Propagation of hemp (Cannabis sativa)

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Cornell CALS
College of Agriculture and Life Sciences

Cornell University Hemp Webinar Series
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Ithaca

LIHREC has specialists in all areas of horticulture:

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Fruit: Viticulture
Vegetables/potatoes
Woody ornamentals/Landscape plants

Cross Commodity Specialists:
Plant disease
Weed science
Entomology
Plant Tissue Culture

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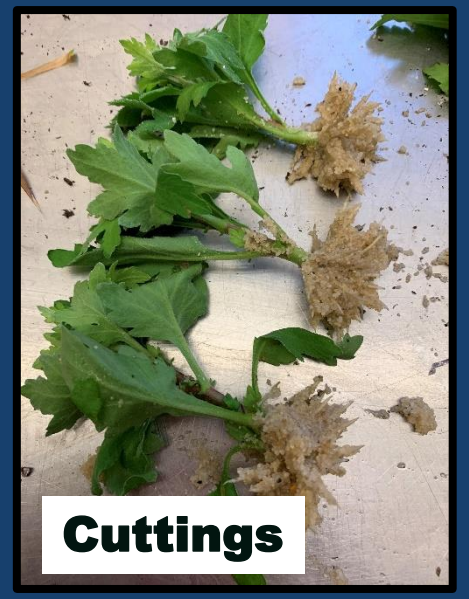
•Plant Propagation

Propagation media

Bud graft

Grafting

Air Layering



Cuttings

Plant Propagation

The multiplication of plants by both sexual and asexual means

IPPS

Sharing Plant Production Knowledge Globally

Hemp plants can be propagated by sexual and asexual means

TODAY:

1. Seed propagation
2. Vegetative cutting propagation
3. Micropropagation

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HOW TO PROPAGATE PLANTS – THE BASICS

- Select **healthy specimens**!

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  - Porous for good drainage
  - Free from weed seeds, pathogens, and insects
  - Easy to wet and maintain moisture
  - Retention of nutrient

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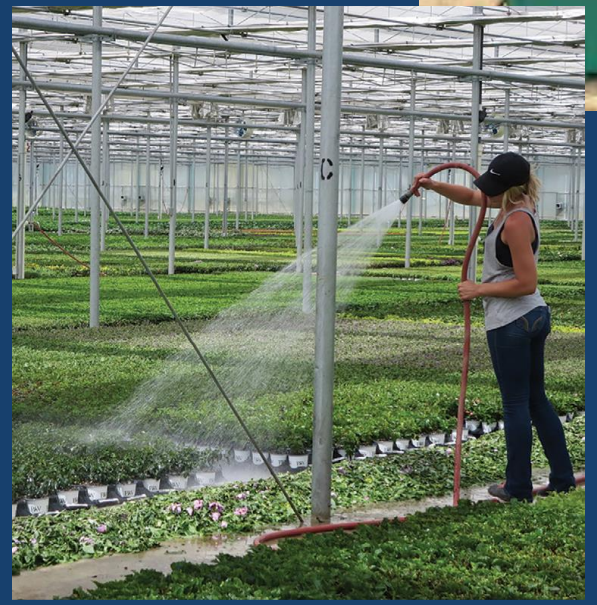
- **Light**
  - Daylength/Photoperiod
  - Intensity
  - Wavelength

HOW TO PROPAGATE PLANTS – THE BASICS

- **Water management and humidity**
  - Intermittent mist?
  - Plastic covers?



HOW TO PROPAGATE PLANTS – THE BASICS

- **Water management and humidity**

- **Greenhouse Temperature**
  - **73 - 77°F is ideal for germination and rooting of cuttings of most plants**
    - **For hemp: up to 80ºF to 82ºF**

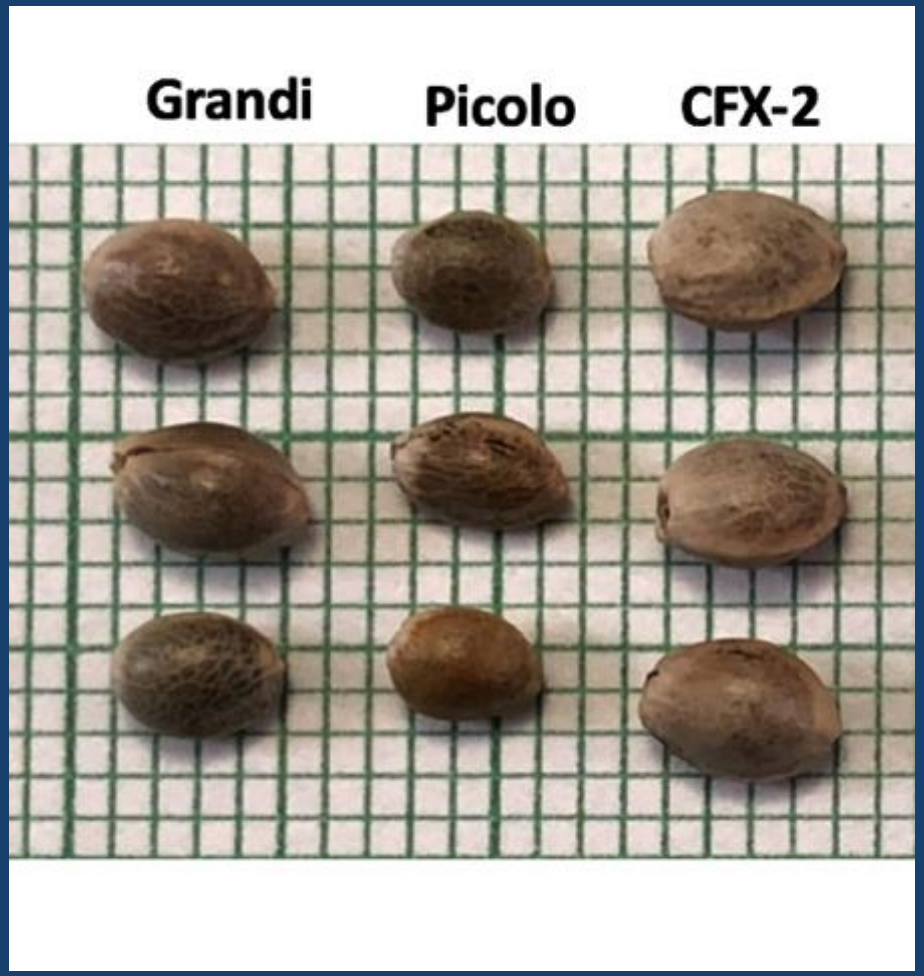
HOW TO PROPAGATE PLANTS – THE BASICS

- Water management and humidity
- Greenhouse Temperature
- Cleanliness
  - Good sanitation

Seed Propagation vs. Vegetative Propagation

- Hemp plants are dioecious
- Pistillate and staminate organs occur on different plants.
  - Pistillate plants are desirable
- Genetic recombination results in segregation of traits & phenotypic diversity
- Do you want clones?
  Use vegetative cutting propagation

Seed Morphology



Dispersal unit is an achene - outer layer is a pericarp as in sunflower seed

Alan Taylor, Cornell

Seed Propagation Hemp

- Start with the correct, viable seed: use GOOD GENETICS
  - Feminized seeds for CBD hemp

Seed Propagation Hemp

- Understand your field soil or greenhouse media:
  - well-draining, low weed pressure, and has plenty of nutrients will lay a healthy foundation
  - Soil tests for field soil

Seed Propagation Hemp

- **Strategically plan** outdoor production to maximize the environment at your location
  - Consider frosts, day length, and rain

Seed Propagation Hemp

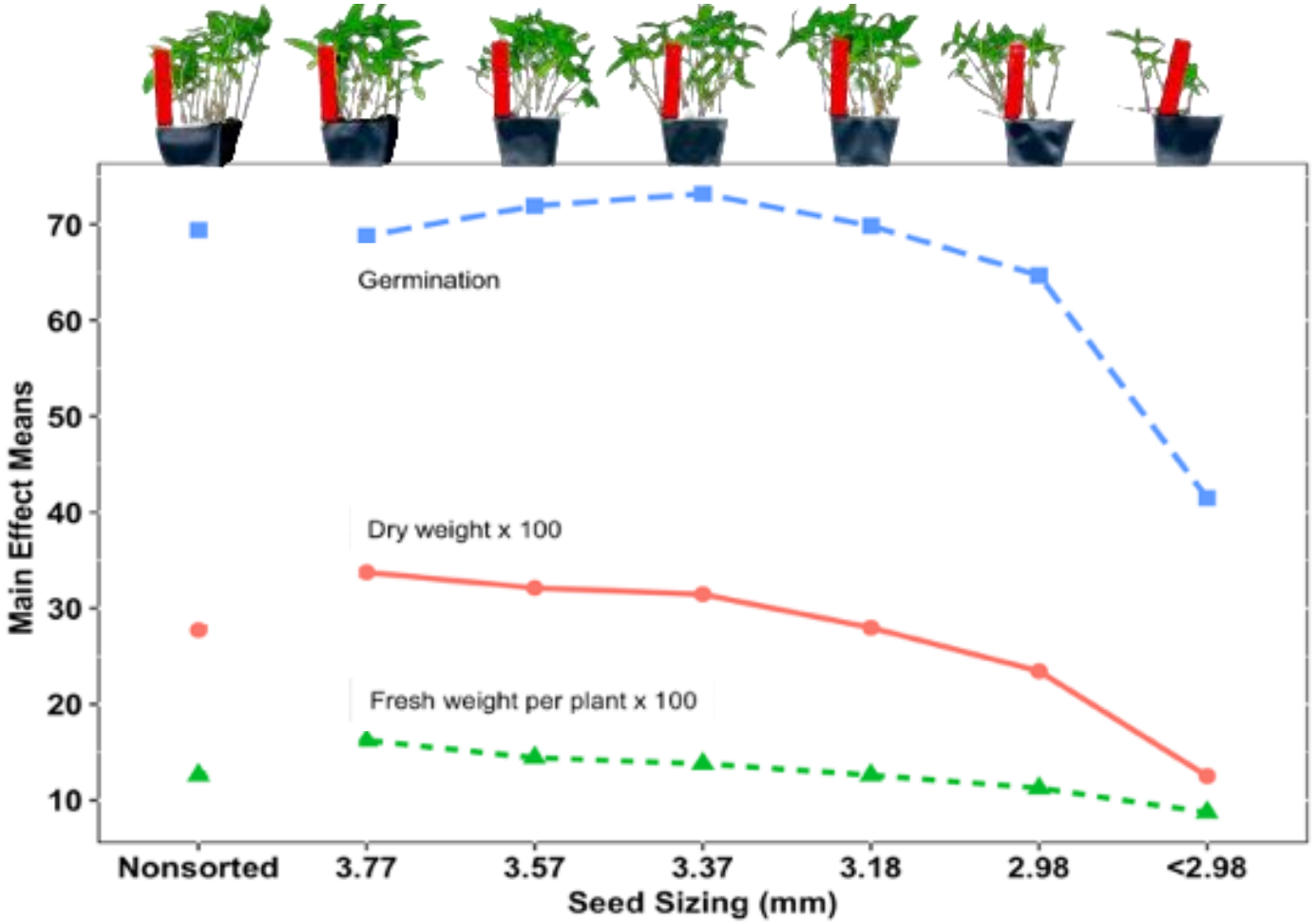
- Pre-soak seeds for 8-12 hours to improve germination (no longer than 24 hours)
  - Helpful, but not necessary
- Ideal seed germination temperature: Between 65º-80ºF
- Plant seeds about 1-inch deep
- Seeds can germinate in light or dark conditions (Small 2016)
- Water thoroughly

Propagation from seed

Alan Taylor, Michael Loos,
Masi Amirkhani, Hilary Mayton
Cornell University, Geneva, NY

SEED SIZE impacts seed quality

Credit Mike Quade 2022



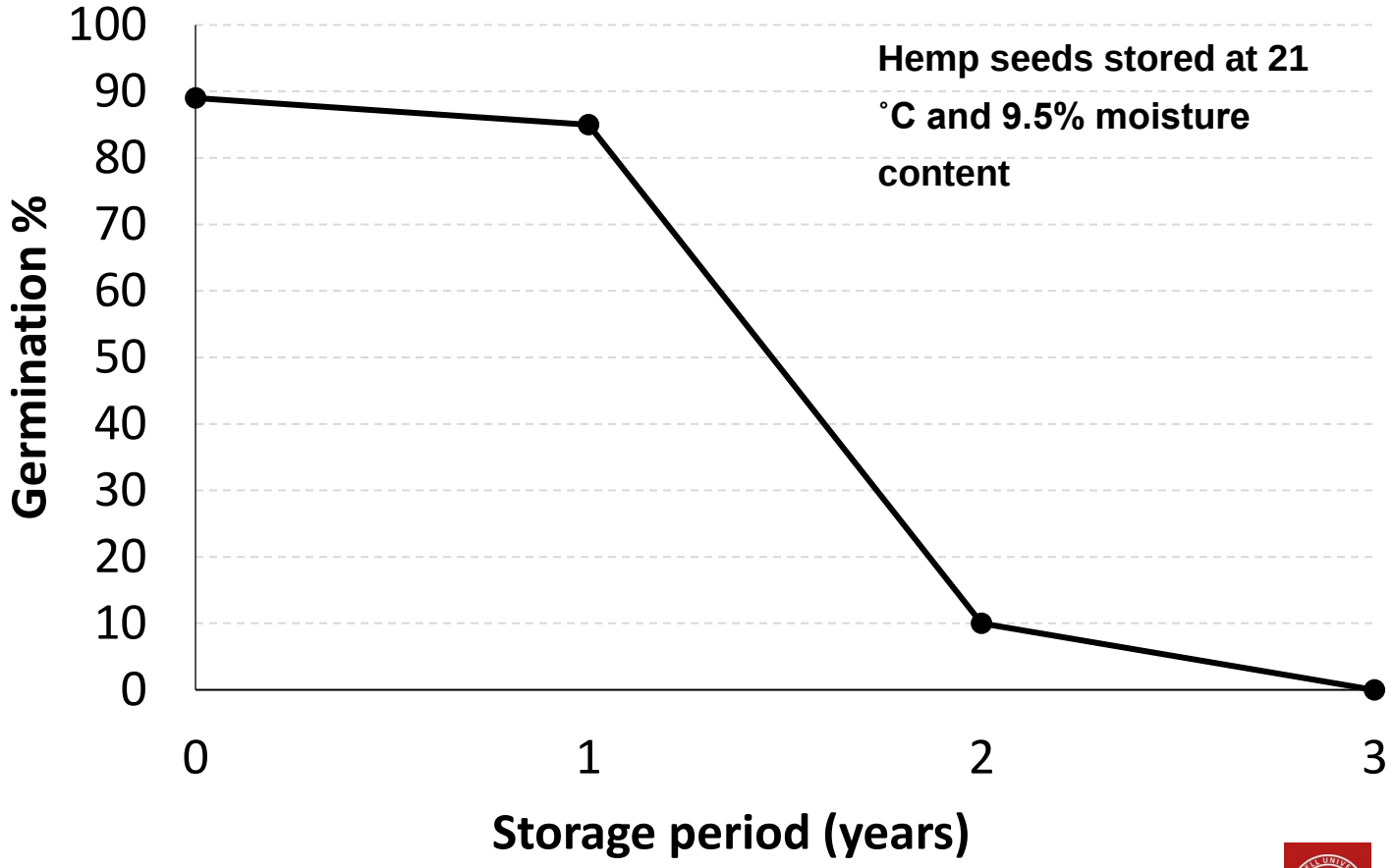
Mi, R.; Taylor, A.G.; Smart, L.B.; Mattson, N.S. 2020, https://doi.org/10.3390/agriculture10120617

Hemp Seed Storage

- **Need to store seeds to maintain viability and vigor**
- **Hemp seed have short-medium longevity in relation to vegetable crop seeds**

Alan Taylor, Michael Loos, Masi Amirkhani, Hilary Mayton
Cornell University, Geneva, NY

Tetrazolium viability test. A, non-viable seeds, seed of hemp *(Photo by S. Elias, Oregon State Univ).*



VEGETATIVE PROPAGATION*

WHY?

- A clone is an exact copy
  - to maintain their genetic and phenotypic characteristics

*Not feasible for grain and fiber hemp

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- Uniformity

*Not feasible for grain and fiber hemp

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- Low cost

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- Clones get a head-start compared to seedlings
- Year-round production in greenhouses

*Not feasible for grain and fiber hemp

What are STEM CUTTINGS?

- **Stem cuttings** = the most common approach to vegetative propagation hemp

- A cutting is a piece of plant that has been cut off from a parent plant and then **given the opportunity to make roots** of its own

- Grow into an **identical plant** as the original stock plant

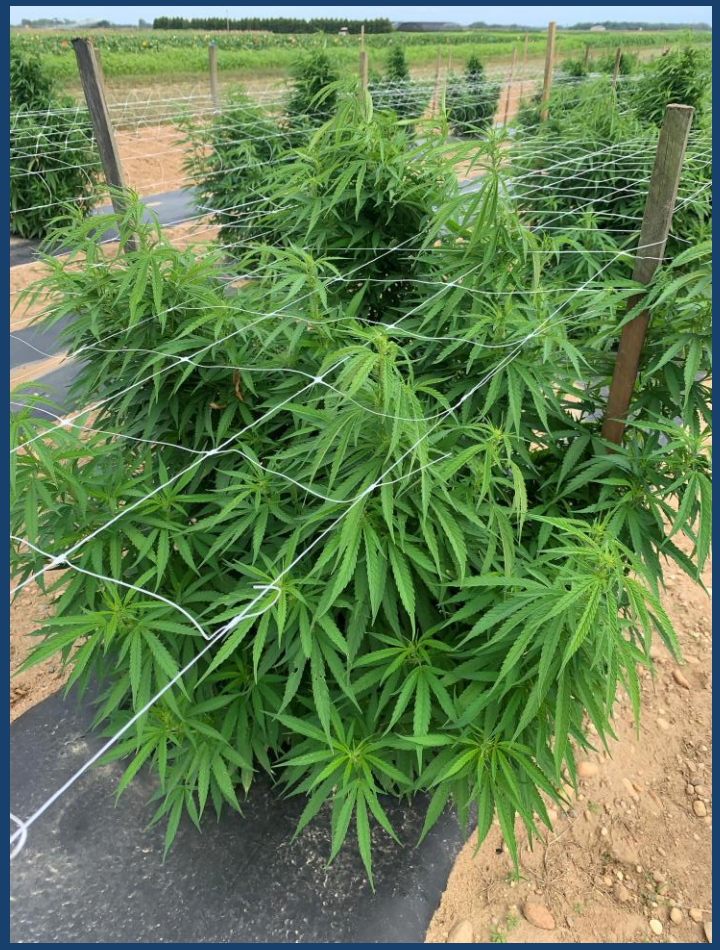
What you will need:

- Sharp knife, clippers (scissors), or a razor
  - clean and disinfect all your tools
- Propagation medium
- Rooting hormone (1,000 ppm)
- Humidity domes or intermittent mist system (greenhouses)
- Take clones from “mature” plants

Hormodin #1 = 0.1% IBA (1,000 ppm)
Hormodin #2 = 0.3% IBA (3,000 ppm)
Hormodin #3 = 0.8% IBA (8,000 ppm)

Stem Cutting Propagation of Hemp

- **Take cuttings* from a well-established and healthy plant (preferably not flowering)**



**Don't forget to label your cuttings*

Nutrition is important

- Stock plants fertigated with 100, 200, & 300 PPM N (15-5-15)
- Greatest fresh root weight and rooting percentages when cuttings are provided with nutrients in propagation
- Stock plants treated with 200 & 300 PPM N showed no chlorosis and produced larger plants (more harvestable cuttings)

From left to right: Stock plants treated with 100, 200, & 300 PPM N (15-5-15)

Alex Carver & Dr. James Faust
Clemson University, South Carolina

Stem Cutting Propagation of Hemp

- Remove apical cuttings from stock plant with 2 to 3 nodes

Approximately 5” – 8”

Stem Cutting Propagation of Hemp

- Trim off any huge lower leaves and clip the top fan leaves if they are big

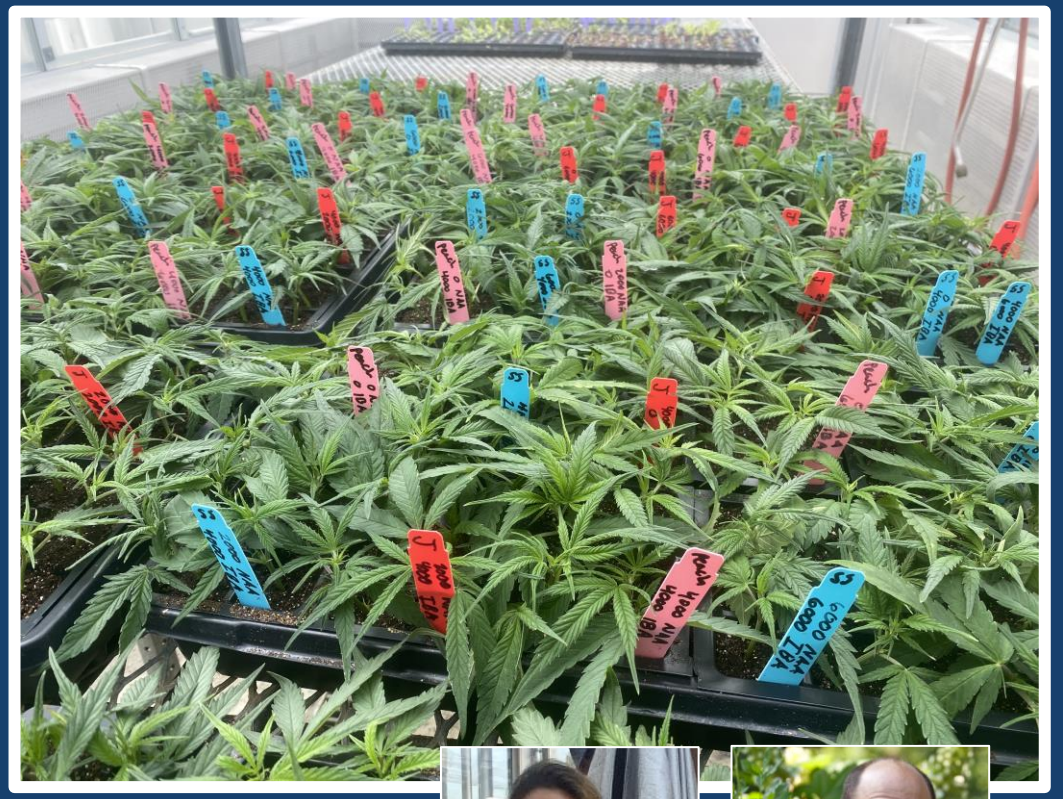
Stem Cutting Propagation of Hemp

- Gently wound the bottom of the cutting

- Treat with a rooting powder
  - 0.1% IBA (1,000 ppm)

Exogeneous Auxin Application

- Results are cultivar specific
  - 7 cv evaluated
- 4 x 4 factorial (16 treatment combinations) of K-IBA and K-NAA liquid dip treatments
  - 0, 2000, 4000, 6000 PPM K-IBA
  - 0, 2000, 4000, 6000 PPM K-NAA
- Overall, 2000-4000 ppm NAA or IBA produced greatest rooting percentages when applied as quick dip



Alex Carver & Dr. James Faust
Clemson University, South Carolina

Stem Cutting Propagation of Hemp

- Insert into a rooting medium

*Campbell et al. (2021) report that **rockwool** offered a 7–13-fold improvement*

Stem Cutting Propagation of Hemp

- Place cuttings in a high humidity environment
  - Cloning domes
  - Intermittent mist

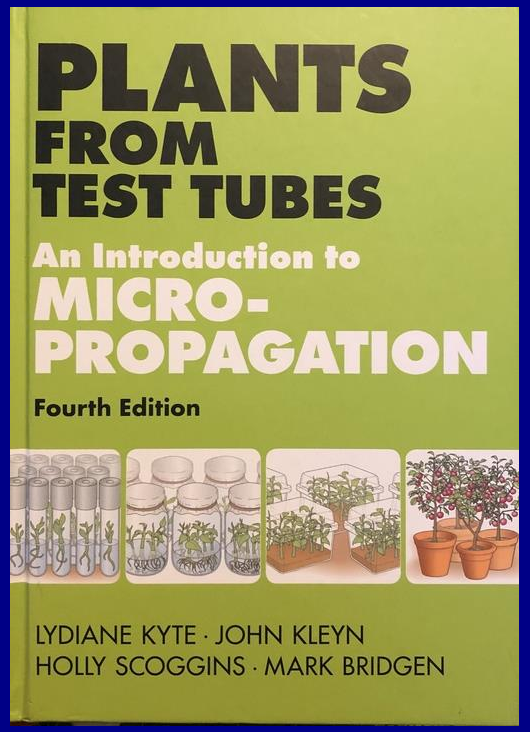
Stem Cutting Propagation of Hemp

- About 72º-75ºF are generally used. Plus 2º-3ºF higher root zone temperature

- Long days: 16/8 (Light/Dark)

Plant Tissue Culture

The growth of plants on a sterile nutrient medium *in vitro* under controlled environmental conditions

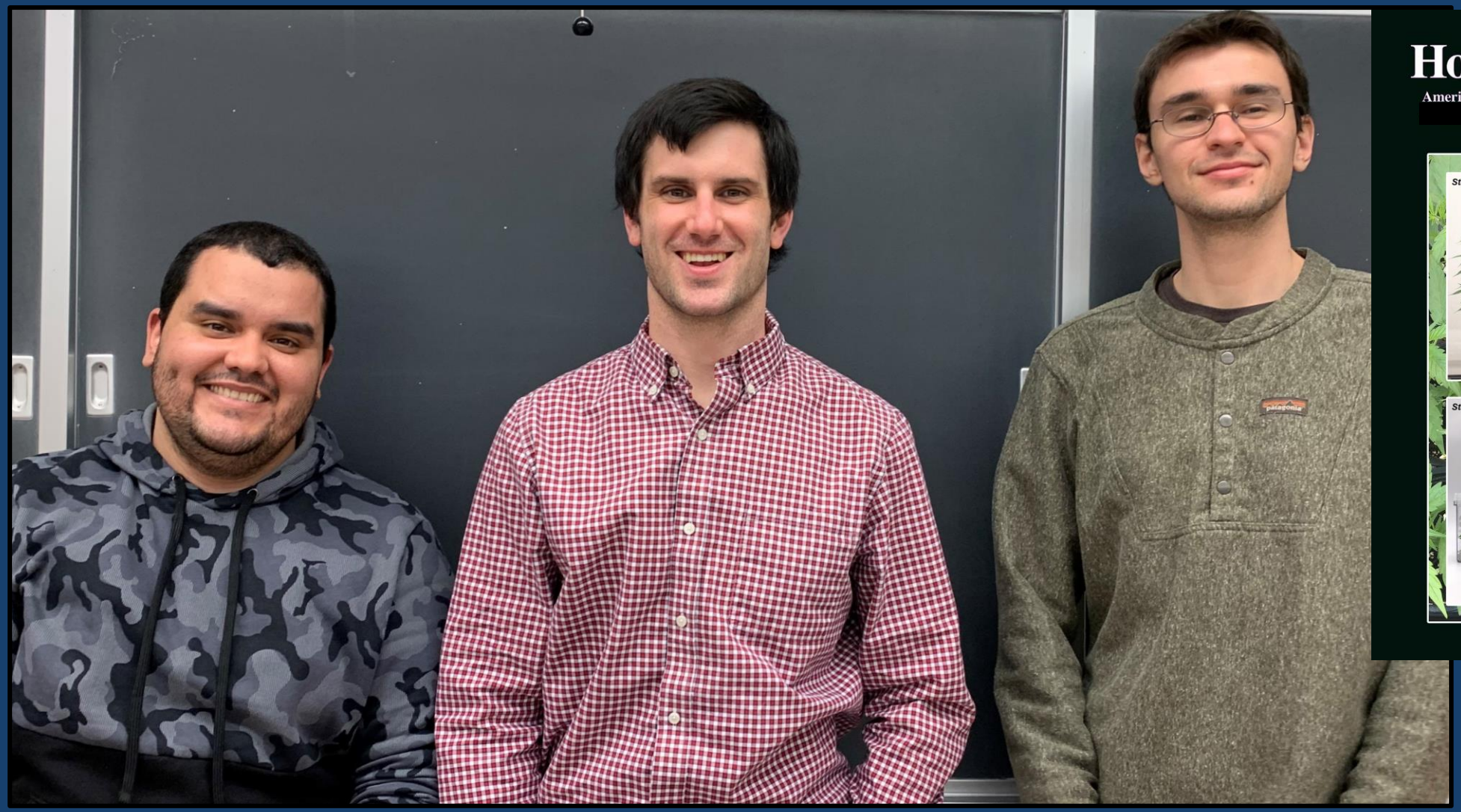


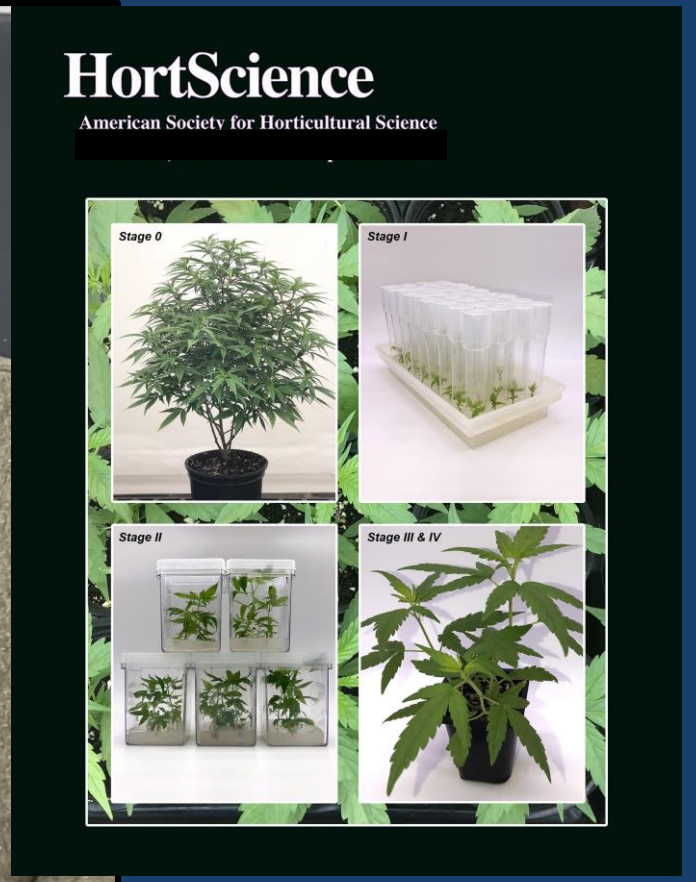
MICROPROPAGATION

The rapid clonal propagation of plants *in vitro*

Micropropagation of Hemp (*Cannabis sativa* L.)

Victor Zayas, Conor Stephen, Andrei Galic

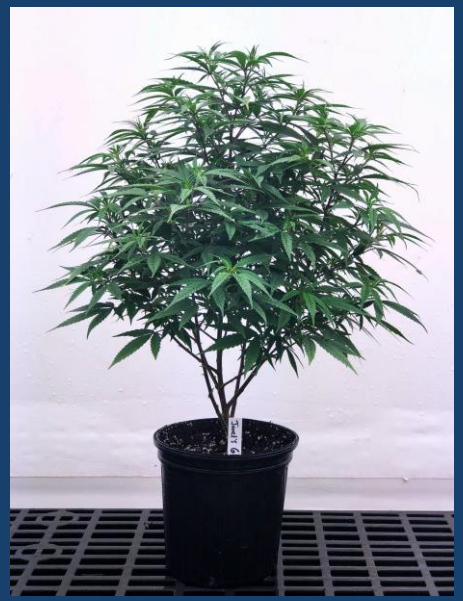




Why Hemp micropropagation?

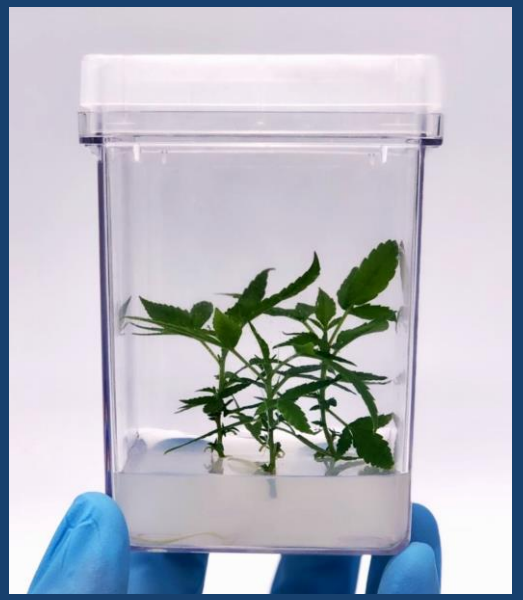
- Clonal propagation
- Clean, vigorous plant material
- Efficient space utilization
- Large numbers
- Less maintenance
- Year-round production
- Controlled environment
- Disease free plants are possible

Stages of Micropropagation



Stage 0:
Proper Growth and Selection

Stage I:
Introduction and aseptic establishment



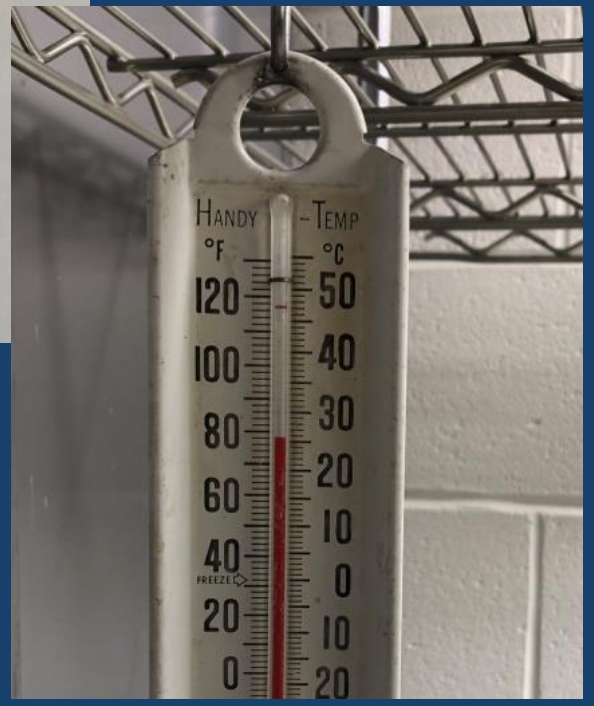
Stage II:
Multiplication

Stage III & IV:
Rooting/Acclimation

Factors Influencing Micropropagation: Growth Medium

- Nutrients + vitamins
- Carbohydrates (Sugar)
- Gelling Agents
- Plant Growth Regulators (Hormones)

Factors Influencing Micropropagation



- Explant type
- Temperature
- Culture pH

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Stage I Materials and Methods

'TJ's CBD'

1 cm shoot tips

Treatments:

- Bleach concentrations (7.5% NaOCl)
  - 20%, 40%, 60%
  - 10 minute duration
- Stock plant environment
  - Greenhouse vs. Growth Chamber

Stage I

Environment	Bleach Concentration	Percent Alive	Percent Contaminated
Greenhouse	20%	75%	90%
	40%	75%	90%
	60%	70%	85%
Growth Chamber	20%	100%	0%
	40%	95%	5%
	60%	100%	0%

KEY POINTS:

- Plant material that is grown in a growth chamber has a better chance for success
- No differences observed between disinfection treatments – all work well
- No bleach concentration damaged plant material – suggesting higher concentrations can be used

Stage II
Nutrient Media

MS LS DKW WPM

Murashige and Skoog (MS)
Linsmaier & Skoog (LS)
Driver & Kuniyuki Walnut Media (DKW)
Lloyd & McCown Woody Plant Medium (WPM)

Results:

- No significant differences between MS, LS, and DKW fresh weight, height, or rating
- WPM resulted in lowest fresh weight, shoot number, and rating

Stage II
Cytokinins

Comparing the cytokinins:

- 6-Benzylaminopurine (BA)
- 6-(γ,γ-Dimethylallylamino) Purine (2iP)
- Thidiazuron (TDZ)

at concentrations of

- 1.0 µM
- 5.0 µM
- 10.0 µM

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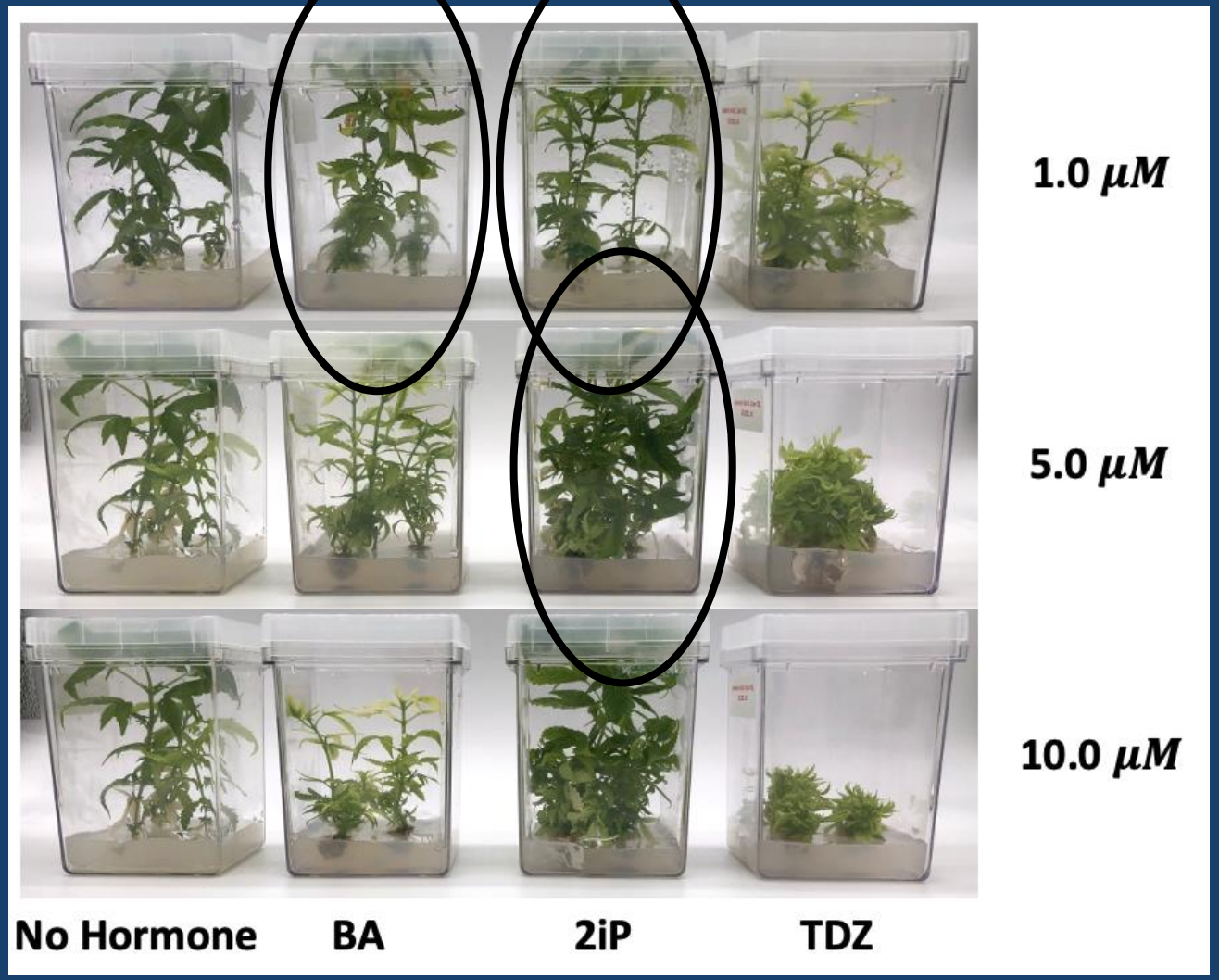
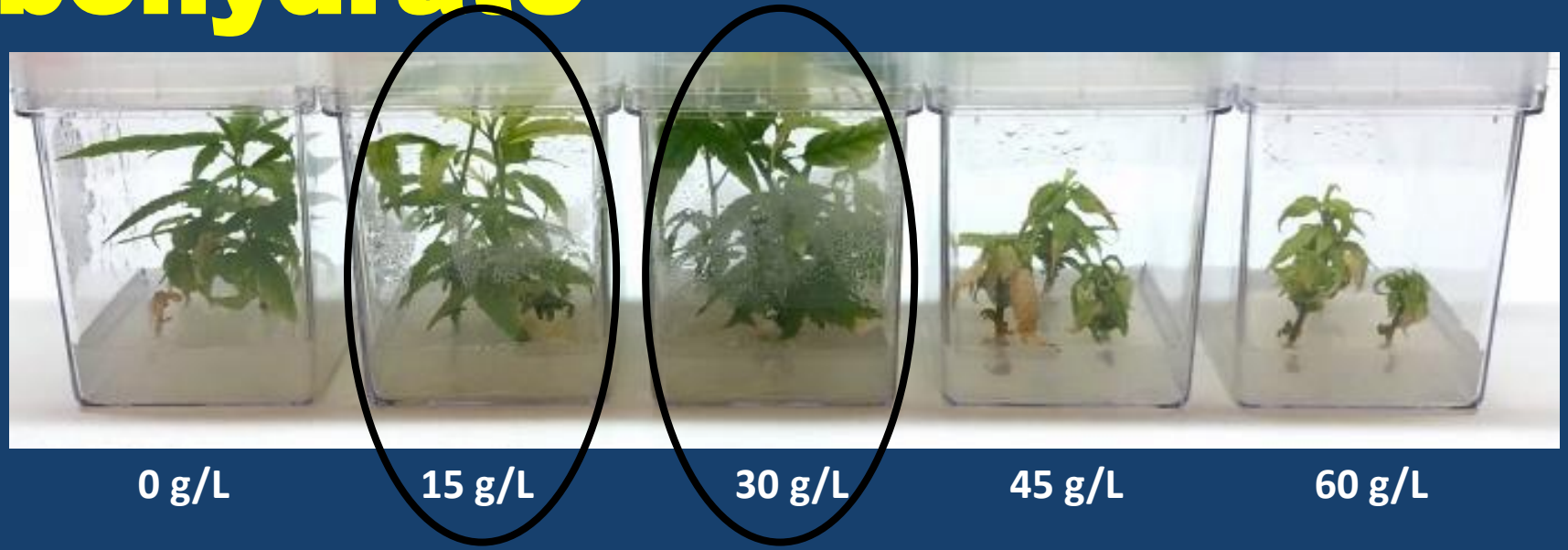
Stage II
Cytokinin

Results:

- 5.0 μM TDZ produced greater fresh weight and number of nodes
  - Also produced shorter shoots and more callus
- 1.0 μM BA, 1.0 μM 2iP, and 5.0 μM 2iP produced significantly greater explant rating
- Low rates of 2iP or BA is recommended



Stage II:
Carbohydrate



Results:

- 15 g/L and 30 g/L sucrose produced greater length, fresh weight, and rating
- 15 g/L showed less hyperhydricity
- 15 g/L sucrose recommended to maintain quality and reduce costs

Stage II
Gelling Agent

Agar | Agargellan | Gellan Gum*

- **Results:**
  - No differences in fresh weight, numbers of nodes, shoot length, and rating between treatments
  - Long term exposure to gellan gum may be detrimental to growth

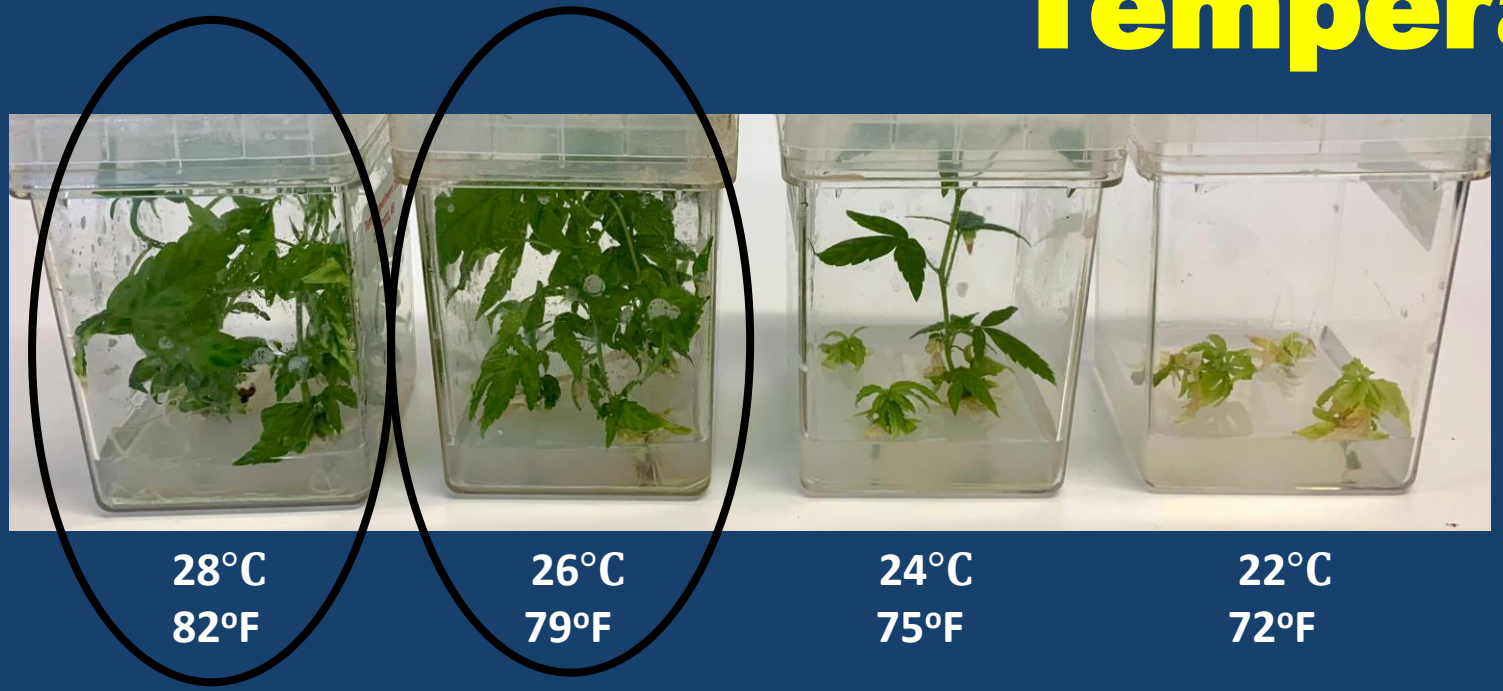
* Gelrite, Phytagel, Gel-Gro, etc.

Stage II
pH

Results:

- 5.8, 6.0, and 7.0 pH produced more nodes and less chlorotic than 4.0 and 5.0 pH
  - No differences between treatment fresh weight, shoot length, and the number of explants that rooted
- The standard pH of 5.8 is recommended

Stage II Temperature



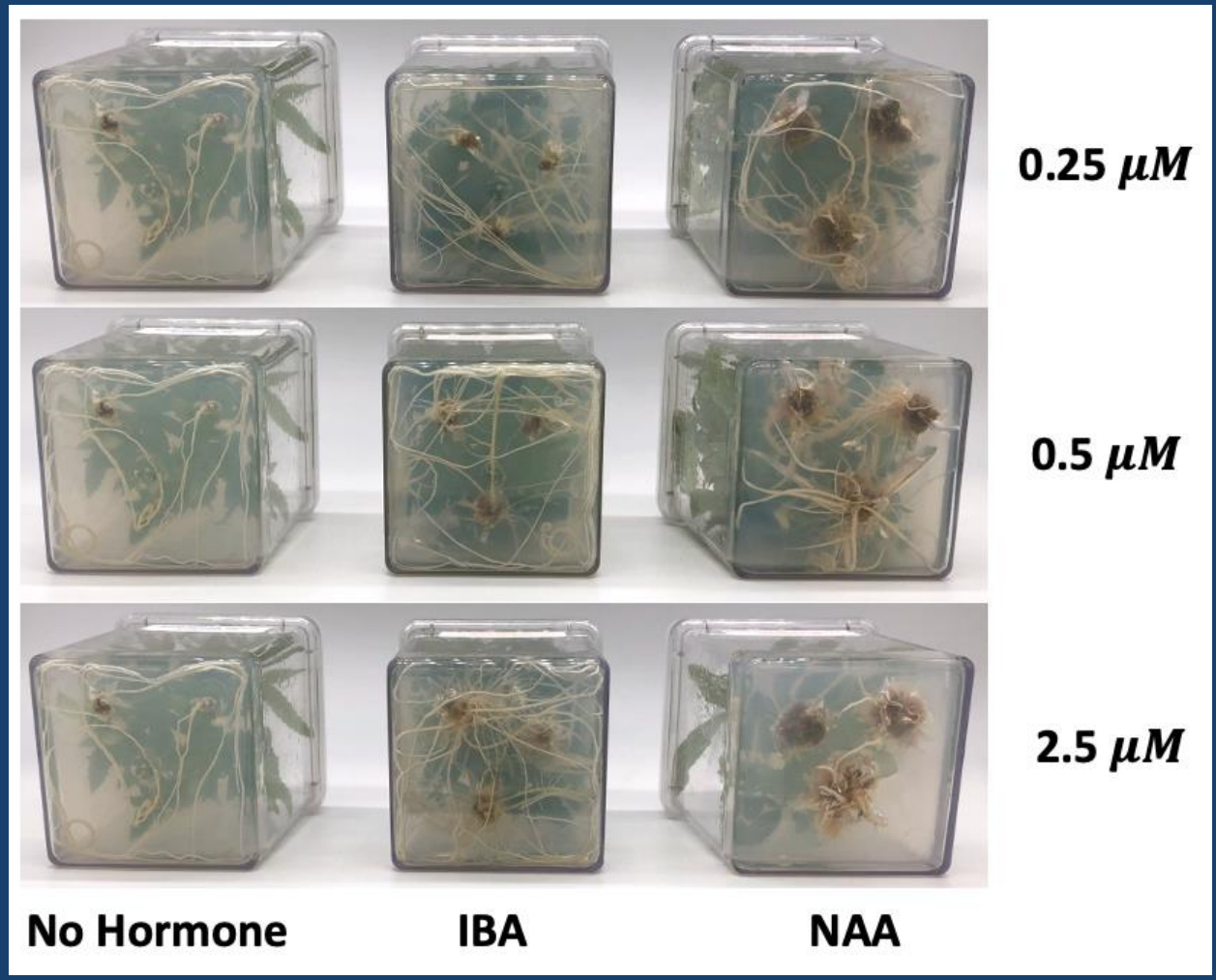
Results:

- 26°C and 28°C produced significantly greater nodes, roots, fresh weight, and rating
- Higher temperatures recommended for hemp micropropagation

Stage III
Auxin

Results:

- 0.25 μM NAA, 0.5 μM NAA, and 2.5 μM IBA produced greater:
  - shoot fresh weight
  - root fresh weight
  - number of nodes
- NAA produced more callus
- 2.5 μM IBA recommended to stimulate root growth while maintaining shoot quality *in vitro*



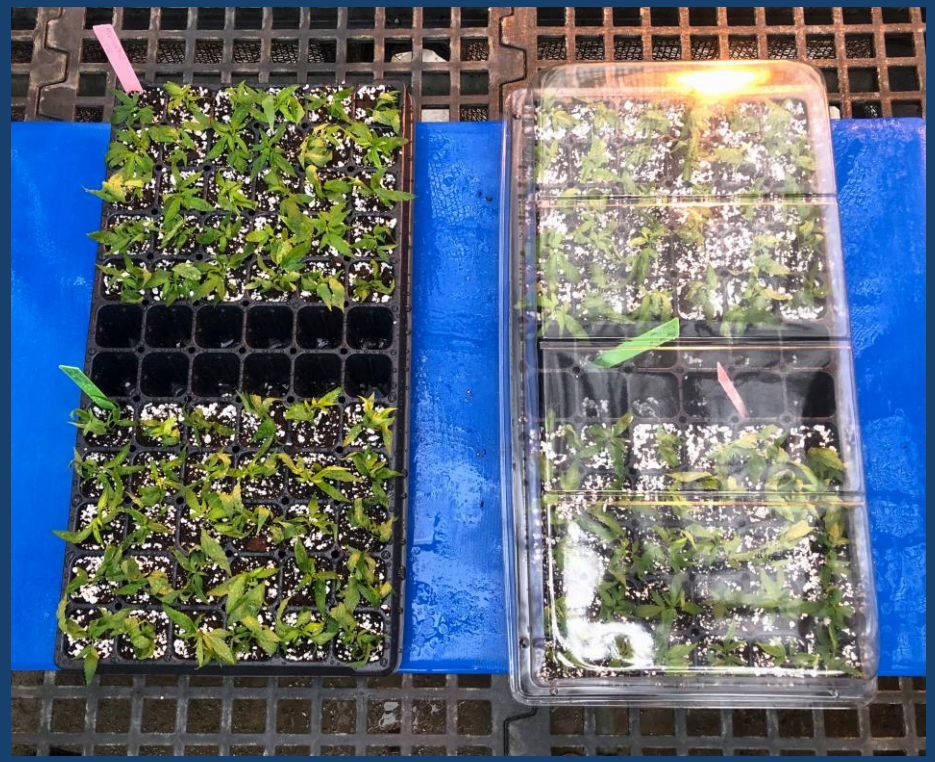
Stage IV
Acclimation

Dome vs. intermittent mist
Auxin vs. no auxin

Auxin

Intermittent Mist

Plastic Domes



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Stage III & IV
Acclimation

Auxin

No Auxin

No Dome

Dome

Conclusions

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- Plants that are acclimated under plastic domes perform better than intermittent mist
- Auxin assists root production, but not necessary for success
- Gelling agent and pH appear to have the least impact on growth compared to other variables that were tested

Examining the stages of micropropagation for hemp

Conor Stephen, MS 2022
Victor Zayas, PhD candidate
Andrei Galic, MPS 2021
Victoria Zeng, BS 2022

QUESTIONS?