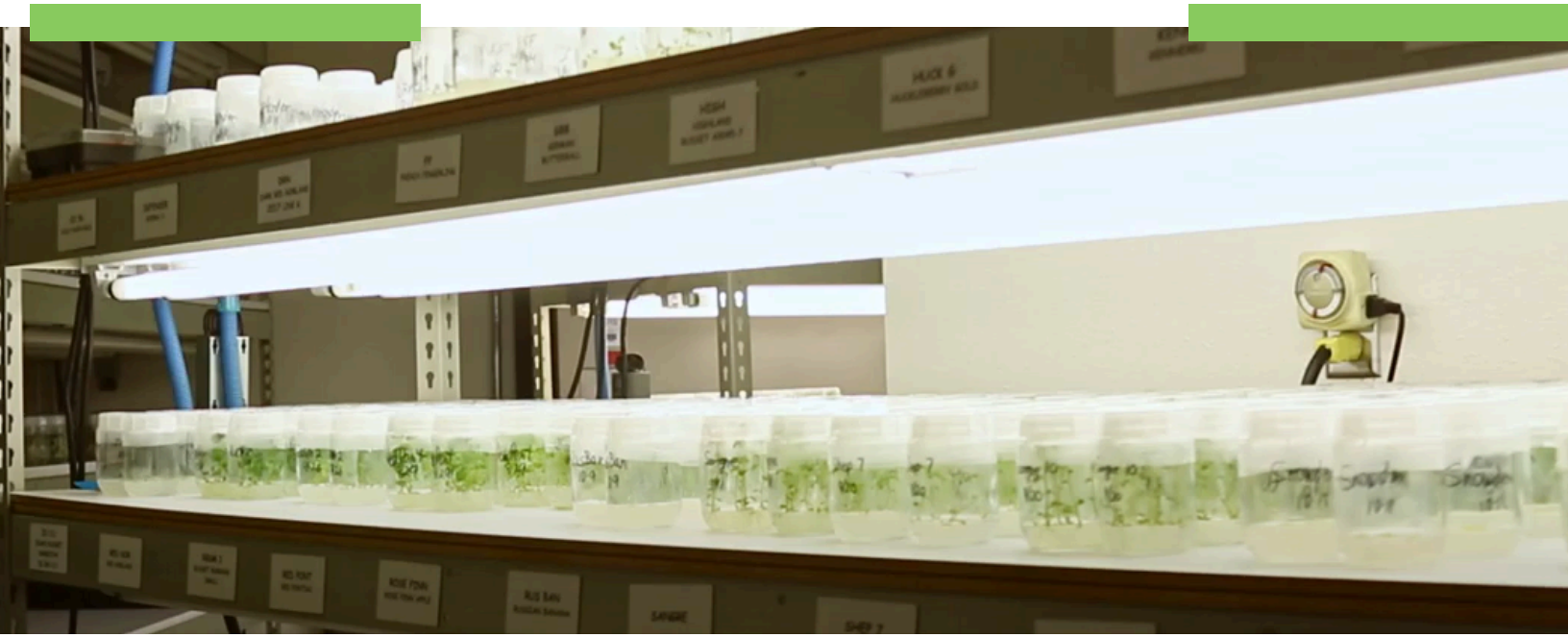
Potato Lab

Tissue Culture Upgrades



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Specialty Crop Block Grant

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In 2021, the potato lab received a Specialty Crop Block Grant through the Montana Department of Agriculture to upgrade the Potato Certification tissue culture program. 7 potato varieties (5 russets and 2 specialty) were evaluated for tolerance to antibiotics and insecticides, growth rate under LED lighting, and growth rate in modified media.

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The funded project identified ways to remediate risks factors that threaten the security of the tissue culture program, and methods that can be used to increase efficiency of maintenance. The project established protocols to reduce microbial and insect contamination, minimize heat risk through LED lighting, reduce labor for maintenance, and establish a secure long-term storage method.

Project Objectives

Objective 1: Improve efficiency and reduce risk in the tissue culture program by updating SOP

How we are reducing required manhours & the possibility of contamination

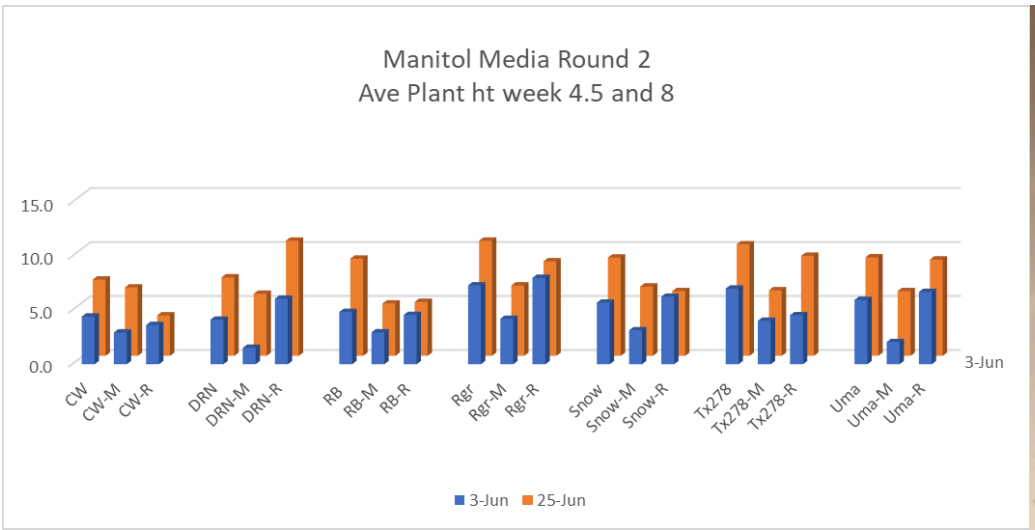
Current maintenance practices require the entirety of the Mother stock to be cut every 4-6 weeks. This equates to ~90 hours a month of highly detailed attention for quality. That time does not include the time needed to refresh the media stock, or the time required to monitor and assess growth quality and catch contamination in its early stages. Contamination can occur with every cutting, even with preventative measures, sanitary practices and aseptic techniques.

>>> Sub-objective A: Increase intervals between maintenance propagation

The goal of this objective is to extend the time needed between maintenance cuttings from 4-6 weeks to 12 or more weeks, with the ability to revive normal growth when replanted in regular media for propagation.

The method chosen for this experiment was a mannitol media which slows plant growth. Mannitol is an osmotic inhibitor, slowing down cell division, which slows down the plantlet growth. While the mannitol media performed as generally expected, stunting the plant growth thus extending time between cutting, abnormal plant growth in some varieties was determined to be unacceptable for the program. The incidence of Callus and unusual growth on some of our most widely used varieties, as well as some poor recovery results did not inspire confidence in this method for preserving our mother stock.

An alternative method was observed on a project-funded trip to University of Wisconsin last April. The method utilizes a media containing succinic acid which reduces plant growth, and maintenance of plants under lighted conditions at 6-8 C . Cutting frequency is increased to 4-6 months reducing cutting frequency. A lighted low temperature incubator has been ordered and the the medium-term storage protocol will be initiated this fall.

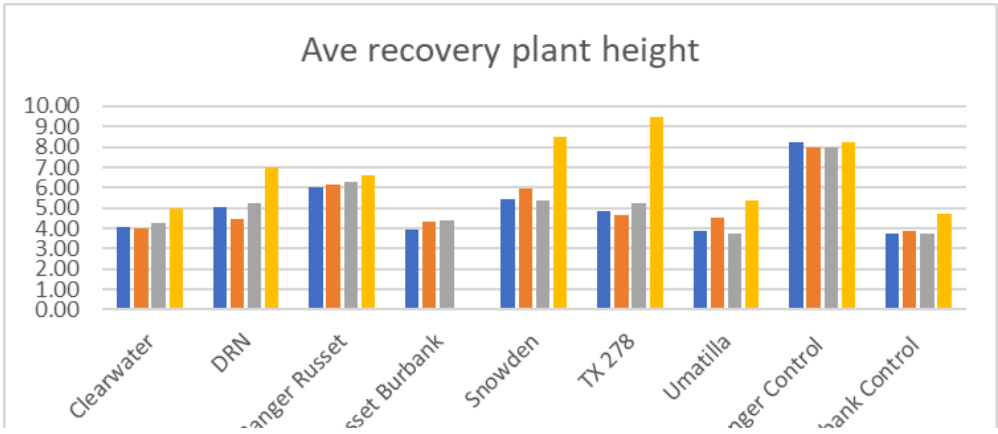


Sub-objective B: Establish a long term mother stock reserve

The goal of this objective is to create a backup mother stock inventory that can be recalled or refreshed without maintenance cutting for up to 2 years

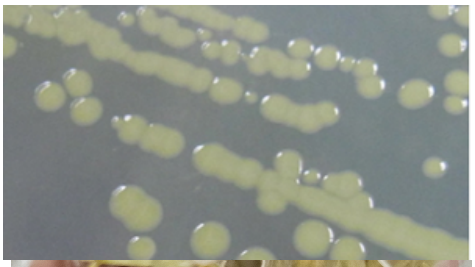
MIM (minituber induction media) is used for storage of mother stock. The process stores plants that develop in darkness to induce micro-tuber formation. The plants are then moved to a cold incubator (7°C), and kept for up to 2 years. Currently 4 recovery events have been evaluated, 3, 6, 9 and 12 months. The plants are removed from the cold room, grown at Room temp with 16/8 hr day night light cycles for 6 weeks, and then cut to normal media. Recovery plants are growing as expected.

This process is vital for asset redundancy. In the case of a catastrophic event (mass contamination, or overheating) we have a system in place to recover all our current varieties without needing to import and quarantine until proven clean.

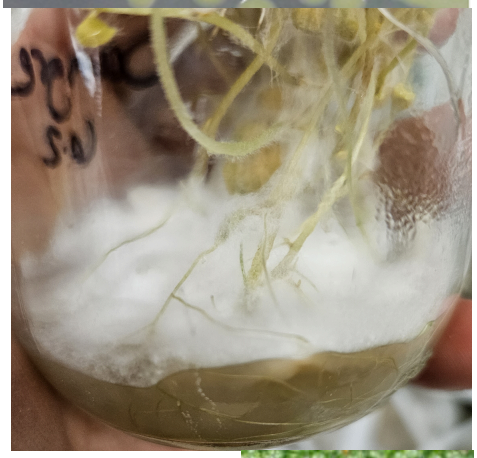


Sub-objective C: Establish a protocol for preventing and treating microbial and insect contamination.

Contamination in tissue culture is one of the largest threats to the maintenance of disease-free mother stock. Even with precautions (aseptic technique, sealing jars, sticky cards, insecticide foggers), thrips can contaminate tissue culture stocks. Thrips spread bacteria and fungus, which can be propagated.

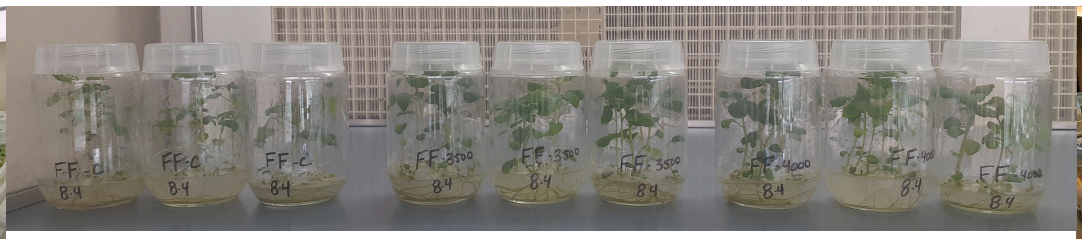


Multiple antibiotics and insecticides were evaluated for their ability to reduce contamination while maintaining normal plant growth. The most effective insecticide is Orthene, a water soluble insecticide that works systemically (through out the plant). We use 1 mL of orthene solution/L of media. The Orthene used in the lab is 13g (75% active ingredient) mixed into 100mL distilled water. It is used cyclically throughout the year to prevent new infestations.



When microbial contamination does appear, antibiotics are added to the media. We tested several antibiotics at multiple concentrations to see where the optimum level of microbial control with the fewest side effects to the plants is. The best antibiotic we have found is **carbenicillin at a rate of 100mg/L.** This antibiotic can be used safely at a fairly high rate with minimal negative effects on the tender plantlets. Other antibiotics tested had much lower tolerances, and in some cases resulted in poor growth and vigor that persisted when plants were cut to fresh media.

Objective 2: Convert plant growth lights from fluorescent to LED



The goal of this objective is to increase efficiency, and reduce long term costs of both energy and cooling costs required in the TC room.

The tissue culture room lighting has been in place for over 20 years. The fluorescent lighting that was originally installed emits a large amount heat requiring an AC unit to run full time to keep the plants at optimal temperatures. It also requires diligence to replace burned out bulbs, and keep the spectrum balance for optimal plant growth.
A Shift to LED lighting has been discussed for a while now, but looking into what the best options are has been daunting. There are so many choices and many of the LED grow lamps designed for greenhouses are purple. It was decided that the purple lighting was not ideal for our program as the color made identifying contamination much more difficult.

MSU facilities sourced adjustable LED lighting, adjustments on the lights include 3 color spectrums and 3 dim levels. Experiments were done on 9 varieties (5 of the most common russets, and 4 specialty lines) to determine if the LED lighting sourced from MSU resulted in optimal plant growth and vigor. The three lighting levels and three color spectra were evaluated.

The LED lights set to spectrum setting 4000 at 4000 lumens produced plants that were greener, stouter, and had overall better vigor than plants that were grown using flourescent lights. Plant height was shorter under fluorescent light early in the experiment, but as the experiment progressed, plants under LED lights caught up and about half of the selected lines were taller.

The Lithonia Lighting CSS series LED lights have been installed in the Tissue Culture room and set to a median color spectrum and median intensity.

Field Trip: University of Wisconsin - Madison

Tissue culture staff got the opportunity to attend a conference in Madison Wisconsin.
While there, we had the opportunity to observe the tissue culture system they have in place.
UW-Madison has successfully utilized both the MIM mother stock Long term storage, and the Succinic acid extension media for maintaining WI's potato mother stock. They utilize Percival cool incubators and a positive pressure growth room for recovery that helps keep contamination to a minimum. We look forward to having similar success with the protocols we are adopting to improve efficiency, cost and manpower to keep our mother stock clean and accessible.