'Weed' be good together: Do Arbuscular Mycorrhizal Fungi form symbiosis with *Cannabis sativa* L.?

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Mitacs

Introduction

- Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that provide benefits to 80% of land plants[1], and can increase plant yield and secondary metabolite production in other crop plants[2].
- Cannabis growers use commercial mycorrhizae products with little evidence of their efficacy.
- Hemp is weakly mycorrhizal[3] but this has not been demonstrated for recreational (drug-type) cannabis.
- Degree of mycorrhizal symbiosis may vary between plants of different genotypes and in different growing conditions[5].

Figure 1. *Cannabis sativa* plants grown in rockwool and coco coir at low fertilizer rate, indoors at Doja Cannabis Company ltd.
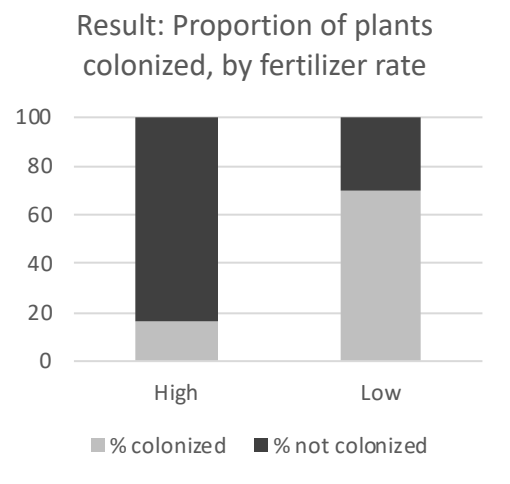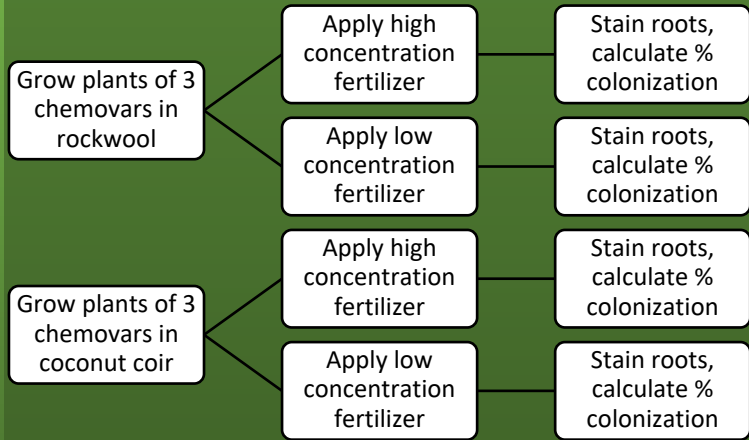

Figure 2. *Cannabis sativa* plants grown in rockwool and coco coir at high fertilizer rate, indoors at Doja Cannabis Company ltd.

Methods

Grow plants of 3 chemovars in rockwool → Apply high concentration fertilizer → Stain roots, calculate % colonization

Grow plants of 3 chemovars in rockwool → Apply low concentration fertilizer → Stain roots, calculate % colonization

Grow plants of 3 chemovars in coconut coir → Apply high concentration fertilizer → Stain roots, calculate % colonization

Grow plants of 3 chemovars in coconut coir → Apply low concentration fertilizer → Stain roots, calculate % colonization

Result: Proportion of plants colonized, by fertilizer rate

■ % colonized   ■ % not colonized

Figure 2. Proportion of cannabis plants that contained AMF and did not contain AMF.

Results

- 40% of plants were colonized with AMF
- Difference in colonization was based on fertilizer application rate
- High fertilizer tended to inhibit AMF colonization

Future Directions

Since we know that AMF can colonize cannabis under certain conditions, we can investigate the effect of AMF colonization on cannabis plant growth, yield, and cannabinoid and terpene production.

References

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Value-addition to haskap berry: development of anthocyanin-rich nanoparticles

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Introduction

- ❑ Haskap (*Lonicera caerulea* L.) is a berry fruit grown in Northern hemisphere including Canada.
- ❑ Ripe Haskap berries are rich in anthocyanins.
- ❑ Anthocyanins degrade during food processing and after ingestion.
- ❑ The entrapment of anthocyanins in polymers is a promising solution to reduce their degradation.

Objectives

- ❑ Determine the phytochemical properties of haskap berry (HB) extracts;
- ❑ Identify a non-toxic, suitable polymer to encapsulate anthocyanins in nanoparticles (NPs).

Methodology

1. Extraction of anthocyanins from haskap berry
2. Encapsulation of anthocyanins in 3 polymers:
   - ▪ Maltodextrin (MDX)
   - ▪ Carboxymethyl chitosan (CMC)
   - ▪ Poly (lactide-*co*-glycolide)-polyethylene glycol (PLGA-PEG)

NPs
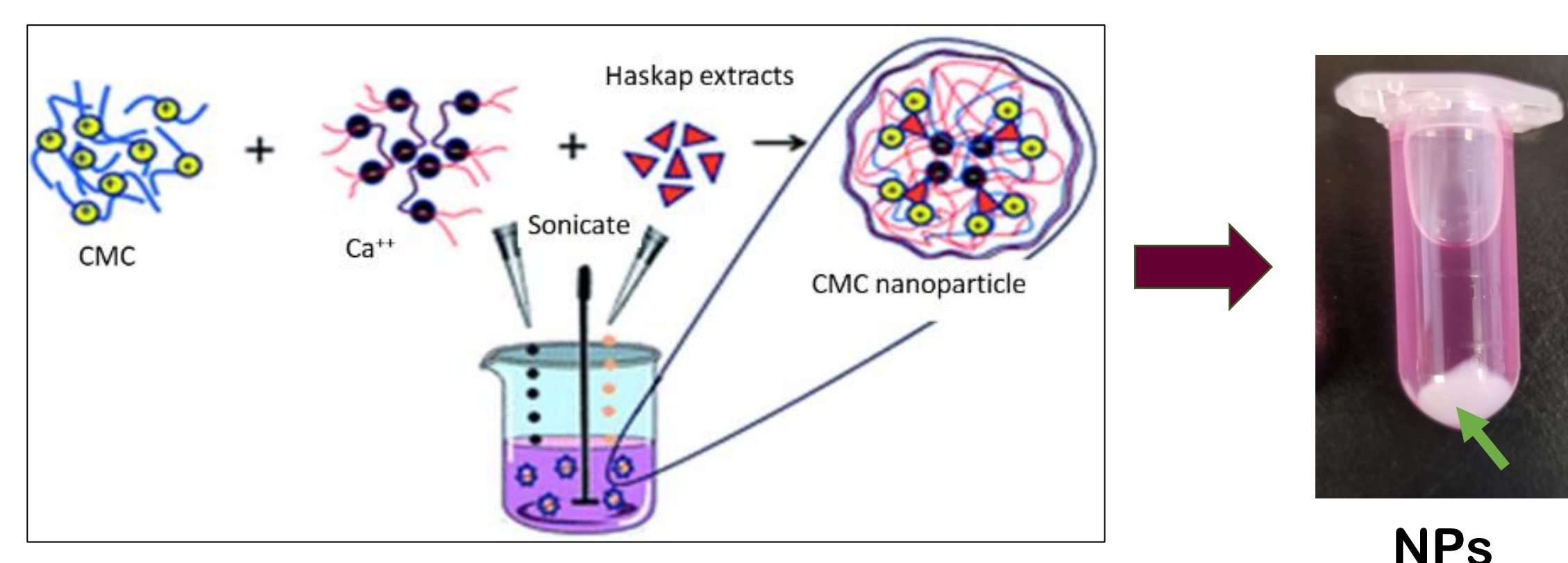
3. Test NPs for physicochemical properties.
4. Determine the effect of NPs on normal lung epithelial cell viability.

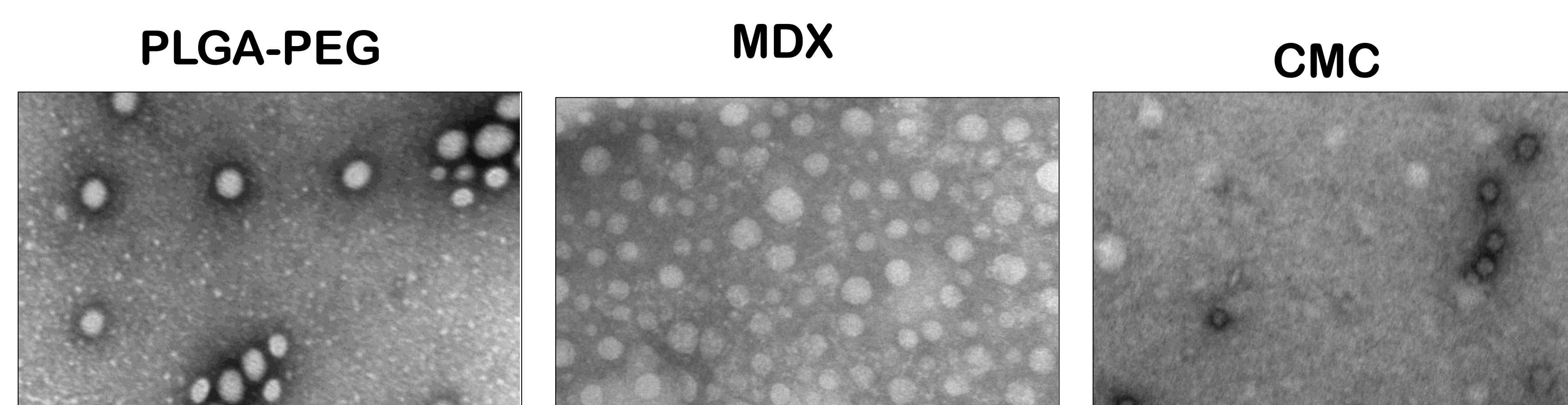NPs 1 - 400 µg/ml for 24 h → Lung cells → >80% viable cells

Results

PLGA-PEG   MDX   CMC
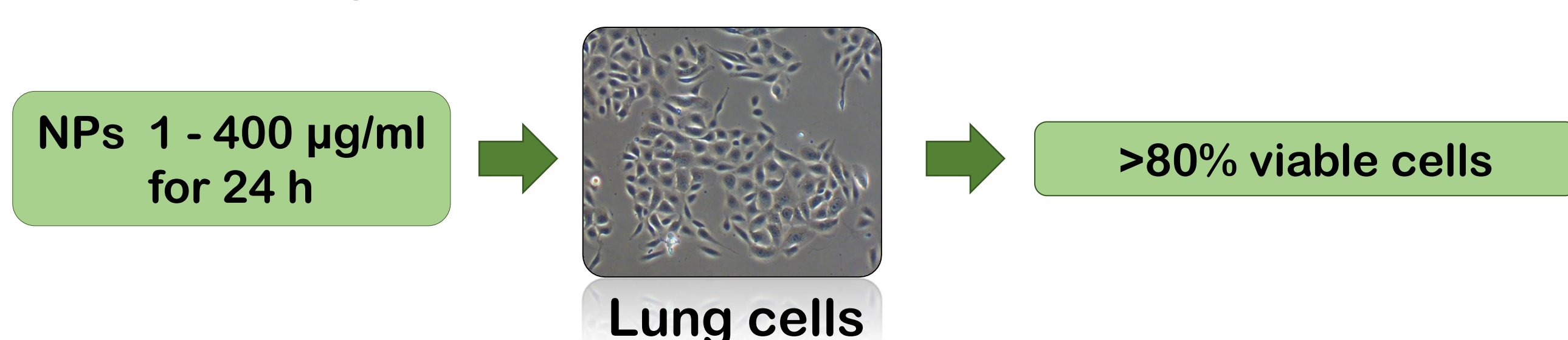
Figure 1. Morphology of the NPs, TEM images
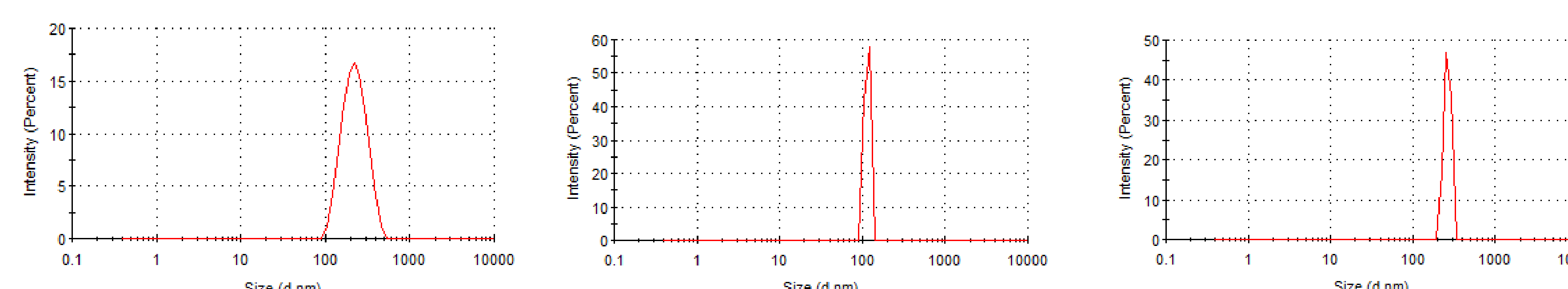
Figure 2. Particle size distribution of the NPs

Figure 3. The encapsulation efficiency of anthocyanins in NPs

Table 1. Physical properties of the NPs

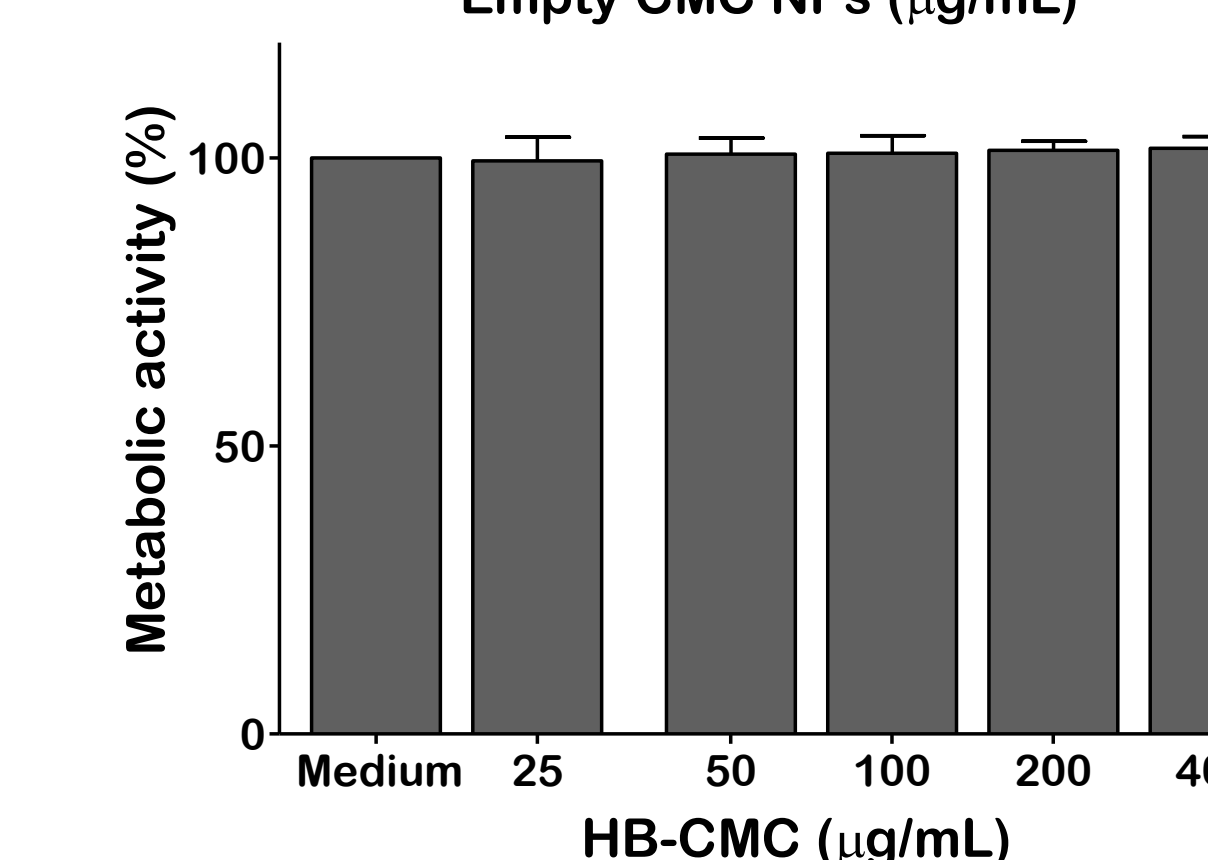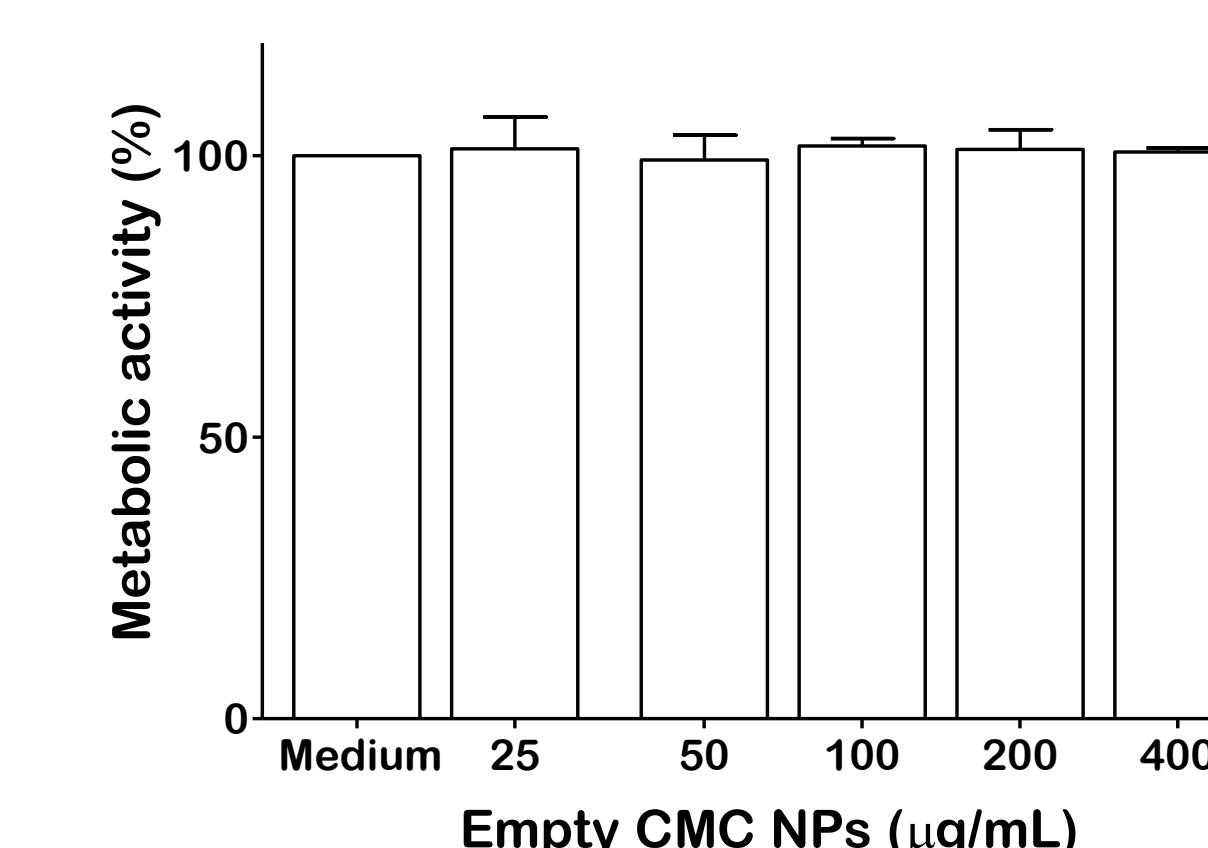| Sample	Particle size (nm)	Zeta potential (mV)	Total yield (%)
HB-CMC	163 – 170	30.4	63.0±5
HB-MDX	116-420	-0.5	94.0±1.0
HB-PLGA-PEG	110-280	-4.2	35.3±1.8

Figure 4. CMC is not toxic to the normal lung cells

Discussion

- ❑ Cyanidin-3-*O*-glucoside is the most abundant anthocyanin present in haskap berries.
- ❑ NPs were spherical shape and had monodispersed particle size.
- ❑ The encapsulation efficiency of anthocyanins was significantly higher in CMC than in the other two systems.

Conclusion

CMC is a promising non-cytotoxic material to encapsulate anthocyanins isolated from haskap berries.

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Analyzing the Role of N and K Fertilizer in After-Dormancy Yield Potential of Day-Neutral Strawberry

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Introduction

Background

- Day-neutral strawberry (DN) have been developed to produce fruit irrespective of photoperiod, allowing for an extended harvest season and higher yields (Durner *et al.,* 1984).
- However, DN variety strawberries tend to suffer in yield potential following winter dormancy compared to short-day (SD) strawberries, especially in northern climates (Gagnon *et al.,* 1990).

Solution

- It has been observed that source and concentration of nitrogen can optimize nutrient storage through in strawberry transplant crowns and facilitate optimal growth after breaking dormancy (Human & Kotze, 1990; Kirshbaum *et al.,* 2010; Lopez *et al.,* 2002).
- The balance between N and K fertilizer was also analyzed as K fertilization can also positively effect yield and nutrient storage within the crown in DN strawberries (Ahmad *et al.,* 2014).
- This study aimed to understand N and K fertilizer's role in DN transplants before, during, and after dormancy to establish a fertilizer guide for pre-dormancy transplants

Research Questions

1. **Which source of N (nitrate, ammonium or urea) at a low, medium of high concentration (50mg/L, 100mg/L, or 150mg/L) results in greatest flower bud induction and carbohydrate storage in DN transplant crowns?**

2. **Does the ratio of N:K at 1:1, 1:2 or1:4 v/v also have a significant effect on flower bud induction and carbohydrate storage within the crown?**

Methods

Experiment 1 considered N source and concentration, testing nitrate, ammonium, and urea supplied at 50mg/L, 100mg/L, and 150mg/L. monitored weekly phenology data and took weekly plant samples for dissection and biomass assays

Experiment 2 compared N:K ratios at 1:1, 1:2, and 1:4 intervals. This experiment also monitored weekly phenology data and took weekly plant samples for dissection and biomass assays
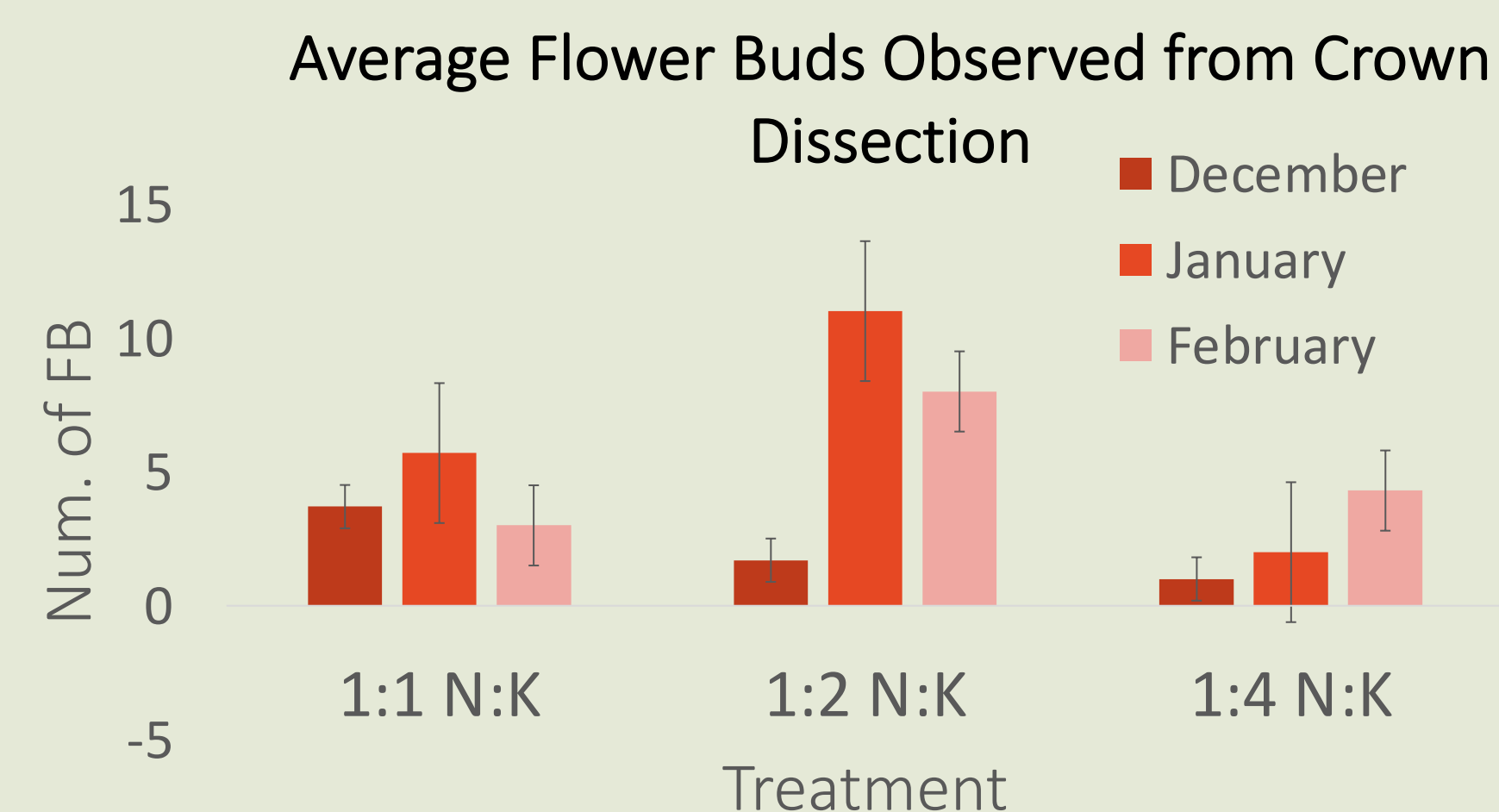
- Transplants for both trials were kept in cold storage in October to initiate dormancy, and samples were taken monthly for the same data parameters. Transplants were again sampled after dormancy.
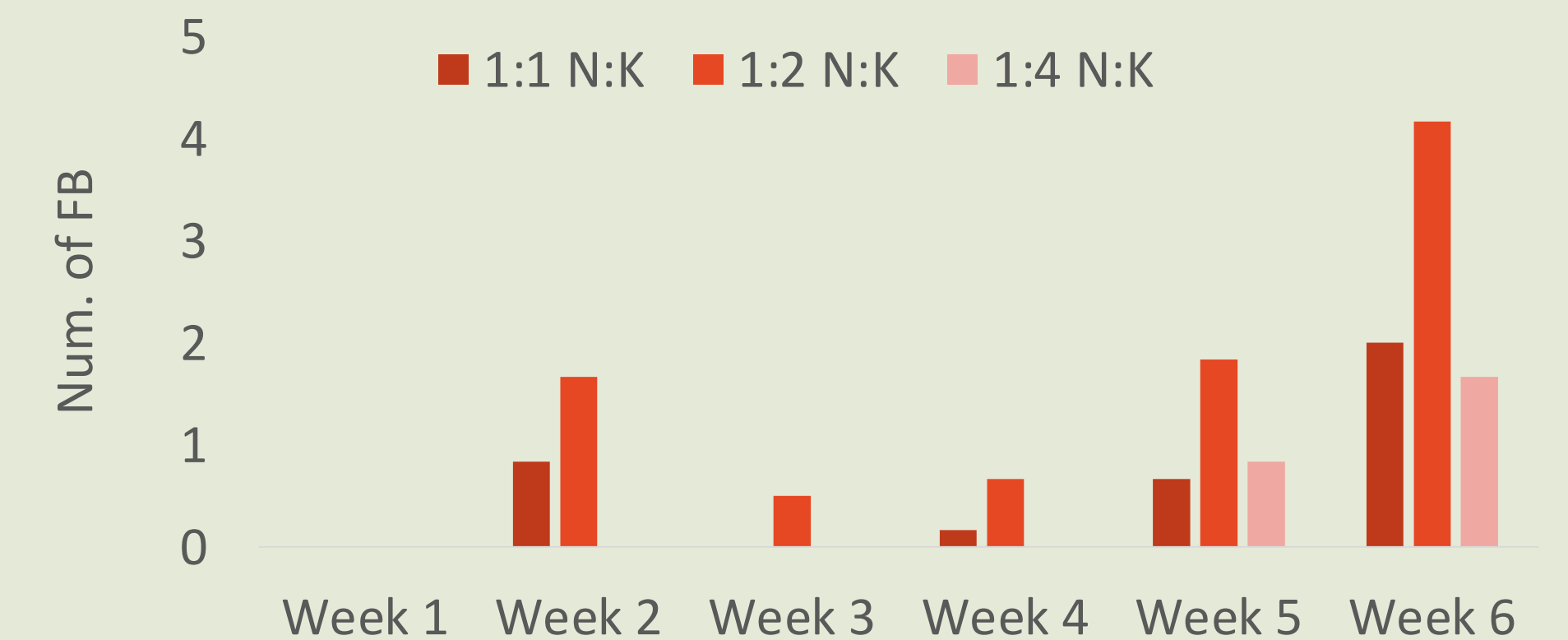
Results

<u>Experiment 1</u>: Results found no significance in data obtained in Experiment 1 before or during dormancy.

<u>Experiment 2</u>:

Average Flower Buds Observed from Crown Dissection

Legend: December, January, February

Y-axis: Num. of FB (-5, 0, 5, 10, 15)
X-axis (Treatment): 1:1 N:K, 1:2 N:K, 1:4 N:K

P- value of treatments:

Weekly Average Flower Buds Observed from Phenology Data

Legend: 1:1 N:K, 1:2 N:K, 1:4 N:K

Y-axis: Num. of FB (0, 1, 2, 3, 4, 5)
X-axis: Week 1, Week 2, Week 3, Week 4, Week 5, Week 6

P-value of treatment:

Conclusions

- Experiment 2 observed that a 1:2 ratio of N:K resulted in and more significant flower buds counted within the crown from dissections taken during dormancy

- Phenology data also saw a significant number of flower buds produced from the 1:2 N:K ratio prior to dormancy

- Results suggest that a 1:2 N:K ratio is optimal for increasing flower bud production in DN transplants after dormancy

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Acknowledgements

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Assessment of postharvest evolution of fruit quality in commercial blueberry varieties and new selections

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Introduction

Blueberries are high-valued fruits popular all over the world due to their pleasant flavors and plentiful bioactive components, vitamins, and mineral elements (Chiabrando and Giacalone 2011; Xu et al. 2016). Fruit quality (*e.g.*, sugar and acid content, texture, and color) is considered as the predominant trait in blueberry, since it drives consumers' appreciation and provides opportunities for packers and marketers to command premium price points. The marketability of blueberry depends not only on the fruit quality as they are harvested, but also maintaining of fruit quality during storage conditions, as blueberries experience long distance travels before going into the international markets.

The development and evaluation of new breeding selections with premium fruit quality is a priority for the blueberry industry in British Columbia (BC). In this study, blueberry samples were collected from 6 commercial varieties and 20 new selections in BC's berry breeding program in summer of 2019, and the effect of storage time on blueberry quality was studied. After being harvested, blueberry samples were stored at 0.5 °C for up to 4 weeks for the analyses of berry weight, water loss, fruit texture, sugar (*i.e.*, total soluble solids, TSS) and acid (*i.e.*, titratable acidity, TA) content at 0, 2, and 4 weeks after cold storage.

Methodology
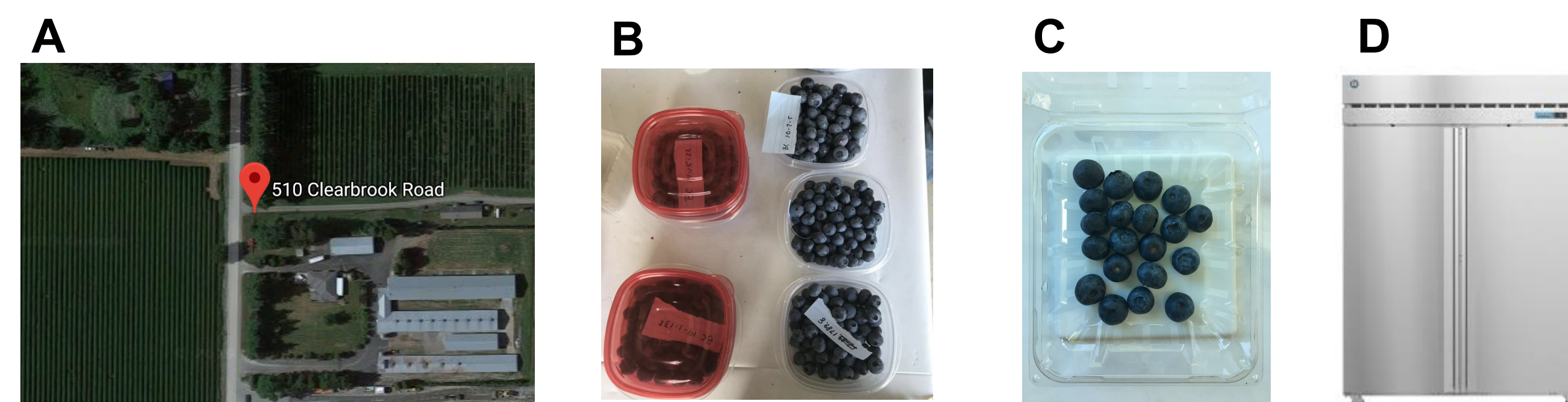
Figure 1. Blueberry sample collection and storage in this study. (A) Blueberry fields in this study. (B) Harvested blueberries of different varieties and selections. (C) An example of clamshell containing blueberry sample for postharvest storage and fruit quality analysis. (D) Blueberry sample storage at 0.5 °C.
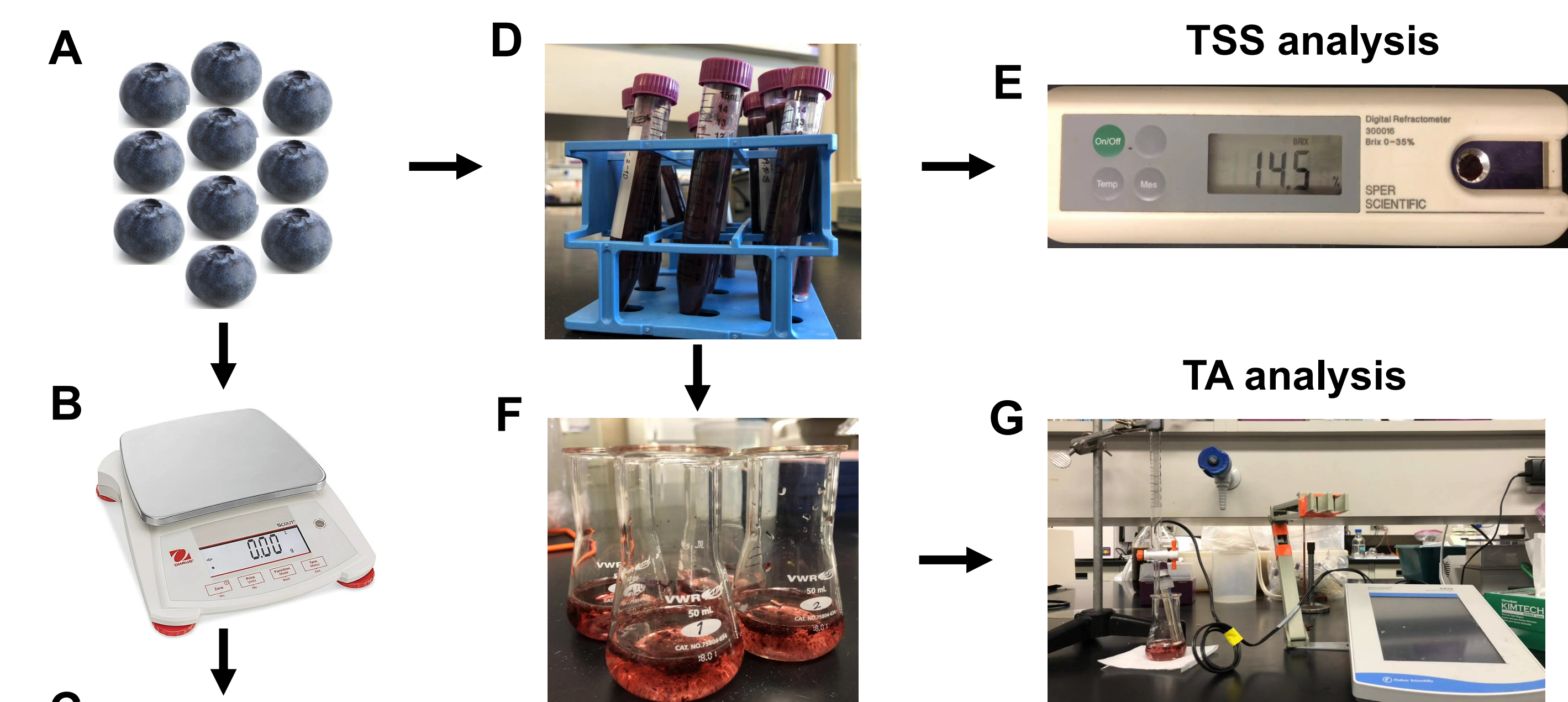

Figure 2. Blueberry sample preparation and analysis for fruit quality. Blueberries were divided into 2 aliquots containing 10 blueberries each. One aliquot was used for berry weight and water loss determination (B) and texture analysis (C, by a Texture Analyzer); the other aliquot was ground by a blender and the puree was used for the analysis of TSS (E, by a refractometer) and TA (G, by titration with NaOH until pH 8.2)

Results
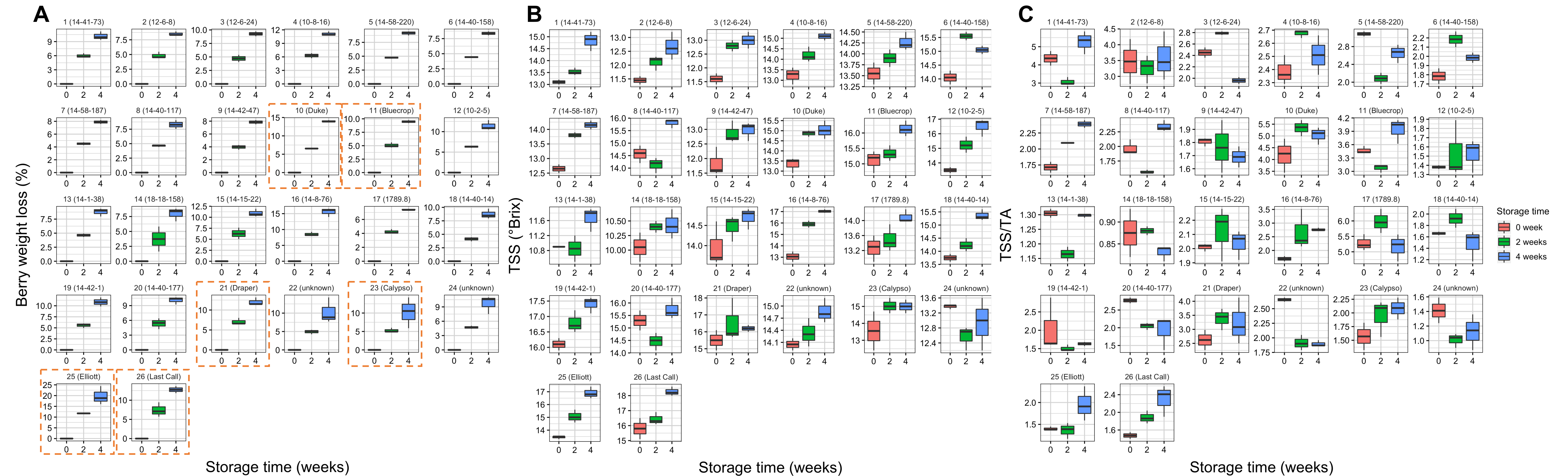
Figure 3. Box plot indicating loss of berry weight (A), changes in TSS (B) and the ratio of TSS to TA (C) in 26 blueberry varieties and new selections, during a storage time of 0-4 weeks.
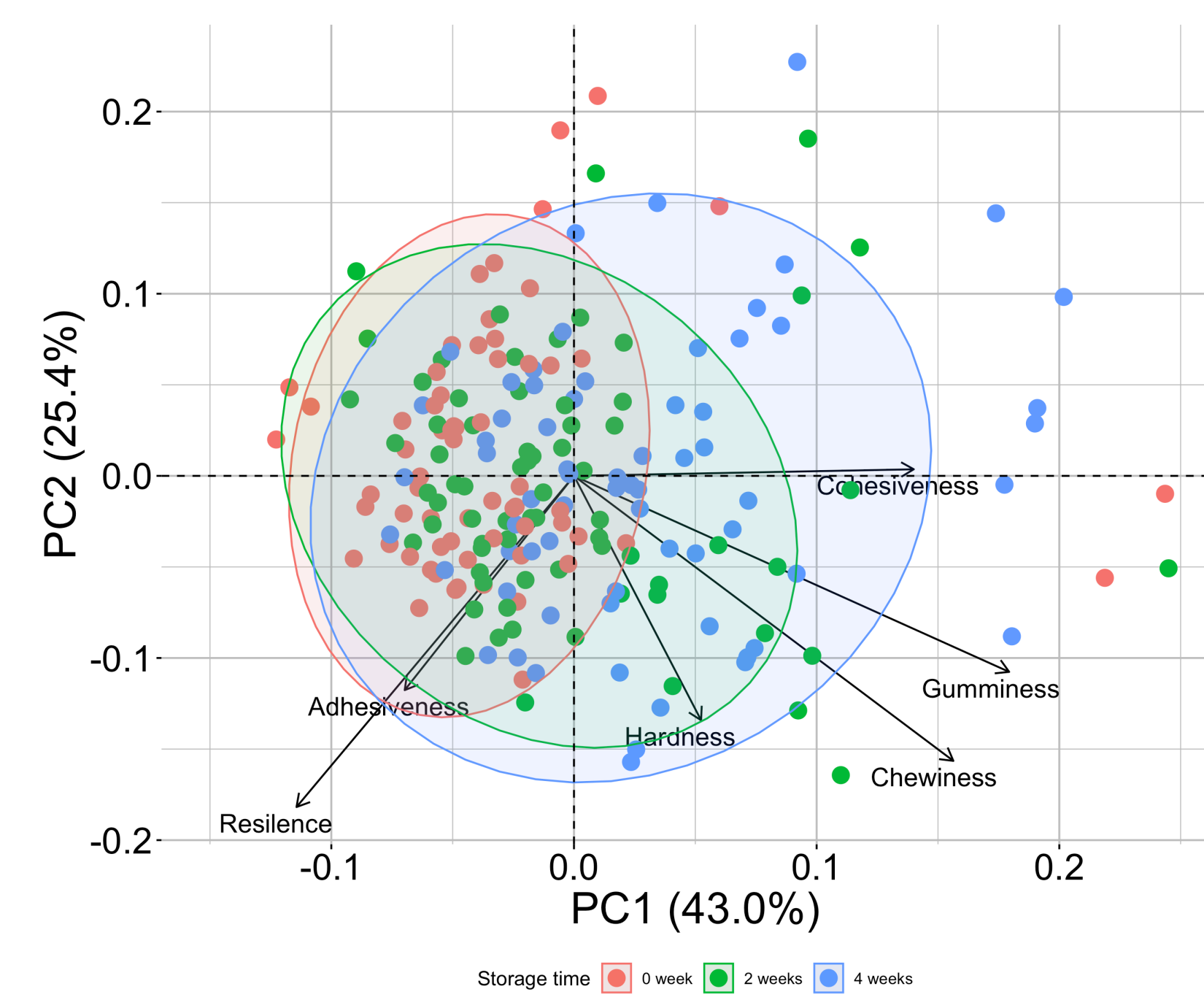
Figure 4. Principal component analysis (PCA) on blueberry texture in 26 varieties and new selections during a storage time of 0-4 weeks. The red dots refer to samples at harvest (0-week storage), the green dots refer to samples after 2-week storage, and the blue dots refer to samples after 4-week storage; the ellipse represent 95% of confidence interval. The arrows refer to all the variables (loadings) analyzed.

Table 1. Pearson correlation between weight loss, total soluble solids change, and hardness change during postharvest storage.

Pearson correlation from 0 to 2 weeks	Berry weight loss	Total soluble solids change	Hardness change
Berry weight loss	1	0.329	0.027
Total soluble solids change	0.329	1	-0.220
Hardness change	0.241	0.027	1

Pearson correlation from 2 to 4 weeks	Berry weight loss	Total soluble solids change	Hardness change
Berry weight loss	1	0.633**	-0.523*
Total soluble solids change	0.633**	1	-0.220
Hardness change	-0.523*	-0.220	1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Conclusions

- Loss of blueberry weight (*i.e.*, water loss) during storage differed among varieties and selections, with most of the new selections showing lower weight loss in comparison with the commercial varieties.

- Total soluble solids (TSS) increased during storage, with the increase between 2 and 4 weeks of storage correlating with water loss.

- The pattern of titratable acidity (TA) change during storage differed among blueberry varieties and selections, but the ratio of TSS to TA was within an acceptable range of 1.0-3.3 for most varieties and selections.

- In most blueberry varieties and selections, fruit hardness increased between 0 and 2 weeks of storage and decreased between 2 and 4 weeks of storage. This decrease in fruit hardness was correlated to water loss.

- These results provide new insights on the evolution of blueberry quality features during postharvest storage, which will be useful for selecting new varieties in BC.

References

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Acknowledgement

POTENTIAL FLAVINS SECRETING ENDOPHYTIC BACTERIA OF APPLE (*Malus domestica*) ROOTS AND THEIR EFFECT ON PLANT GROWTH PROMOTION

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1. Abstract

Flavins (FLs) are essential molecules to carry out numerous flavoprotein-mediated redox reactions in a variety of metabolic pathways. This study is focused on isolation and characterization of FL secreting endophytic bacteria from apple (*Malus domestica*) roots and determine their **P**lant **G**rowth **P**romoting (PGP) effect. Minimal mannitol ammonium (MMNH$_4$) media will be used to isolate FL secreting endophytic bacteria. Determination of FL secretion in growth media will be done by measuring relative fluorescence at excitation wavelength of 470 nm and emission wavelength of 530 nm using Bio-Tek Synergy H1 Hybrid Multi-Mode Reader and the Gen5 software application. The readings will be normalized to OD$_{600.}$ The isolates with highest 470/530 fluorescence will be selected. Those potential FL secreting isolates will be assessed for other PGP functions. Phosphate solubilization test will be done using Pikovskaya's (PKV) agar medium, containing insoluble tricalcium phosphate (TCP). Nitrogen fixation assessment will be done by using Jensen media (N free solid media) and I*n vitro* 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity test will be done in M9 minimal media supplemented with 3 mM ACC, production of indole acetic acid (IAA) will be assessed by the colorimetric method using Salkowski reagent (0.5M FeCl$_3$ + 70% perchloric acid). Alfalfa (*Medicago sativa*) plant will be used to test the ability of isolate to promote plant growth in laboratory condition. The harvesting will be done after 5 weeks and dry mass of the plants will be evaluated to assess the plant growth promotion. Our hypothesis is that FL secretion might be a novel PGP bacterial function, which can enhance the plant growth and development. If our hypothesis is correct, FL secreting PGP bacteria could be considered as eco-friendly and greener alternative to chemical fertilizers and pesticides and could act as the biofertilizer.

Key words: Plant Growth Promotion, Flavin secretion, Phosphate solubilization, N fixation, ACC deaminase and Indole Acetic Acid production

2. Introduction

The interactions of beneficial microbe with plants are vital to plant development, health, and stress resistance (Kumar and Verma, 2018). This interaction, especially root-associated bacteria is one of the powerful tools to increase crop productivity and reduce production costs in agricultural practices by reducing the usage of fertilizers and pesticides. Microbial inoculants act in an eco-friendly way to create better quality and healthy agricultural products. Plant growth-promoting bacteria (PGPB) can enhance plant growth and protect plants from abiotic stresses and diseases through the facilitation of nutrients uptake and production of specific compounds like siderophores, and assisting plants to tolerate or resist pathogens (Hayat *et al*., 2010). Several important bacteria characteristics such as biological nitrogen fixation (BNF), phosphate solubilization, ACC (1-aminocyclopropane-1-carboxylic acid) deaminase activity, and production of indole acetic acid (IAA) are the plant growth-promoting (PGP) functions. Recent researches focus on FL, which is one of the potential bioactive molecule secreted by PGPB. FL secretion would be a novel trait in PGPB (Yurgel *et al*., 2014).

FLs are identified as one of the most chemically diverse prosthetic groups of biochemistry. Riboflavin (RF, 7, 8-dimethyl-10-ribitylisoalloxazine), commonly known as vitamin B2. It is essential for all organisms and playing a vital role in oxidative metabolism. RF is an essential constituent of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). FMN and FAD are necessary to carry out numerous flavoprotein-mediated redox reactions in a variety of metabolic pathway. They act as essential cofactors for a multitude of mainstream metabolic enzymes and electron transfer reactions (Jordan *et al*., 1999). RF is easily converted enzymatically or photochemically into lumichrome (Yurgel *et al*., 2014). This compound also considered as potent molecules that stimulates plant growth and development (Matiru and Dakora, 2005).

3. Materials and Methods

Experiment_1: Isolation of FL secreting bacteria from roots
Sample collection and Root tissue preparation

⬇

❖ Culturing and Isolation of bacteria associated with roots

⬇

Screening of FL secreting bacteria (Relative fluorescence measurement - Hybrid Multi-Mode Reader and the Gen5 software application)
Experiment_2: Analysis of other Plant Growth Promotion Functions
Phosphate solubilization test by using Pikovskaya's Media

⬇

Nitrogen fixation assessment by using Burk's media

⬇

In *vitro* 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity test by using DF salt minimal media supplemented with 3 mM ACC

⬇

Indole acetic acid (IAA) production test by using Colorimetric method using Salkowski reagent (0.5M FeCl$_3$ + 70% perchloric acid)
Experiment_3: Plant Growth Test with inoculation of FL secreting bacteria
Alfalfa seeds sterilization and germination on water agar

⬇

Inoculate after transplanting of pre-germinated seeds into magneta boxes

⬇

The plants will be harvested after 5 weeks to take dry mass for statistical analysis

4. Expected Outcomes

❖ Develop a reliable and efficient method for isolation of FL secreting bacteria, which will be used to create a collection of microorganisms with novel mechanisms of PGP

❖ Isolated potential FL secreting bacteria do not exhibit any other PGP function and their PGP effects might be attributed to FL secretion, which might improve growth of host plant

5. Future Direction

❖ In order to evaluate the role of bacterial derived FL act as PGP molecule, create a mutant which will loose the ability to secrete FL

❖ According to the genetic characterization, find the gene responsible for the FL secretion in potential FL secreting bacterial isolates

❖ Apply the microbiome studies on the role of FL secreting bacteria on plant growth and development

6. References

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Hybrid Multi-Mode Reader

Plant growth test in Magneta boxes

Genetic Diversity and Population Structure Analysis in Asparagus

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Introduction

- Asparagus is one of the most predominant and commercially significant species of genus asparagus with impressive nutrients and medicinal properties.
- Asparagus is thought to have a narrow genetic base although breeders have selected for diverse uses and adaptation to different climates.
- Understanding genetic diversity in asparagus germplasm, especially the occurrence of common or distinct alleles among or within the different use or adaptation groups would offer useful insight for breeding programs.

Objective

➢ To analyze the genetic variability among different accessions of asparagus using SNPs.
➢ To determine the genetic relationship between globally distributed asparagus germplasm based on environmental adaptation and commercial uses.

Material and Methods

- **Germplasm:** a total of 68 asparagus genotypes were used in this study.
- **DNA extraction:**
  - Using commercially available NucleoSpin Plant II kits from Machery-Nagel.
- **Genotyping:** The GBS approach was used to identify SNPs.
- **Data analysis**
  - **Structure analysis:** The model based genetic clustering algorithm in STRUCTURE v 2.3.3 software package was used to determine the population structure of all the accessions.
  - **Principal Component Analysis (PCA):** PCA analysis was conducted in TASSEL 5.2.38 and plots were generated in R package.
  - **Genetic Diversity Analysis:** To compare and examine the diversity among various asparagus genotypes distinct indices such as allele frequency, observed heterozygosity, gene diversity and polymorphism information content were calculated using PowerMarker v 3.25.
  - **Phylogenetic tree:** Dendrograms were constructed using Neighbor-Joining method in TASSEL and plots were visualized with Dendroscope 3.2.10
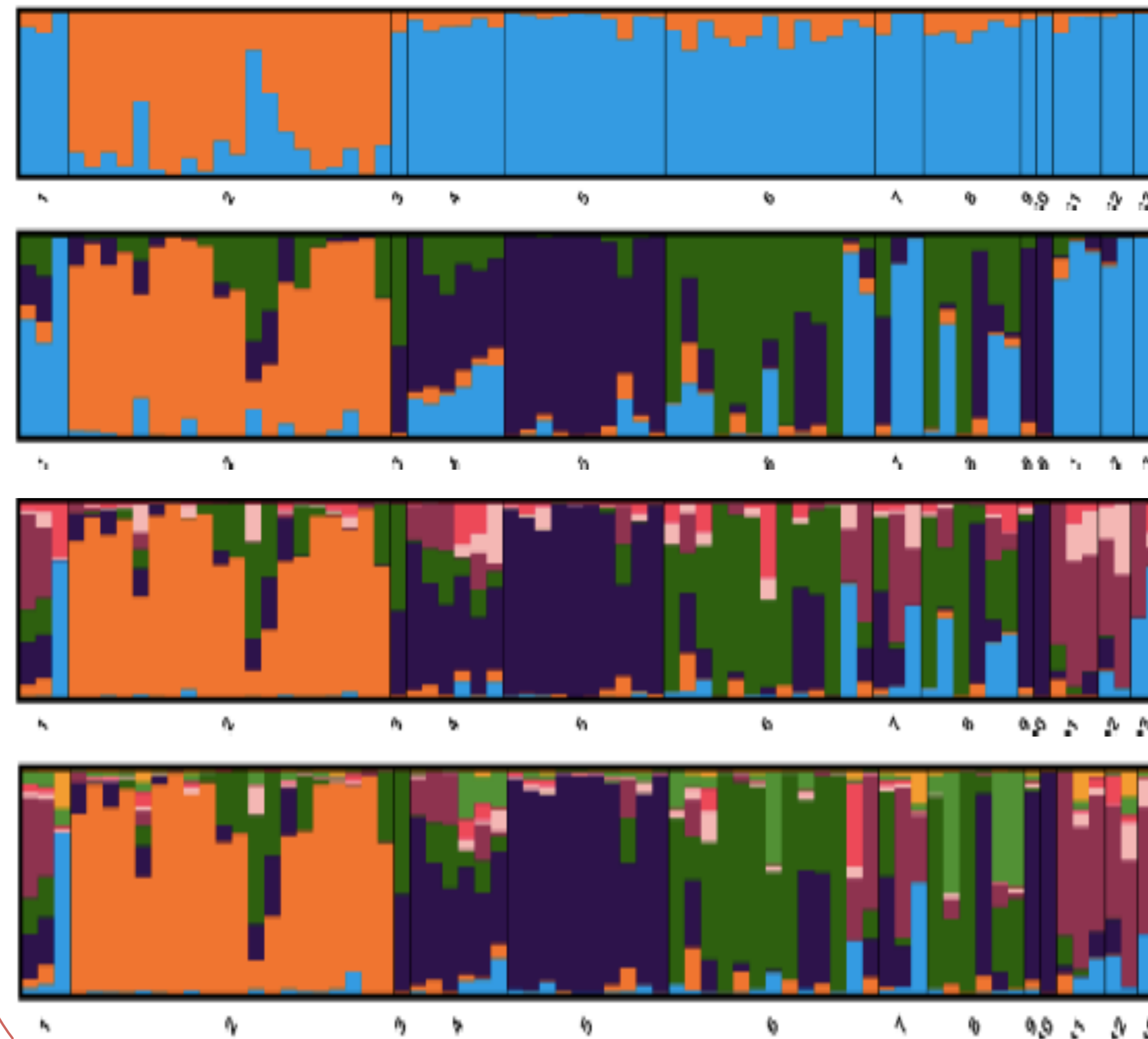

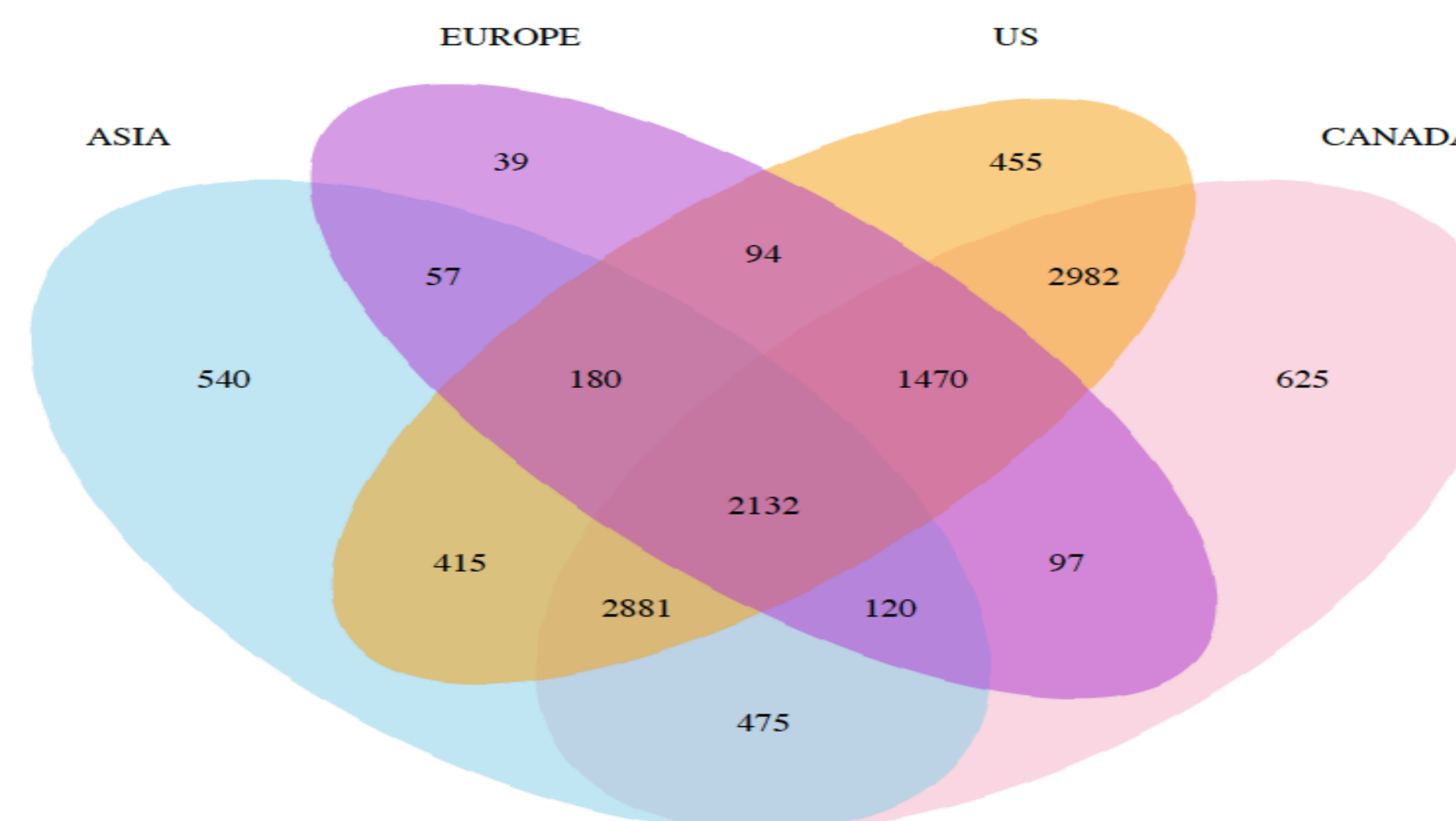Fig 1: Population structure of 68 asparagus lines from K=2, K=4, K=7 to K=9

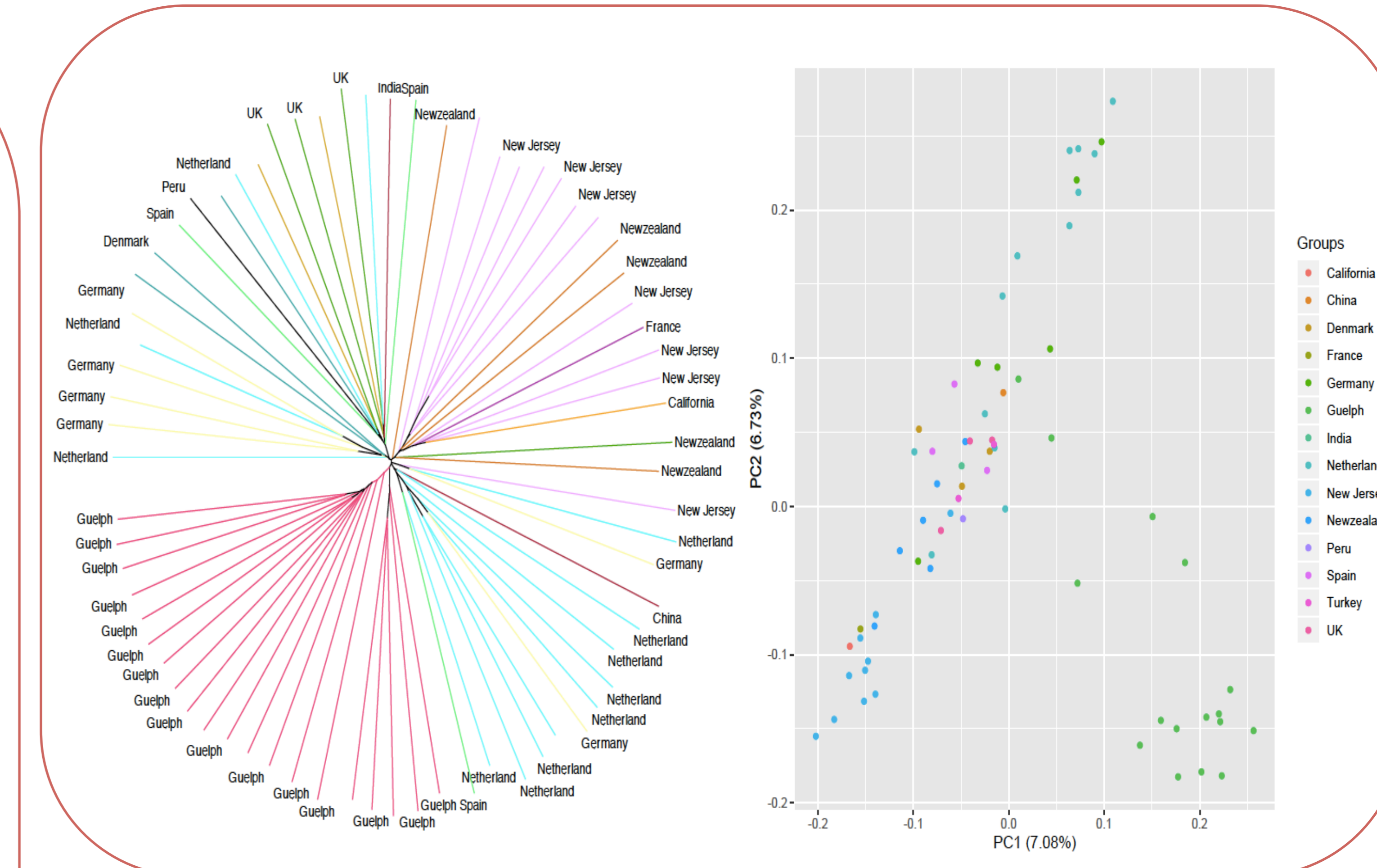Fig 2: Venn diagram displaying distribution of alleles in four defined groups of asparagus

Fig 3:NJ phylogenetic tree and PCA plot of 68 asparagus genotypes

Results

- Population STRUCTURE analysis results revealed that all 68 genotypes were grouped into 2,4,7,and 9 clusters (Fig 1). The results were further confirmed by PCA and phylogenetic tree method (Fig 3).
- To develop hybrids in future, crosses can be made between parent lines from different subpopulations, specifically between lines from subpopulation 7 (UK), subpopulation 6 (Netherland) and 8 (Guelph).
- The cultivars from Canadian subgroup exhibited to possess maximum number of unique alleles, whereas European cultivars possessed least number of unique alleles (Fig 2).

Conclusion

- The extent of genetic diversity determined in this study can be used to develop new asparagus cultivars in the future with desired traits.
- It will also assist in association mapping, MAS and genomic selection studies in the future.

Do different Brassica cover crops differ in their effects on the soil fungal and nematode communities in a vineyard?

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Background

Studies that look at the response of soil communities to biofumigants have mainly focused on direct incorporation of plant biomass into the soil (ie. green manure). Biofumigant cover crops are commonly used in vineyards, although little is known about how they affect the soil microbial community without additional processing. Using both greenhouse and field trials, we are testing four Brassicas as vine row biofumigants. White mustard, Tillage radish, Shepard's purse, and Rockcress.
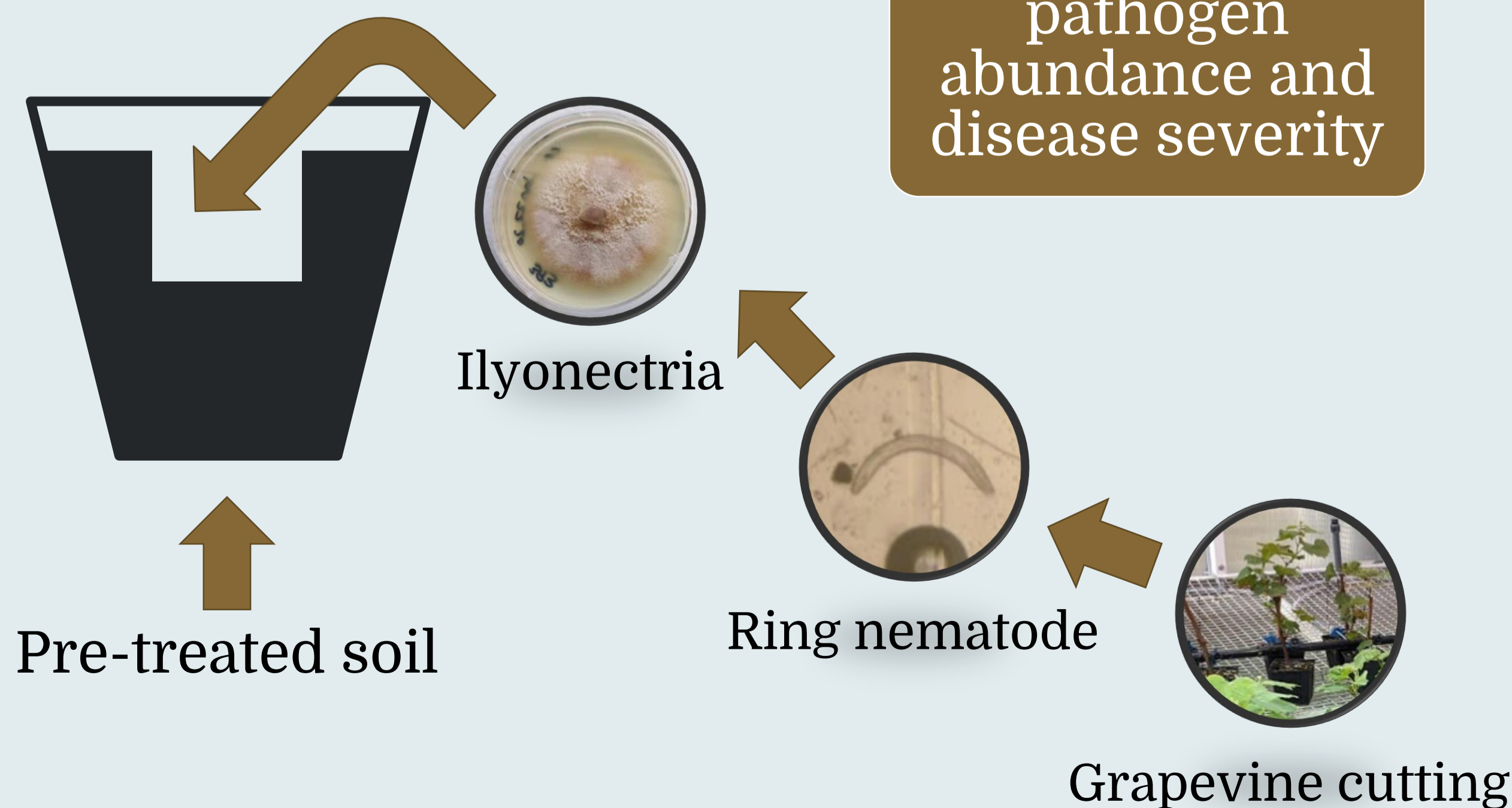
Greenhouse Study

Pre-treat soil by growing cover crops

↓

Inoculate soil with pathogens and plant a grapevine

↓

Analyze for pathogen abundance and disease severity

Pre-treated soil
Ilyonectria
Ring nematode
Grapevine cutting

Field Study

White mustard (*Sinapis alba*)

Shepard's purse (*Capsella bursa-pastoris*)

Tillage radish (*Raphanus sativus*)

Holboell's rockcress (*Boechera holboelli*)

Grow cover crops for two growing seasons → Collect soil samples at the end of season two → Analyze and compare fungal and nematode communities

Objectives

1. Field – Compare effects of different Brassica cover crops on the soil microbial community in a vineyard

2. Greenhouse – Test the suppressiveness of soils treated with different brassica species.

Predictions

We predict if there is any difference between the brassicas, the species with greater biomass (Tillage Radish and White Mustard) will have a greater effect on the soil community as more biomass would mean more glucosinolate production.

What does this mean?

The results of this experiment will help us provide recommendations about biofumigation cover crops for growers in the Okanagan.

tantalus vineyards
OKANAGAN VALLEY

Agriculture and Agri-Food Canada

Determining the role that abiotic and biotic stress factors play on the grapevine trunk disease latent pathogen *Phaeomoniella chlamydospora* development in young vines

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[1]*Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC V0H 1Z0, Canada.* [2]*Department of Biology, University of British Columbia – Okanagan, Kelowna, BC V1V 1V7, Canada.*

INTRODUCTION/GOALS

• Petri disease is caused primarily by *Phaeomoniella chlamydospora* and is a component of the young vine decline complex (Gramaje and Armengol, 2011).
• Petri is an economically important disease in vineyards worldwide, and is of significant concern in British Columbia which has a younger wine industry.
•Young vine decline has been observed in 7.8% of young vines monitored in British Columbia, and in certain vineyards incidence of infected plants was as high as 55% (Úrbez-Torres, et al., 2014).
• Petri disease pathogens were identified in 50.3% of vineyards surveyed in British Columbia and in 43.7% of samples collected in young vineyards (Úrbez-Torres, et al., 2014).
• Petri disease pathogens are thought to be latent pathogens and are hypothesized to transition to a pathogenic phase under stress conditions (Hrycan et al., in press). The goal of this study is to determine the role abiotic and biotic stress has on disease development in young vines.
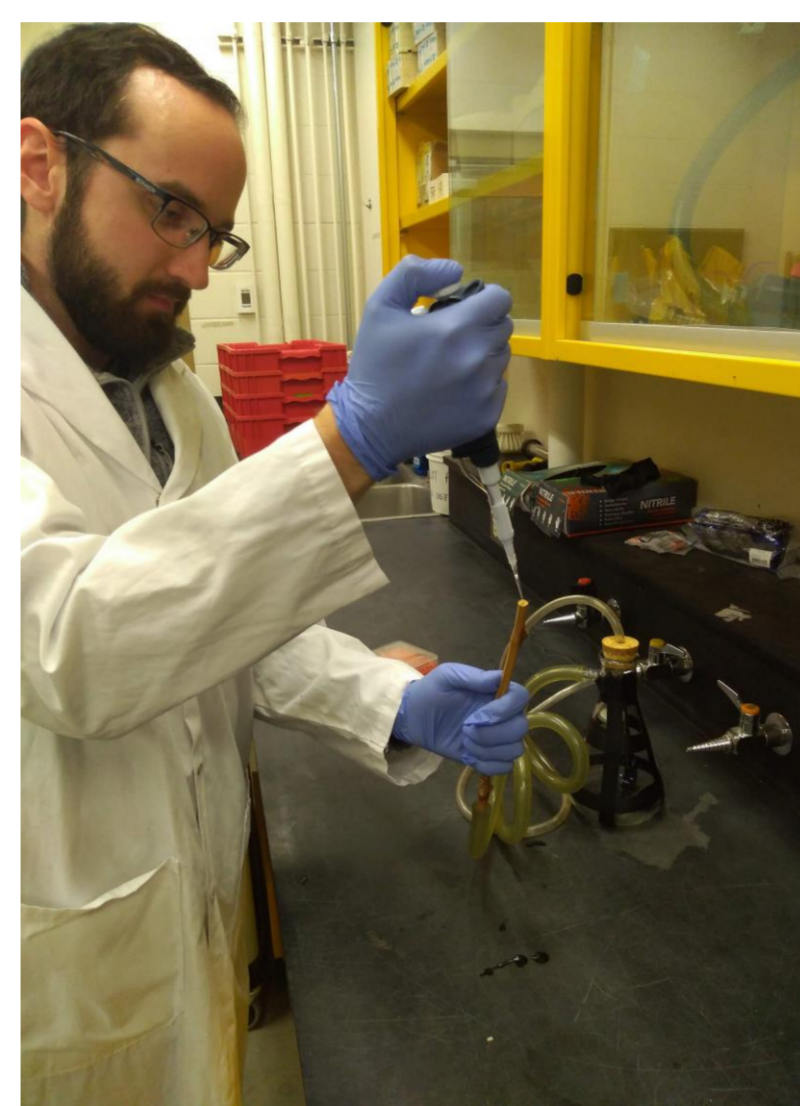
Cross section showing necrosis found in Young Vine Decline

Foliar symptoms of Young Vine Decline

J.R. Úrbez - Torres

J.R. Úrbez - Torres

OBJECTIVE 1: Abiotic Stress on Petri Disease Development

• *Phaeomoniella chlamydospora* will be vacuum inoculated into grapevine canes using the technique developed by Rooney and Gubler (2001). The spores were vacuum inoculated into the cane at either a concentration of 25,000 spores, 5,000 spores, and 1,000 spores.
• The effect of drought stress on symptom expression and pathogen development within the cane is Currently being tested in a greenhouse trial. Leaf gas exchange will be conducted to monitor plant stress and soil tensiometers will be used to monitor the soil matric potential.
• At the end of the experiment, the canes will be harvested and internal necrosis will be measured, re-isolations will be performed to verify active infection, and droplet digital PCR will be conducted using *P. chlamydospora* specific beta-tubulin primers developed by Pouzoulet et al. (2013) to determine the pathogen copy numbers in different sections of the cane.
• Little is known about the effect that abiotic stresses have on pathogen development in young grapevines. The main goal of this experiment is to determine the effect water stress has on Petri disease pathogen development.
• *Rhizophagus intraradices* an arbuscular mycorrhizal fungi (AMF) is known to reduce plant stress, in particular water stress. AMF will be added to the soil to determine whether AMF will reduce plant stress, and in turn reduce pathogen development and disease expression.

Methodology:

• Merlot canes were vacuum inoculated with 10uL of a *P. chlamydospora* solution, or 10uL of water amended with Tween 80 for 1 second.
• Canes were rooted in water and planted in 3000 grams of sandy loam in 1 gallon pots.
• Plants from objective 1 are hand watered to ensure even watering across all plants.
• Shoot and leaf pruning's collected for leaf and shoot dry weight. Leaf gas exchange and soil tensiometers will be used to measure plant stress and soil matric potential throughout experiment.

Gramaje, D., & Armengol, J. (2011). Fungal trunk pathogens in the grapevine propagation process: Potential inoculum sources, detection, identification, and management strategies.*Plant Disease, 95*(9), 1040-1055.
Hrycan, J., Hart, M., Bowen, P., Forge, T., Úrbez-Torres, J. R. (In press). Grapevine trunk disease fungi: Their role as latent pathogens and stress factors that may favor disease development and symptom expression in grapevines. *Phytopathologia Mediterranea.*
Pouzoulet, J., Mailhac, N., Couderc, C., Besson, X., Daydé, J., Lummerzheim, M., & Jacques, A. (2013). A method to detect and quantify phaeomoniella chlamydospora and phaeoacremonium aleophilum DNA in grapevine-wood samples. *Applied Microbiology and Biotechnology, 97*(23), 10163-10175.

Vacuum inoculation of *P. chlamydospora*

Re-isolation of *P. chlamydospora* at 15cm, 7.5cm, and 0.5cm from the base of the cane

OBJECTIVE 2: Biotic Stress on Petri Disease Development

• *Phaeomoniella chlamydospora* will be vacuum inoculated into grapevine canes using the technique developed by Rooney and Gubler (2001). The spores were vacuum inoculated into the cane at either a concentration of 25,000 spores, 5,000 spores, and 1,000 spores.
• Ring nematodes will be inoculated into each pot at a rate of 1,000 nematodes per 1,000 grams of soil.
• Foliar symptom expression will be monitored throughout the trial to determine the effects of nematode infestation on disease development. Leaf gas exchange will be conducted to monitor plant stress.
• At the end of the experiment, the grapevines will be harvested and internal necrosis will be measured. Re-isolations will be performed to verify infection, and droplet digital PCR will be performed to determine pathogen copy numbers in different sections of the cane.
• Little is known about the effect that biotic stress (ring nematode damage) has on pathogen development in young grapevines. The main goal of this experiment is to determine the effect biotic stress has on Petri disease pathogen development.
• AMF will be added to the soil to determine whether AMF will reduce plant stress, and in turn reduce pathogen development and disease expression.

OBJECTIVE 3: Abiotic and Biotic Stress on Petri Disease in the Field

• Four field experiments are currently underway to determine the effect abiotic and biotic stress has on Petri disease development in the field.
• Vacuum inoculation was conducted using 25,000 and 2,500 *Phaeomoniella chlamydospora* spores in self-rooted merlot or merlot grafted onto SO4 rootstocks. Canes were rooted in the greenhouse until 2 weeks prior to planting when they were transferred to the shadehouse.
• The experiments will determine the effect of
i) Drought stress
ii) Nematode infestation
iii) J-rooting
iv) Overcropping
on symptom expression and pathogen development in young vines in the field.

Planting of J-rooted vine

Rooney, S. N., & Gubler, W. D. (2001). Effect of hot water treatments on eradication of phaeomoniella chlamydospora and phaeoacremonium inflatipes from dormant grapevine wood. *Phytopathologia Mediterranea, 40*, S467-S472.
Úrbez-Torres, J. R., Haag, P., Bowen, P., & O'Gorman, D. T. (2014b). Grapevine trunk diseases in british columbia: Incidence and characterization of the fungal pathogens associated with esca and petri diseases of grapevine. *Plant Disease, 98*(4), 469-482.

Canada

Light Characterization of Cannabis Using Controlled Environment Technology

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UNIVERSITY of GUELPH

IMPROVE LIFE.

Introduction

Controlled environment agriculture (CEA) is an advanced method of plant production that can to be used to study the physiological responses of plants. A high quality controlled environment system will optimize the productivity of a plant when placed under homogenous environment conditions. This results in consistent and stable genetics between multiple plant generations.

The characterization of any plant is mediated by key variables, these include:

- Light (quality, quantity, photoperiod)
- Temperature
- Humidity
- CO_2/O_2
- Water/Nutrients
- Pest/pathogens

The utilization of high performing growth chambers allows for precise whole plant in situ measurement of photosynthesis and evapotranspiration (Dixon *et al.* 2017). Using response curves (RC) to quantify the relationship between photosynthesis and a specific environmental variable provides information on the maximum photosynthetic capacity and assimilation rates. These systems can be designed to evaluate the light spectral quality and quantity throughout the plant's life cycle. Maximizing the unique capabilities of light emitting diodes, advantages which include energy efficient utilization and fine tuning the desired spectral composition, allows for growers to target photoreceptor pigments for targeted physiological response.

Figure 1. Four different lighting spectrums set up in a two tiered modified PGC Flex chamber.

Objective

Due to the legalization, it is of increasing importance to *Cannabis spp.* producers to identify the best growing methodology. The aim of this research is to characterize the optimal light recipe using narrow bandwidth light emitting diodes at various stages of the plant life cycle.

Specifications

Chambers

5 Conviron/Intravision PS1000 Photosystem Chambers, and 3 modified Conviron PGC Flex chambers

Control

- Temperature control range (15-35°C)
- VPD control (0.2-1.5 kPa)
- CO_2 enrichment (0-10,000 ppm)
- Remote access control system by Argus Control

LED

- 1600 Watt water-cooled LED lighting system with seven channels
- UV (365nm), violet (410nm), blue (440nm), green (530nm), red (660nm), far-red (735nm), white (5650K)
- Adjust intensity of individual LED channels
- Narrow band/wide spectrum

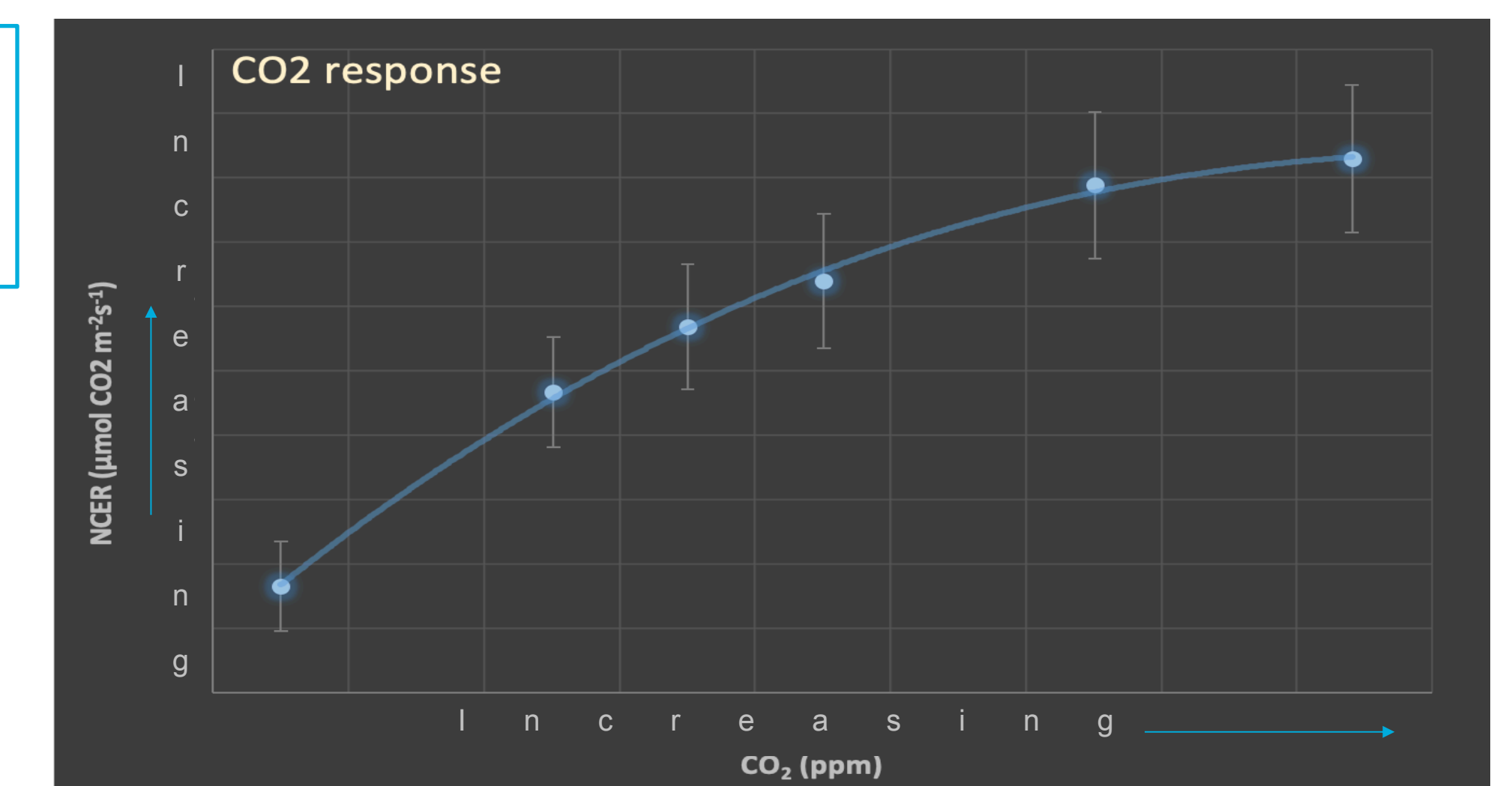**Figure 2.** Cannabis growing in a PS1000 chamber

System capabilities

Previous experiments of light (quality and quantity), CO_2 and temperature have investigated the optimal parameters.
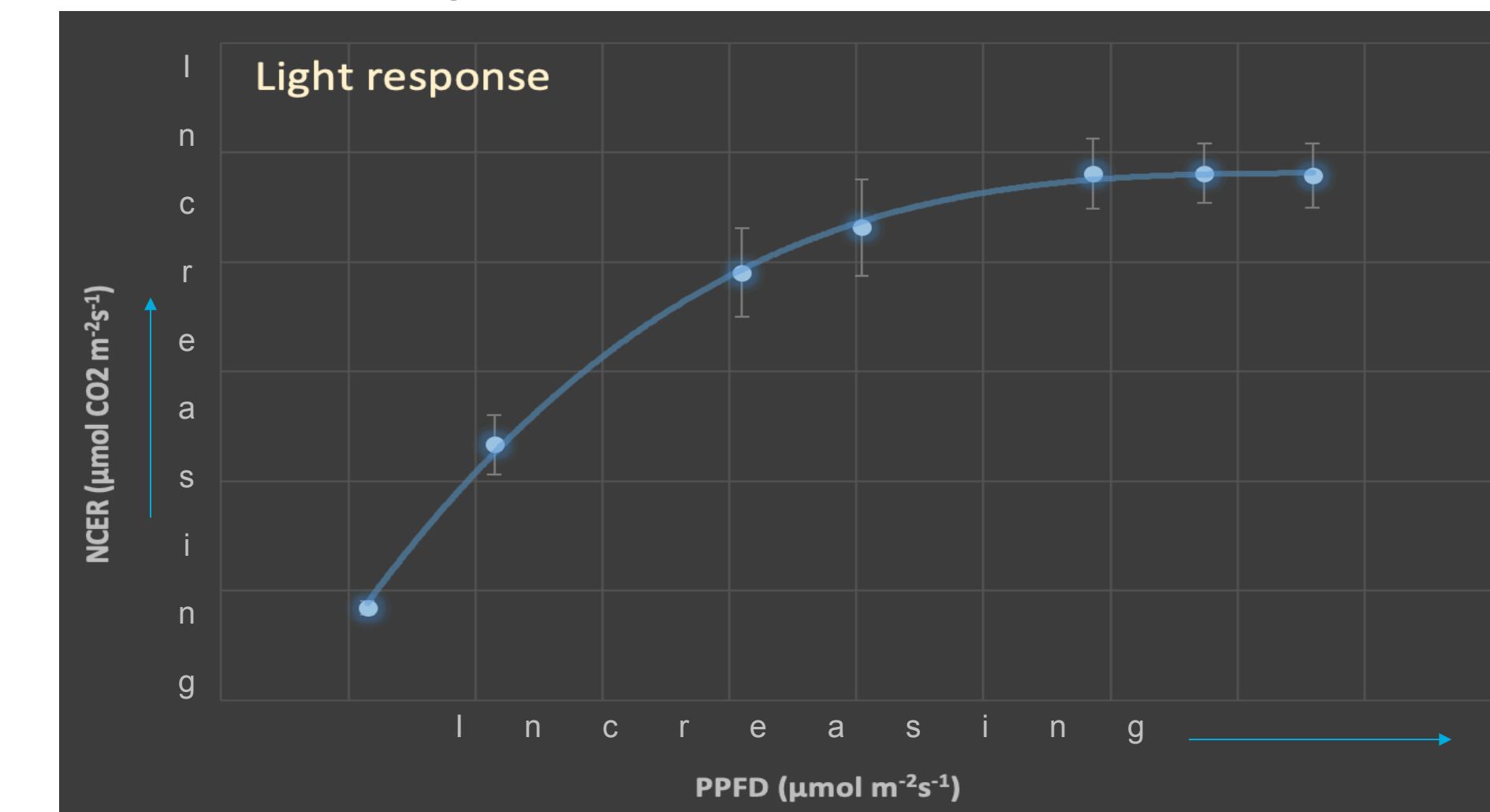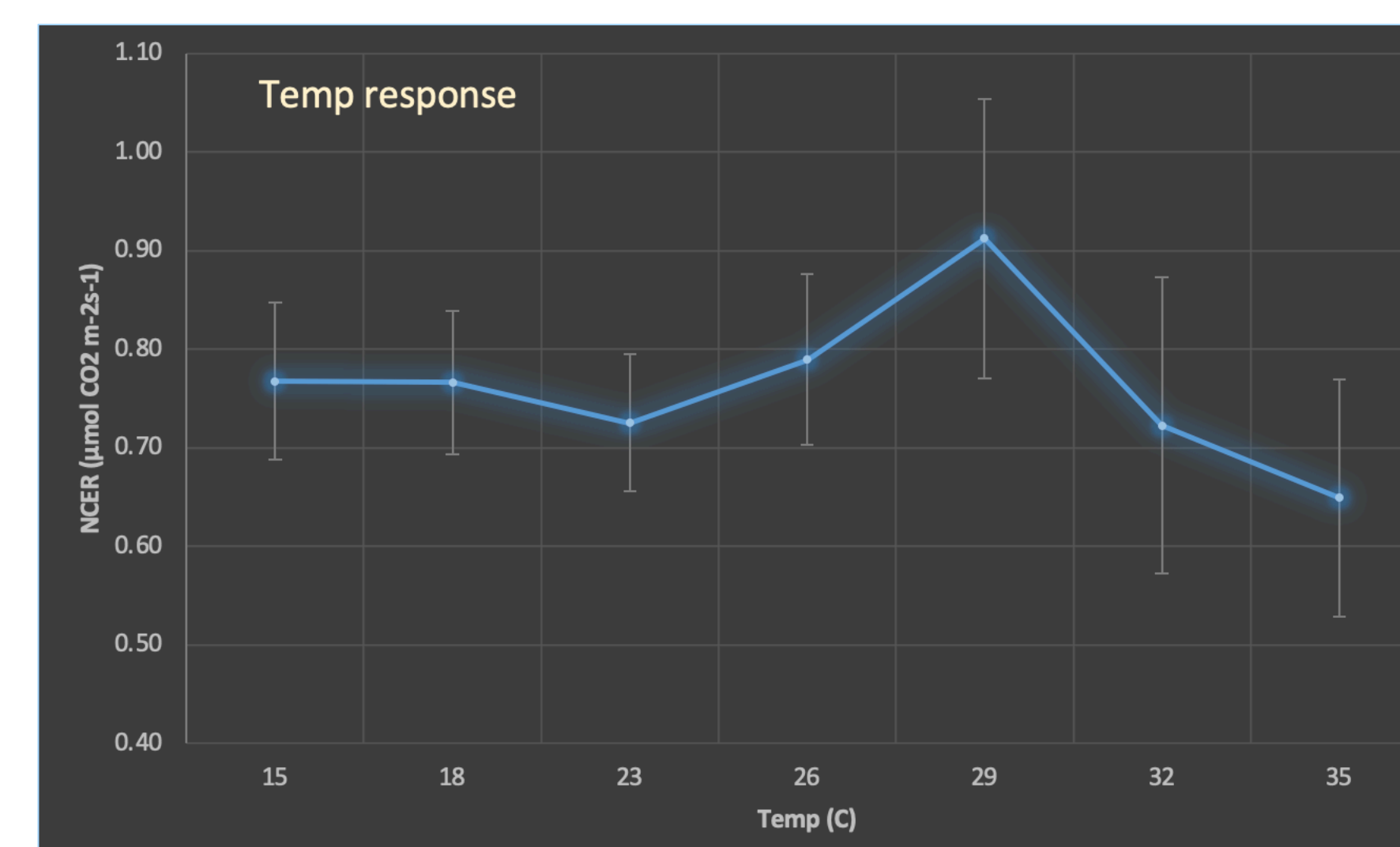

Figure 3. Net carbon exchange rate (NCER) in response to increasing light intensity.
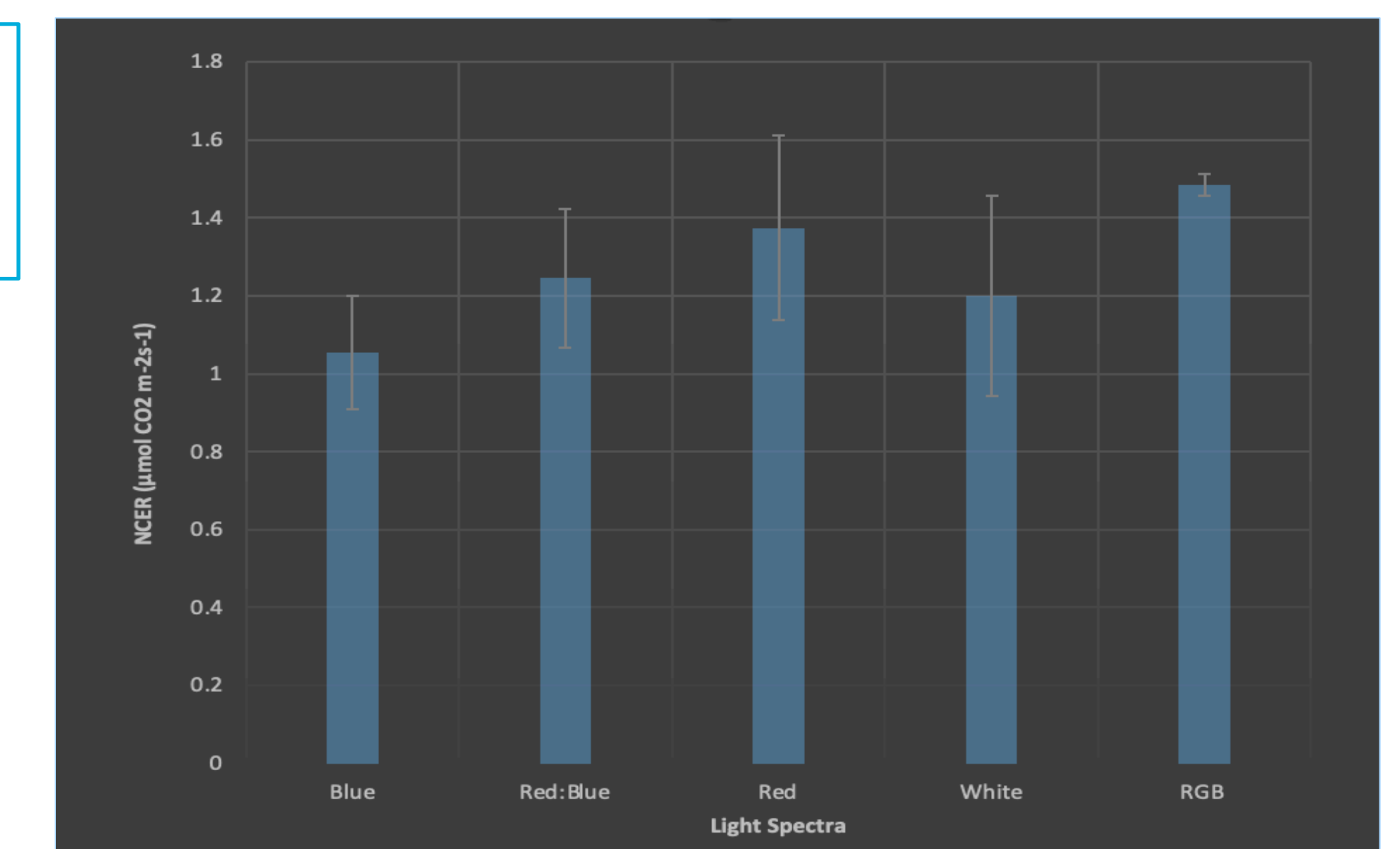

Figure 4. Net carbon exchange rate (NCER) in response to increasing CO_2 concentration.

Figure 5. Net carbon exchange rate (NCER) in response to increasing temperature.

Figure 6. Net carbon exchange rate (NCER) in response to blue, red:blue, red, RGB and white light.

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Dixon, M., Stasiak, M., Rondeau V. T., & Graham, Thomas. (2017). Advanced Life Support Research and Technology Transfer at the University of Guelph. *Open Agriculture, 2,* 139-147

Controlled environment cultivation for better plant-based medicines: An investigation of *Withania somnifera* (Ashwagandha)

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Controlled Environment Systems Research Facility (CESRF) Advisors: Thomas Graham and Mike Dixon

Background

Withania somnifera (Ashwagandha) is a medicinal herb with endangered status, high market value and clinically proven uses.

Germination of field grown *Withania* is poor, having both low germination rates and slow germination time.

Overview

CEA can allow growers to cultivate plants with increased uniformity, consistency and valuable traits.while helping to conserve wild populations.

Manipulating environmental factors, specifically light quality (wavelength in nm) and light quantity (photosynthetic photon flux density-PPFD in $\mu mol/m^2/s$) can influence plant development and medicinal traits.

Germination success is key to seedling establishment. By increasing the rate and success of germination growers can shorten production time and increase the number of harvests per year.

Objectives

I. Determine the germination potential of *Withania somnifera* seeds when subjected to scarification or 24hr hydropriming treatment under two different light qualities.

II. Create growth stage parameters (germination through harvest) to enhance and standardize plant material for medicinal use.

Fig.1. Depiction of one block within the growth chamber. Two shelves (A_1 and A_2) have separate light qualities, 660nm and 660/470nm mix respectively. Each shelf contains 12 petri dishes with four dishes from each pre-germination treatment. In this graphic white represents control (B_1), blue represents hydroprime (B_2) and grey represents scarification (B_3).
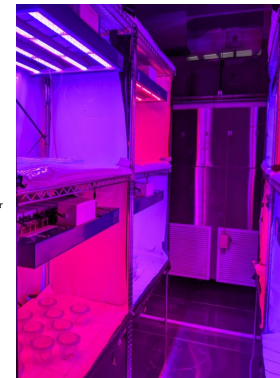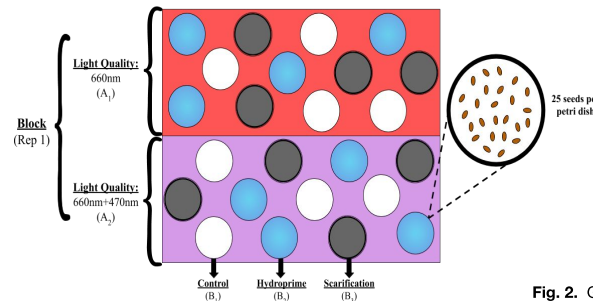
Fig. 2. Growth chamber with 8 shelves. Split-plot experiment in a randomized complete block design. Two light qualities (A_1, A_2) will be randomized to main plots, Pre-germination treatments (B_1,B_2,B_3) will be randomized to subplots

Methodology

Trial first will take place at the University of Guelph's Controlled Environment Research Facility (CESRF) in Guelph, Ontario.

Plant material: Withania Seeds provided by ReHeva Botanicals Inc.

Environment: Walk-in growth chamber, temperature 28°C, water-cooled LED shelving (adjustable UV, Blue, Green, Red, Far-Red)

Lighting: LED arrays (Intravision ltd.) provide pure red light (660nm) and an equal mixture of red (660nm) and blue light (470nm) with the same PPFD of $200\mu mol/m^2/s$ and 16hr photoperiod.

Treatments: 24 hr hydropriming or mechanical scarification

Data collection: Germination rate and %

Data collection Ojective II: leaf area, root and shoot fresh/dry weights, concentration of secondary metabolites

Results: Experiment ongoing

Righting a Wrong: Can Enhanced Efficiency Products help Reduce N$_2$O Emissions from Fertilizer?

Trang Phan[1], Rich Farrell[2] and Kate Congreves[1*]

[1]Dept of Plant Sciences, [2]Dept of Soil Science, University of Saskatchewan

UNIVERSITY OF SASKATCHEWAN

INTRODUCTION

Fertilizer management is one of the major challenges in sustainable horticultural production systems (1). Many vegetable and fruit crops require high N inputs for desirable crop yields. Farmers may choose to apply fertilizer in fall to help lesson the workload in spring. But, high N available in moist soils, especially during the spring thaw, can promote denitrification and increase the risk of soil nitrous oxide (N$_2$O) emissions. This important greenhouse gas has a significant impact on climate change (1-3).

Using an enhanced efficiency N fertilizer (EENF) product might be an effective way to balance agronomic performances and environmental outcomes (4,5). For example, eNtrench^{TM} has active ingredient of *Nitrapyrin* which controls nitrate (NO$_3^-$) production and indirectly retards the production of N$_2$O (Fig. 1).
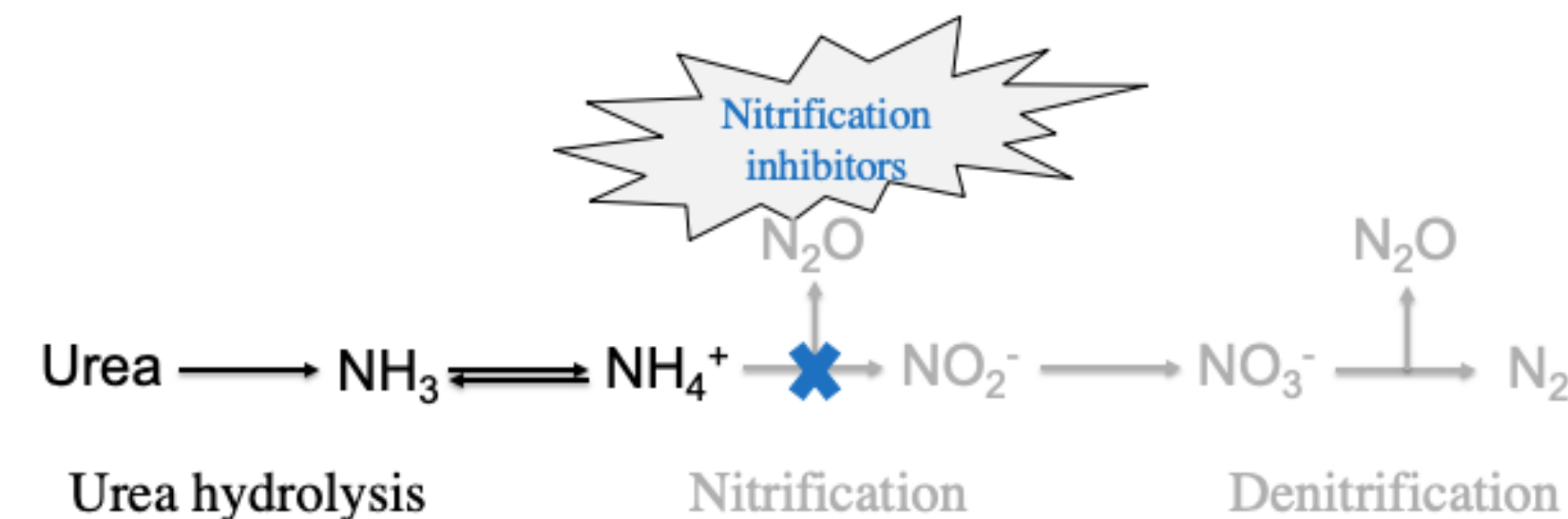

Fig.1. Mechanisms for how eNtrench^{TM} influences the production of N$_2$O

RESEARCH OBJECTIVES

A seven-week soil incubation study was conducted to evaluate the effects of urea-N fertilizer with and without enzyme inhibitors (*Nitrapyrin*), before and after soil freezing on:
- the production mechanisms (nitrification *vs.* denitrification)
- reduction potentials of N$_2$O

METHODOLOGY

Soil collection and preparation:
- Soil samples (0-10 cm depth) from the Saskatoon field site (table 1) were collected in Sept 2019, air-dried and sieved (2 mm) in preparation for an incubation study.
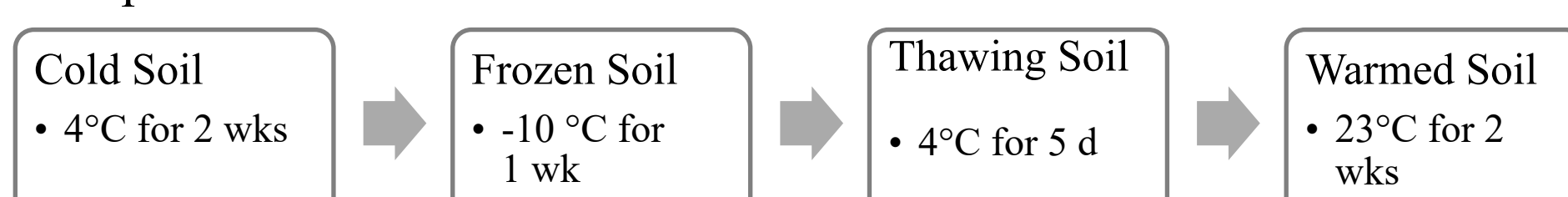
Table 1. Soil characteristics and cropping history for three soil types studied.

Soil association	Texture	pH	Field capacity (θg)	Organic matter (%)	Soil mineral N (μg g^{-1})
Asquith	Sandy loam	8.2	0.40	4.0	16

- Soils were pre-incubated at 23ºC for 2 weeks at 40% water-filled pore space (WFPS).

Soil incubation study:
- Randomized complete block design with 4 replicates
- Treatments:
  - Moisture treatments established based on gravimetric moisture levels at 55, 70, and 80% water filled pore space (WFPS)
  - N treatments included non-fertilized control, urea, eNtrench^{TM} mixed with Urea. N rate established based on soil test N-rate recommendation
- 21.87 cm^3 petri dishes was completely filled with 25.0 g of soil with bulk density of 1.14 g cm^{-3}
- Soil microcosms sealed inside 1L mason jars, lids fitted with septa for gas sampling
- Soil microcosms incubated in dark and subjected a sequential change in temperature as follows:

Cold Soil		Frozen Soil		Thawing Soil		Warmed Soil
• 4ºC for 2 wks	→	• -10 ºC for 1 wk	→	• 4ºC for 5 d	→	• 23ºC for 2 wks

- Frequent gas sampling during each phase
- Gas sampled for N$_2$O and ^{15}N$_2$O; analyzed concentrations via GC (Bruker 450) and CRDS (Picarro G5131-*i* isotopic N$_2$O analyzer)
- Isotopomers are used to calculate site preference (SP) – this indicates nitrification vs denitrification
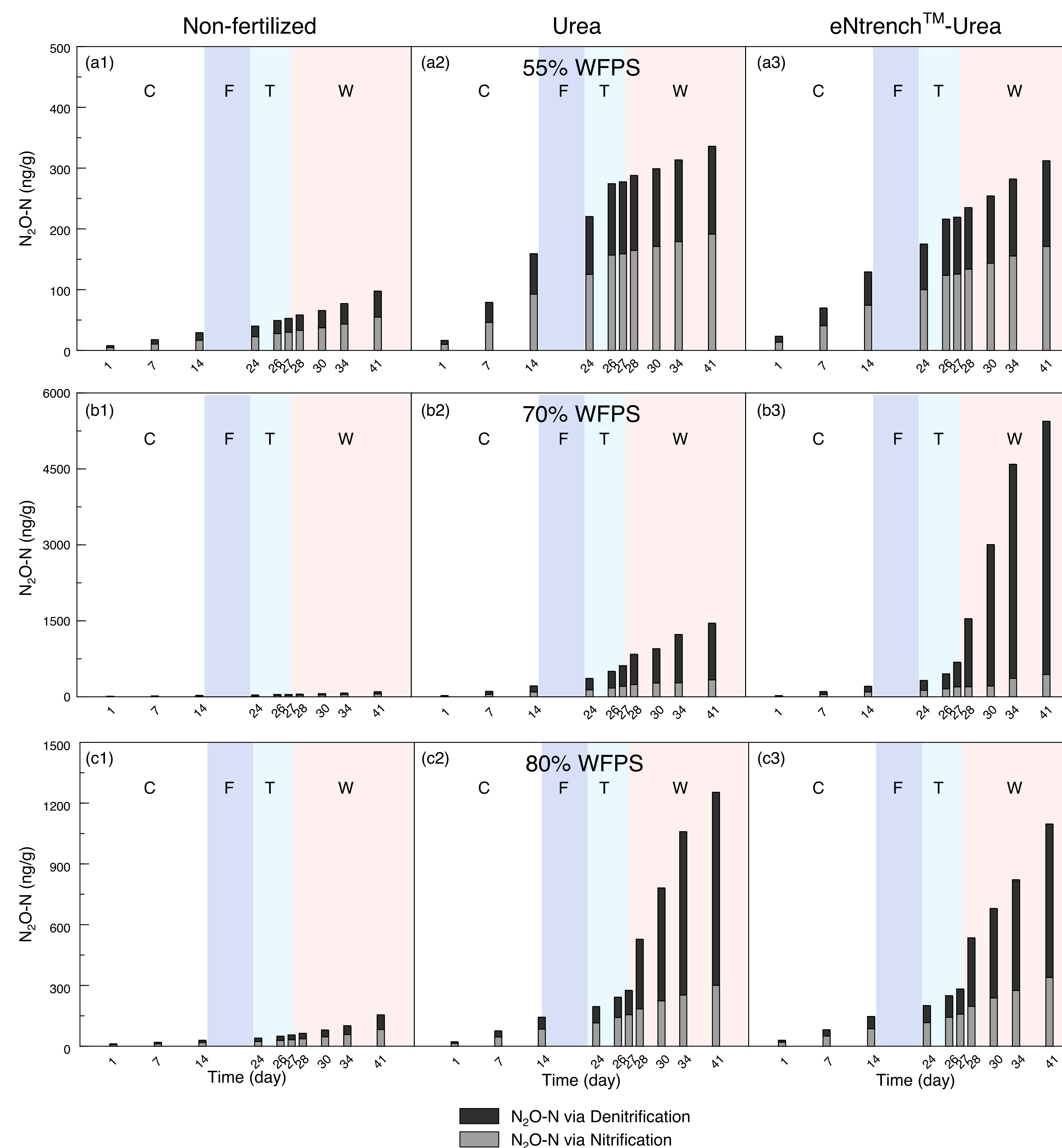
RESULTS & DISCUSSION

Fig. 2. N$_2$O-N production and the source partitioning of N$_2$O derived from nitrification and denitrification as influenced by fertilizer sources [non-fertilizer (**a1, b1, c1**); urea (**a2, b2, c2**); eNtrench^{TM}-urea (**a3, b3, c3**)], soil water-filled pore space [WFPS; 55% WFPS (**a1, a2, a3**); 70% WFPS (**b1, b2, b3**); 80% WFPS (**c1, c2, c3**)], and temperature (C: cold at 4ºC; F: freeze at -10ºC; T: thaw at 4ºC; W: warm at 23ºC) throughout the incubation duration. Note: y-axes were set at different scales to accomodate the comparision of fertilizer treatment effects on N$_2$O-N emissions at different soil moistures.

Soil moisture, fertility levels, and temperature are key regulators of N$_2$O production

Prior to freezing:
- Total N$_2$O fluxes from three N sources remained relatively low (Fig. 2)
- Nitrification outcompeted denitrification (Fig. 2)

Once the soils were thawed:
- Regardless to fertilizer sources, an increase in total N$_2$O fluxes was observed across soil moisture levels (Fig. 2)
- The magnitude of N$_2$O fluxes were more intense from the N-treated soils compare to non-fertilized soils (Fig. 2)

- The contributions of production pathways to N$_2$O emissions were equally important (Fig. 2)

As temperature reached 23°C:
- N$_2$O fluxes from N-treated soils were incrementally magnified over the rest of the incubation duration (Fig. 2)
- N$_2$O production primarily attributed to denitrification when soil was moist (beyond 70% WFPS) (Fig. 2)

RESULTS & DISCUSSION (continued)

The addition of *Nitrapyrin* to Urea did not yield a significant reduction in N$_2$O emissions

Prior to freezing:
- The magnitude of N$_2$O fluxes were similar across the soil moisture and fertility levels (Fig. 2)

Once the soil was thawed:
- The reduction potential of N$_2$O from eNtrench^{TM}-Urea treated soils were insignificant (Fig. 2)

As temperature reached 23°C:
- Regardless to the magnitude of N$_2$O emissions, there was no difference in nitrification rates between Urea and eNtrench^{TM}-Urea treatments (Fig. 2)
- At 55% and 80% WFPS, a reduction potential of N$_2$O was observed. However, similar effect of *Nitrapyrin* on N$_2$O reduction was not found when soil moisture was at 70% WFPS (Fig. 2)

CONCLUSIONS

- Freezing-thawing stimulates the microbial activities of both nitrifiers and denitrifiers which results in a rapid increase in cumulative N$_2$O emissions as the soils warm up.
- The use of *Nitrapyrin* products for spring N application to minimize N$_2$O emissions may be worthwhile for dry-land farming. However, applying *Nitrapyrin* in the fall may not help lower soil N$_2$O emissions in the following spring, due to the interplay of soil moisture and denitrification during soil freeze-thaw.
- Since the N$_2$O-producing microbes may maintain some activity under cold soil conditions, *Nitrapyrin* may not entirely suppress this biological processes prior to thaw; applying N fertilizer during the fall may still present a high risk of N$_2$O loss. Therefore, more research is needed to find better ways of minimizing N$_2$O loss with fall fertilizer—otherwise fall applications should be discouraged.

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ACKNOWLEDGEMENTS

Effect of Ratios of Far Red to Blue Lights on *Colletotrichum acutatum*

Zahra Charkhzarrin and Valérie Gravel - Department of Plant Science, McGill University

McGill UNIVERSITY

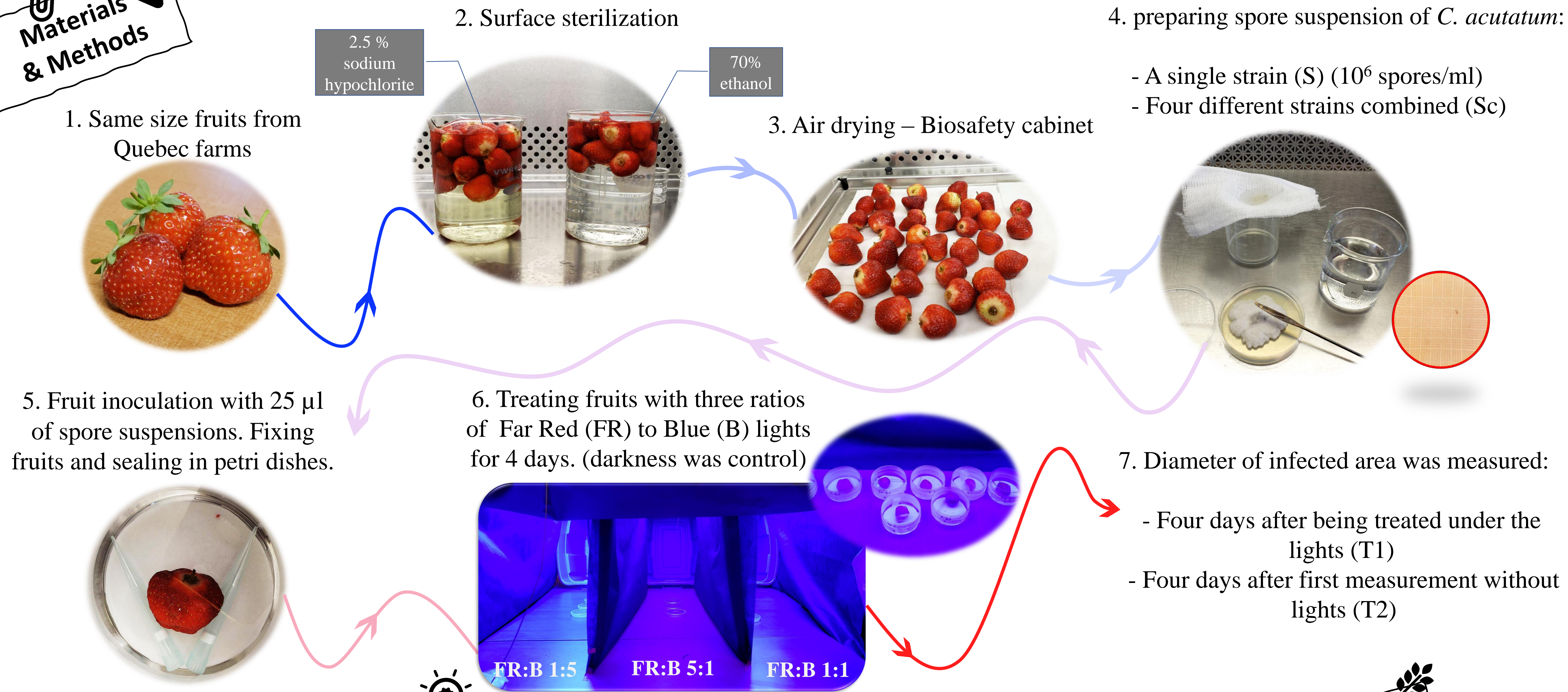Agriculture, Pêcheries et Alimentation Québec

INTRODUCTION

- *Colletotrichum acutatum* (J.H. Simmonds) is a very important ubiquitous fungal pathogen responsible for anthracnose fruit rot (AFR) of strawberry [1 &2] causing millions of dollars of loss annually in strawberry nurseries and production fields around the world [2] [Fig.1].

Fig.1. Anthracnose fruit rot of strawberry - Symptoms

- Other than chemical fungicides with their known side effects [3], eco-friendly and effective AFR management methods are required to be discovered, as it has been also emphasized on by Agriculture and Agri-food Canada [4].

- Light is an environmental factor affecting both pathogen and plant which makes it a potential good candidate for disease management [5&6].

Materials & Methods

1. Same size fruits from Quebec farms

2. Surface sterilization
   - 2.5 % sodium hypochlorite
   - 70% ethanol

3. Air drying – Biosafety cabinet

4. preparing spore suspension of *C. acutatum*:
   - A single strain (S) (10^6 spores/ml)
   - Four different strains combined (Sc)

5. Fruit inoculation with 25 µl of spore suspensions. Fixing fruits and sealing in petri dishes.

6. Treating fruits with three ratios of Far Red (FR) to Blue (B) lights for 4 days. (darkness was control)

FR:B 1:5   FR:B 5:1   FR:B 1:1

7. Diameter of infected area was measured:
   - Four days after being treated under the lights (T1)
   - Four days after first measurement without lights (T2)

Results

✓ Light qualities effect on pathogenicity and growth ability of the pathogen varied among S and Sc [Table 1].

✓ Strains had significant effect on the size of the infected area [Table 1]. In fact, S caused bigger lesions compared to Sc [Fig.2].

✓ All light treatments significantly limited the pathogen activity on the fruits, the ratios of 1:1 and 1:5 totally prevented the symptoms from appearing [Fig.2].

✓ **Effect of light treatments on the pathogen was permanent** [Fig.2] [Table 1].
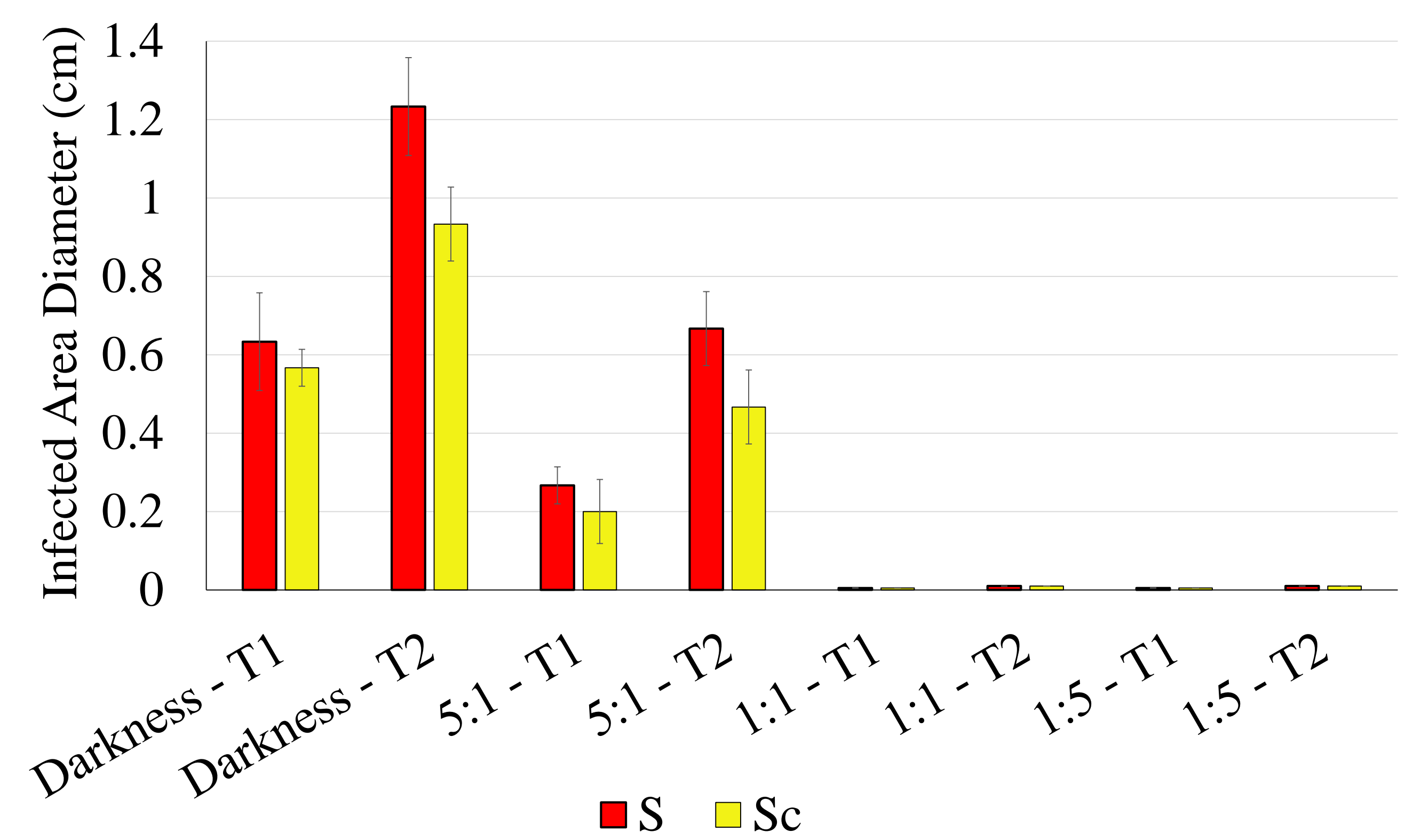
Fig.2. Effects of light treatments on size of infected area caused by *C. acutatum* on strawberry

Table 1. Repeated measure ANOVA results

Within Subject Effects	
Source	P-value
Strains	0.0057
Lights	<.0001
Time	<.0001
Time×Strains	0.0933
Time×Lights	<.0001
Time× Strains×Lights	0.3349

Between Subject Effects	
Source	P-value
Strains	0.0013
Lights	<.0001
Strains×Lights	0.0102

Conclusions

➤ All the ratios of FR to B lights have **permanent limiting** and **fatal** effect on *C. acutatum*, and they can reduce its **pathogenicity**. Both ratios of 1:1 and 5:1 were equally fatal for the pathogen while the ratio of 1:5 had significant limiting effect on **disease expansion**.

➤ The complex of strains used in this experiment did not improve the overall pathogenicity of *C. acutatum*.

➤ These ratios of lights might be a good replacement for UV treatments used for surface sterilization of the fruits for longer storage life [7].

➤ Besides the negative impacts of these lights on *C. acutatum*, positive effects of red, blue, and a combinations of both lights on strawberry plants in increasing plant growth, advancing flowering, and improving plant production and fruit yield have been proven in previous studies [6, 8 , & 9].

➤ **Therefore, these light treatments could be employed in nurseries to eliminate the main inoculum source of AFR in strawberry field, in greenhouses to limit the pathogen activities and improve plant productions, and in cold storage to maintain fruits healthy and marketable.**

➤ All these information show that there is a potential for invention of a **new management strategy** which could significantly reduce **chemical fungicide application** and help build a **sustainable agriculture system**.

Acknowledgements

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