

On Farm Tissue Culture Production of Lingonberries

Abstract

Nearly all lingonberry cultivars available commercially in the United States are from Europe and exhibit two flowering periods per season. In Alaska, the first flowering occurs too late in the summer to produce mature fruit. Locally collected plant materials provide for development of lingonberry cultivars adapted to regional growing conditions. While lingonberries root readily from stem cuttings, there are several problems associated with stem cutting propagation: large numbers of stock plants are necessary to provide enough stem cuttings for commercial production; plants propagated from stem cuttings rarely produce rhizomes and do not form productive matted rows; and research in Sweden has suggested that plants from stem cuttings may have a limited life span that will eventually necessitate replacement of entire fields. Tissue culture provides an alternative for rapid propagation of large quantities of local cultivars. No commercial tissue culture labs exist in Alaska, so we developed a small on-farm tissue culture laboratory that is used to propagate local material for our own needs and eventually other local growers. The most significant challenges included: minimizing culture contamination, adequate ventilation and cooling in a very small laboratory space, defining optimum light and temperature environment in a location where typical experimental design was impossible, developing a system of microshoot rooting that minimized space, and maintenance of rooted cuttings for several months in winter prior to planting. Microshoot production has been extremely successful using Woody Plant Medium and Plant Preservative Mixture biocide to minimize contamination. Rooting was accomplished in thin layers of a peat-based medium rolled jelly roll fashion in plastic film. Although microshoot production was successful, rooting failures have been unacceptably high and require additional research.

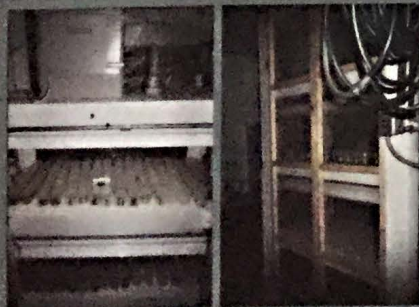
Introduction

The first cultivated fields of lingonberries, *Vaccinium vitis-idaea*, in Alaska, were established using the European cultivars, 'Red Pearl' and 'Sanna' as well as 'Regal' and 'Splendor' from Wisconsin, USA. All of these cultivars have two flowering and fruiting periods per season. In Trapper Creek, Alaska, fruit ripening occurs in July, and the second flowering begins in October. After three years of field growth, none of these cultivars was deemed acceptable because the length of Alaska's summers is too short to support the development of fruit from the second, and largest flowering period. Consequently, selection from wild populations of lingonberries in Alaska was begun to identify superior strains of locally adapted plant materials. One significant problem has been the lack of propagation facilities for increasing selected strains. Tissue culture has been shown to be the superior method of vegetatively propagating lingonberries. The objective of this project was to develop an on-farm tissue culture facility for rapid propagation of selected Alaska strains of lingonberries.

Facilities

A 4.3m by 3.7 m storage area in the farmhouse was converted to a laboratory. It included:

1. four tiers of shelves, 46cm x 1.3m, to accommodate standard nursery flats for growing cultures and for growing stock plants
2. work benches and shelves for lab supplies large laundry sink and dishwasher,
3. transfer room: (2.5m x 1.8m) with a 45cm x 90cm enclave and a portable sterile hood originally used for mushroom culture,
4. growth cabinet: one 4-tier shelving unit enclosed in clear plastic sheeting equipped with a circulating fan, heater and air conditioner, average temperature, 21-24°C,
5. and other necessary equipment: 1/1000 gram scale, hotplate stirrer, pH meter, refrigerator, dissecting microscope, large capacity pressure cooker for sterilizing equipment and cultures, and various lab glassware and equipment.



Culture chamber for microshoot production

Plant Selections

Stock plants were collected from two Alaska locations. Plants with large fruit size and upright growth habit were collected 440 km N of Anchorage, Alaska. Plants with small berries but large clusters (7 to 12 fruit per cluster) and upright growth habit were collected from Moose Creek Farm, 185 km N of Anchorage. These selections were transplanted to flats of peat and grown under fluorescent lights in the lab.

Culture Trials

Unreplicated trials were conducted using the most recent experimental research on lingonberries. They included:

1. determining an appropriate culture medium: Anderson's Rhododendron Medium versus Woody Plant Medium both with 20mg l⁻¹ sucrose and pH 4.8 for explant initiation,
2. use of Plant Preservative Mixture® as an experimental biocide
3. determining optimum levels of 2iP growth regulator, range 2mg l⁻¹ to 20mg l⁻¹
4. and determining best concentration of agar from 3.5 g l⁻¹ to 6.0 g l⁻¹

Best Medium

Woody Plant Medium with 15 mg l⁻¹ 2iP, 20 mg l⁻¹ sucrose, pH 4.8, 3.75g l⁻¹ agar. Plant Preservative Mixture® as biocide.

Best Protocol

1. Culture jars: baby food jars with 20ml of solution per jar, sterilized in a pressure cooker at 6.5 kg of pressure for 18 minutes.
2. Explants surface sterilized in 5% bleach solution with 2 drops Tween 20 in 500 ml solution. Full strength Woody Plant Medium plus 20ml l⁻¹ Plant Preservative Mixture® as soak for cleaned explants prior to transfer.
3. 4cm to 6cm explants, trimmed to expose lateral buds and scrubbed with a soft toothbrush in the bleach solution, rinsed three times in sterile water, then soaked for 72 hours in Plant Preservative Mixture® solution.
4. Explants horizontally in the medium, six to eight explants per jar.
5. Culture environment: 22.5°C average temperature, 55% relative humidity. Cool white fluorescent lights, 30 minutes alternating light/ dark cycles for 16 hours, and 8 hours of dark.
6. Subculture every four to five weeks.



Young microshoots under high light intensity



Young microshoots grown under low light conditions

Rooting of Microshoots

1. Microshoots: 6cm - 8cm length
2. Rooting medium: any of the following: perlite, vermiculite, peat moss (avoid coconut husk fibers)
3. Propagation rolls: sheets of plastic film covered with 10cm height and 2.5cm depth rooting medium, rolled jelly roll fashion into an 18cm round propagating roll.
4. Rolls enclosed in plastic bags and placed under the same light conditions as cultures.



Side view and top view of lingonberry microshoots 99 days (left) and 39 days (middle) from insertion into propagation rolls and newly planted cuttings (right).



Construction of propagation rolls



Propagation rolls enclosed in plastic bags in the growth cabinet

Cold Storage

A 2.5m by 3.7m cold room with shelves and heater was built to fulfill the chilling requirement for lingonberries. Once rooting was established and new plantlets began to set a terminal bud plants were transferred to the cold room for 720 hours of chilling at 10°C, then returned to the lab light tables for a second growth cycle. Stock plants were also transferred to the chilling room after setting a terminal bud and returned to the lab after 720 hours.

Major Problems

Contamination

Trials with high levels (10%) bleach as well as longer periods of scrubbing did not reduce contamination. Plant Preservative Mixture® biocide, reduced contamination levels to less than five percent of jars. It was also possible to salvage new explants that showed signs of contamination by re-cleaning and soaking in the Plant Preservative Mixture® solution.

Too Much Light in Culture Room

Under the 16-hr continuous light and 8-hr continuous dark photoperiod, the microshoots were mostly rusty red colored with very little green. The new leaves emerged green then quickly turn rusty red as they continued expanding. By alternating the light and dark photoperiods into 30 segments (30 minutes light/dark for 16 hours; 8 hours dark) we were able to maintain a very satisfactory green color for the new microshoots.

Poor Microshoot Rooting in Propagation Rolls

The propagation rolls used for rooting allowed for handling of large quantities of shoots in very small space. Rooting success has been inconsistent with this method because of difficulty maintaining high humidity within the bags and also avoiding disease.

Rooting and plant establishment were satisfactory on peat, perlite, and vermiculite but a shredded coconut husk fibers. Microshoots rooted in coconut husk fiber, but subsequent growth was poor; cuttings became rusty red and did not show vigorous above ground growth. Root cuttings transferred from the coconut husk fibers to a peat base medium recovered fully a continue development.

Abstract

Nearly all lingonberry cultivars available commercially in the United States are from Europe and exhibit two flowering periods per season. In Alaska, the first flowering period is insignificant and results in small yields. The second flowering occurs too late in the summer to produce mature fruit. Locally collected plant materials provide for development of lingonberry cultivars adapted to regional growing conditions. While lingonberries root readily from stem cuttings, there are several problems associated with stem cutting propagation: large numbers of stock plants are necessary to provide enough stem cuttings for commercial production; plants propagated from stem cuttings rarely produce rhizomes and do not form productive matted rows; and research in Sweden has suggested that plants from stem cuttings may have a limited life span that will eventually necessitate replacement of entire fields. Tissue culture provides an alternative for rapid propagation of large quantities of local cultivars. No commercial tissue culture labs exist in Alaska, so we developed a small on-farm tissue culture laboratory that is used to propagate local material for our own needs and eventually other local growers. The most significant challenges included: minimizing culture contamination, adequate ventilation and cooling in a very small laboratory space, defining optimum light and temperature environment in a location where typical experimental design was impossible, developing a system of microshoot rooting that minimized space, and maintenance of rooted cuttings for several months in winter prior to planting. Microshoot production has been extremely successful using Woody Plant Medium and Plant Preservative Mixture biocide to minimize contamination. Rooting was accomplished in thin layers of a peat-based medium rolled jelly roll fashion in plastic film. Although microshoot production was successful, rooting failures have been unacceptably high and require additional research.

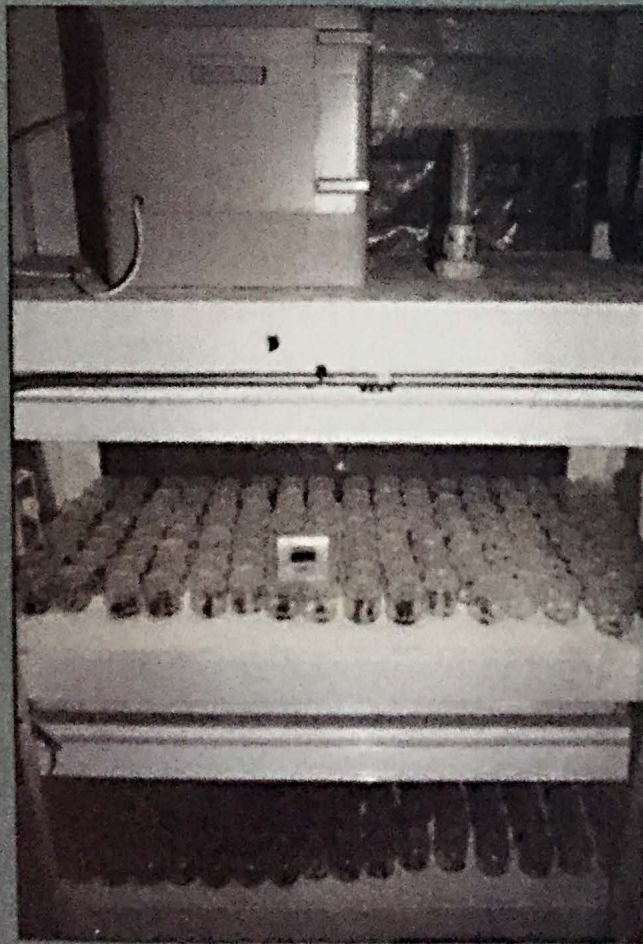
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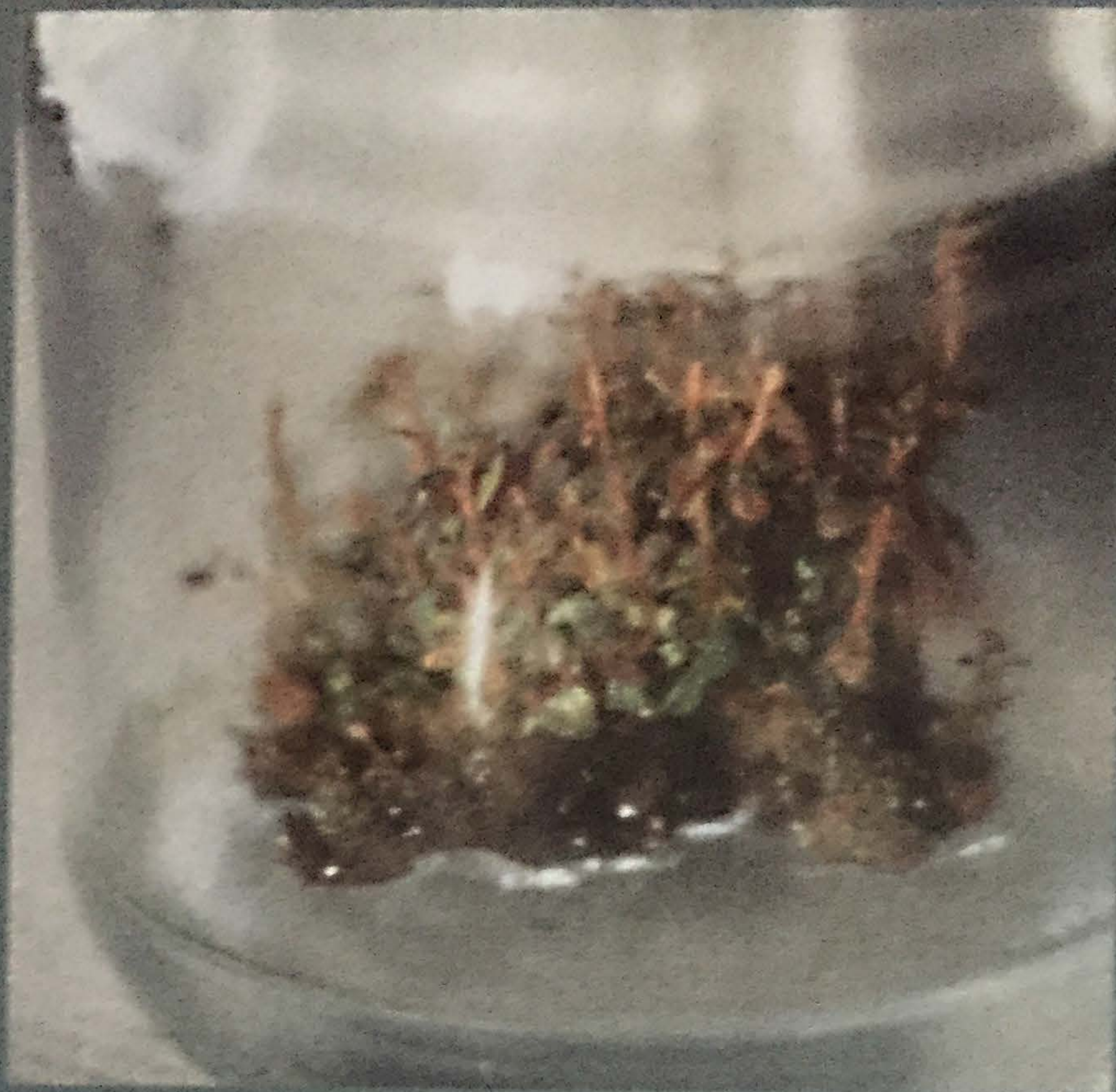
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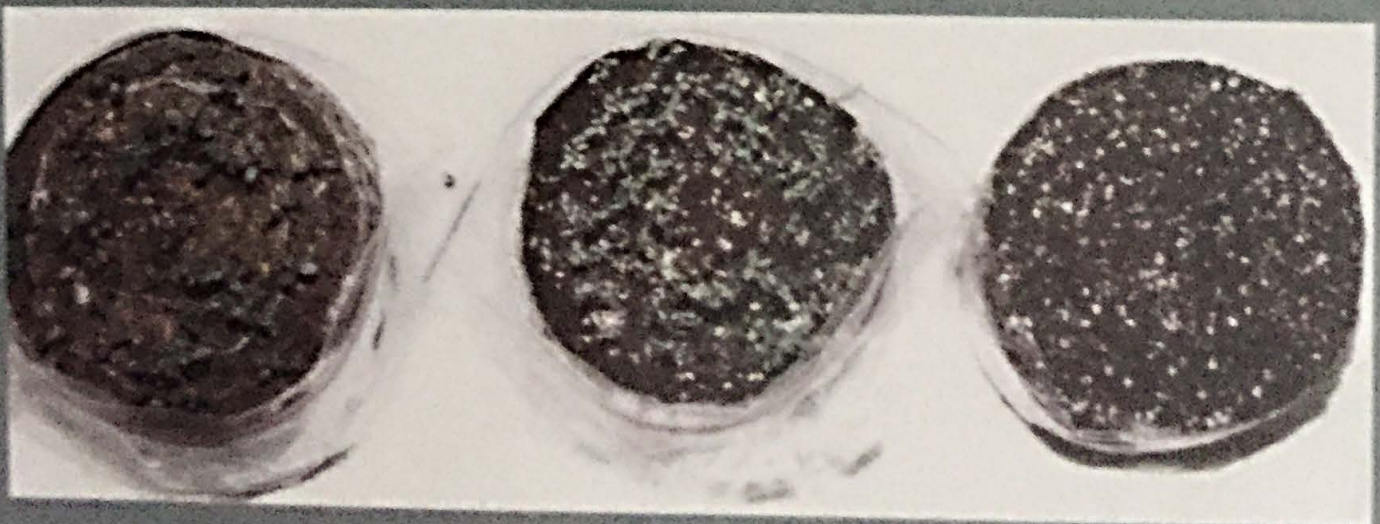
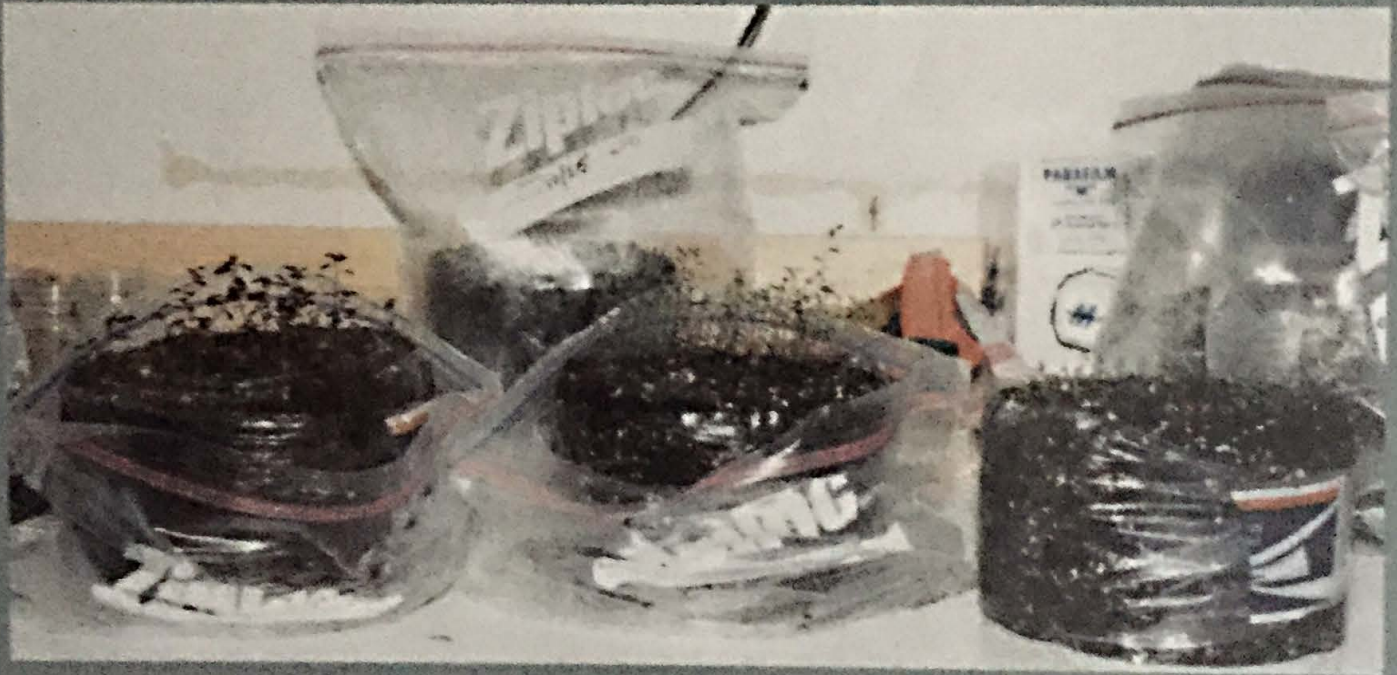
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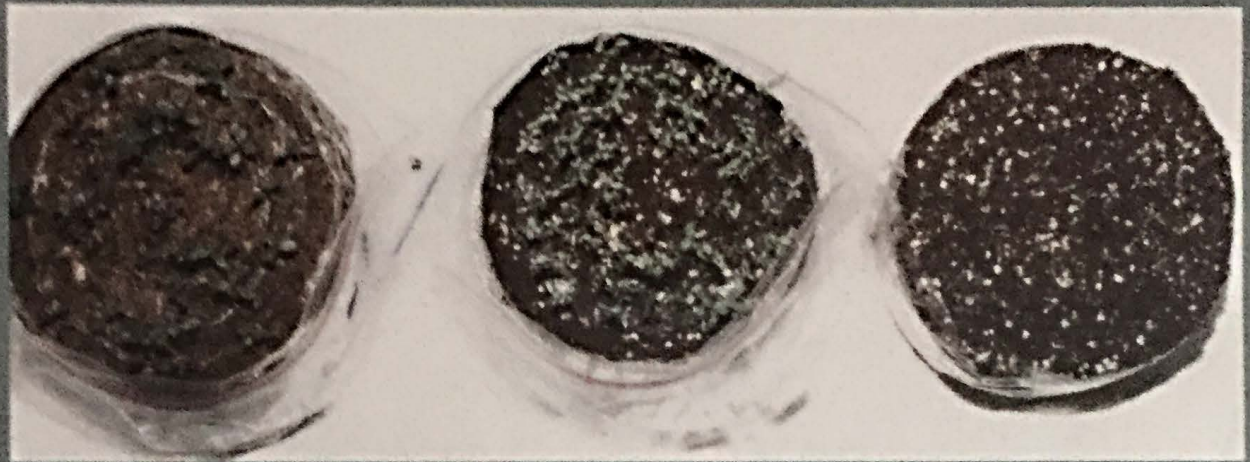
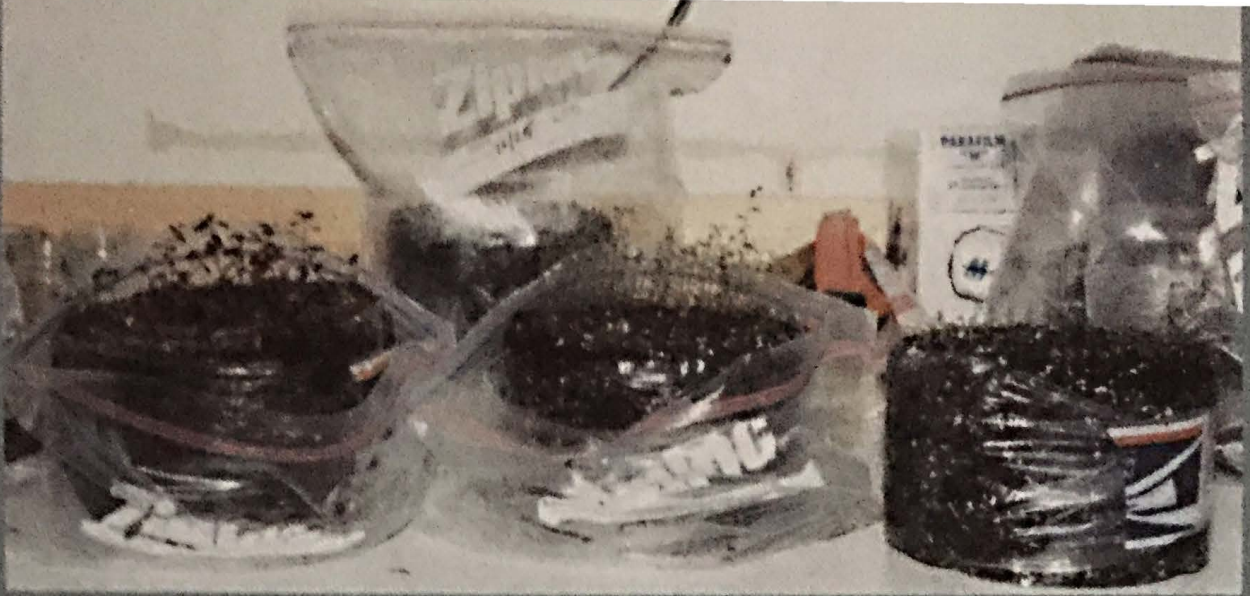
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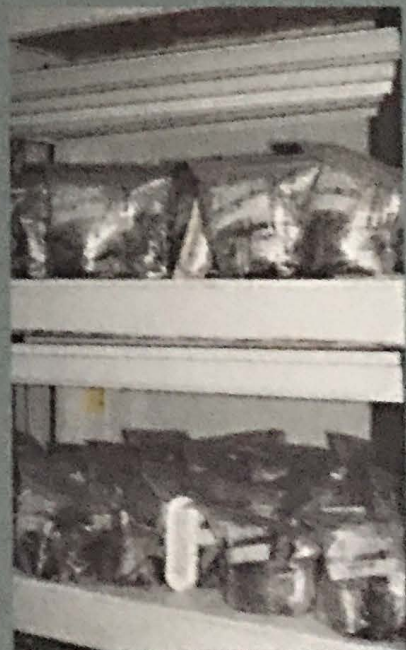
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