for each of the 4 cultivars. Samples were processed as described in figure 8. Spore load and presence of the pathogen in the tuber skin and roots was tested using PCR.

Conclusions:

There are three different types of root galls in terms of size and color that were observed all four cultivars. We tested whether all of them release spore balls and whether there was any relation with the size of root galls. All galls showed presence of the pathogen irrespective of size and color (Figure-9).

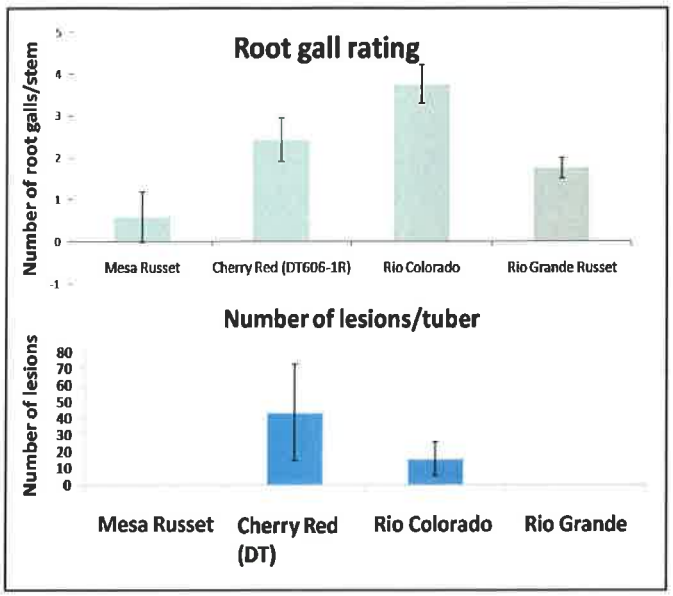

Figure-10

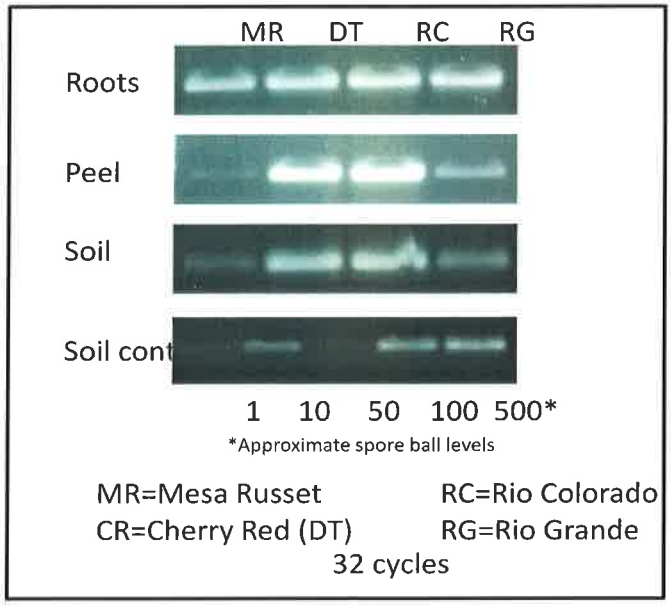

Figure-11

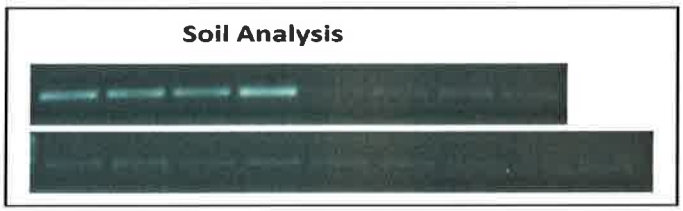

Figure-12

We counted the number of root galls for each cultivar. Rio Colorado is highly susceptible for root galling. Russets are less prone to root galling. We did not observe any lesions on tubers of russet cultivars. Cherry red tubers have the highest number of powdery scab lesions on the tuber skin surface among all the cultivars tested (Figure-10).

Russets tested positive for the presence of the pathogen in their peel sample even though they did not show any symptoms on their skin (Figure 11). We planted these russets tubers in a powdery scab free soil containing pots in a green house to test whether these tubers can spread inoculum. We tested soil collected from these pots using PCR same specific primers for powdery scab pathogen (Figure-12). Only four out of 17 samples tested showed positive with intense PCR band of target size(Figure-12).

Our results suggest that Rio Grande Russet and Mesa Russet cultivars are less susceptible to root galling compared to the smooth skin cultivars, Rio Colorado and Cherry Red. Both russet cultivars did not develop powdery scab lesions on the skin but tested positive with PCR. Preliminary results suggest that russet seed tubers from infected field can spread inoculum

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