

Healthy hop planting stock for Wisconsin:

Virus eradication Greenhouse propagation

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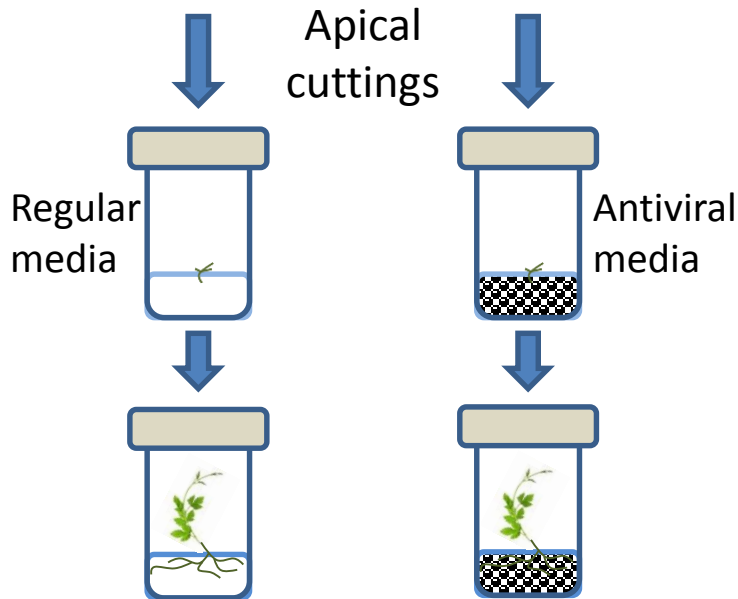


Approaches to virus eradication

- Meristem culture
 - Excise cells from growing tip and grow in aseptic culture
 - Virus less likely to be present in youngest cells
- Thermotherapy (heat treatment)
- Cryotherapy (cold treatment)
 - Both seek to slow virus multiplication
 - Generally followed by meristem or apical tip culture
- Antiviral compounds in tissue culture media
 - Sometimes combined with thermotherapy and meristem or apical tip culture

Our approaches to virus eradication

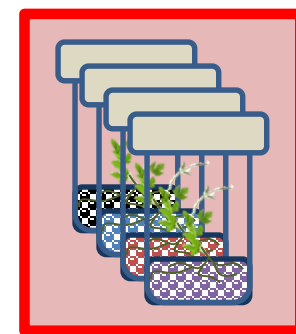
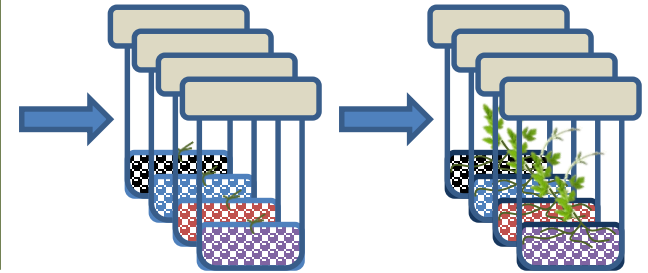
Start with potted plants



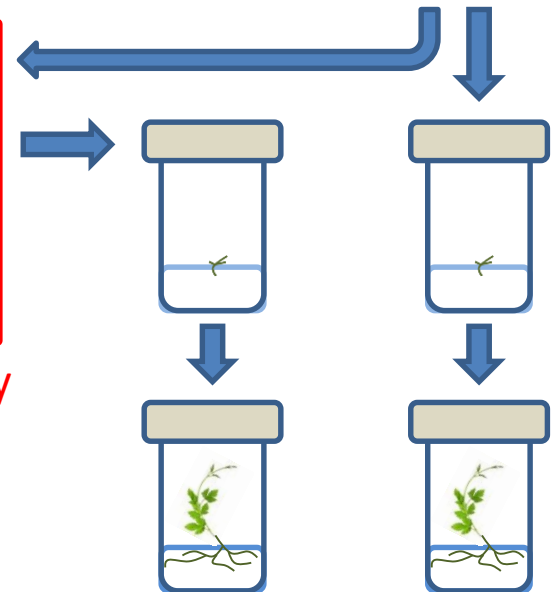
Start with tissue cultured plants



Grow on antiviral-containing media



Thermotherapy



Thermotherapy on potted plants

- Brewer's Gold, Centennial
- Infected with:
 - ApMV
 - carlaviruses
- Thermotherapy method following Postman et al (2005):
- 6 week old plants
- 8 hour cycle:
 - 4 hrs lights on, 38 C (100 F)
 - 4 hrs lights off, 30 C (86 F)
- Apical tip culture after 3 weeks



Thermotherapy on potted plants



- Developed multiple fine bines, overall bushy appearance
- 1 of 9 plants died

- Fewer bines and less robust than BG
- More delicate apical tips
- 5 of 9 plants died

Thermotherapy and apical tip culture

Variety	Media	Transfer date	Alive, clean	Alive, contaminated	Discarded (contaminated or no growth)
Brewer's Gold	HUM-STOR (no antivirals)	9/22/2015	8	6	3
	HUM-STOR (no antivirals)	10/22/2015	8	0	1
	HUM-STOR +DHT	9/22/2015	1	0	17
TOTAL - Brewer's Gold			17	6	21
Centennial	HUM-STOR (no antivirals)	9/22/2015	0	0	1
	HUM-STOR (no antivirals)	10/22/2015	6	0	10
	HUM-STOR +DHT	9/22/2015	0	0	5
TOTAL - Centennial			6	0	16

Test for ApMV, carlaviruses

Tissue culture plants on antivirals

- Crystal, Brewer's Gold, Centennial
- Antivirals added to "storage" media (low iron)

			Plant growth on antivirals	
Ribavirin (15 mg/L)	DHT (50 mg/L)	ASA (1.8 mg/L)	Crystal	Brewer's Gold/ Centennial
+			Stunted	Small, but active growth
+	+		Stunted	Small, but active growth
+		+	Very stunted	Small, but active growth
	+		Small, but active growth	Small, but active growth

Survival of apical tips after antiviral treatment (+/- thermotherapy)

- Apical tips excised from 5 week old plants onto HUM-STOR media
- Subset of Crystal plants given 2 weeks thermotherapy, then apical tips excised. Placed onto HUM-REGEN media due to poor condition of parent plants.

Antivirals added	Crystal	Crystal (thermo)	Brewer's Gold	Centennial
Ribavirin	0	0	6/6	5/6
Riba+DHT	0	0	5/6	7/7
Riba+ASA	0	0	5/6	5/5
DHT	0	0	5/6	6/6
TOTALS	0	0	21/24	23/24

Test for ApMV, carlaviruses

Conclusions and future work on virus eradication

- Variety differences in sensitivity to antivirals and heat stress
- Combination of antivirals and thermotherapy is very stressful to plants
 - Optimize temperature, heat-cool cycling, antiviral concentration
 - Other varieties?
- Contamination in tissue culture is a problem when taking apical tips from heat-treated potted plants
 - Does DHT counteract the surface sterilization compound (PPM)? Could other antivirals be used instead of DHT? Would bleach work better than PPM?
 - Use fungicides/antibiotics in tissue culture media?
 - Transfer from tissue culture to potting soil as quickly as possible

Greenhouse propagation

- Cut single nodes with ~1 inch stem to either side, and trim leaves to ~1 inch square if necessary
- Soak in rooting hormone for 2 minutes (indole butyric acid: boric acid solution, 10 ppm each in 50% ethanol)
- Insert into moist potting media so that nodes are barely covered; ensure correct orientation
- Cover with plastic dome. Dome should allow some ventilation and be placed out of direct sunlight.
- Water gently. Re-cover nodes with soil if exposed by watering.

Varietal differences noted:

- Poor survival when started in winter/spring (even with supplemental light)
 - Mt. Hood, Saazer 38, Hallertauer Tradition, Willamette
- Differences in growth rate in greenhouse:
 - Slow: Mt. Hood
 - Medium: Saazer 38, Hallertauer Tradition, Willamette
 - Rapid: Cascade, Fuggle, Nugget, Galena, Yakima Gold



Test of rooting hormone concentration

Cuttings taken March 13 2015; assessed 4 weeks later

Indole butyric acid: boric acid in 50% ethanol

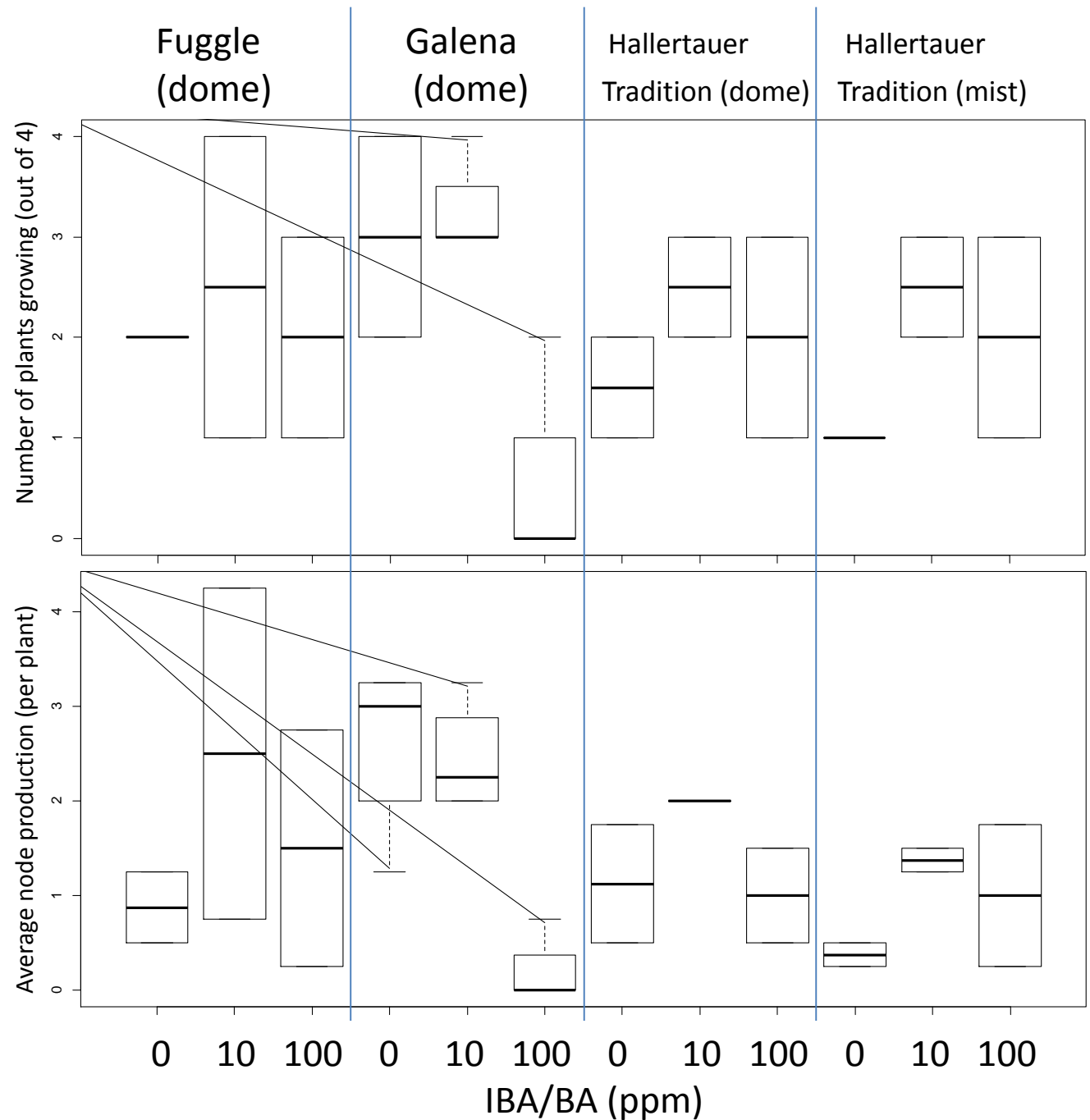
Results

Galena propagation impaired by higher levels of IBA/BA

Other varieties less sensitive

10 ppm IBA/BA acceptable for all varieties

Note, HCPN recommends 1000 ppm but this is not successful for us.



Comparison of domes vs. mist

Cuttings taken March 27 2015; assessed 4 weeks later

IBA/BA 10 ppm

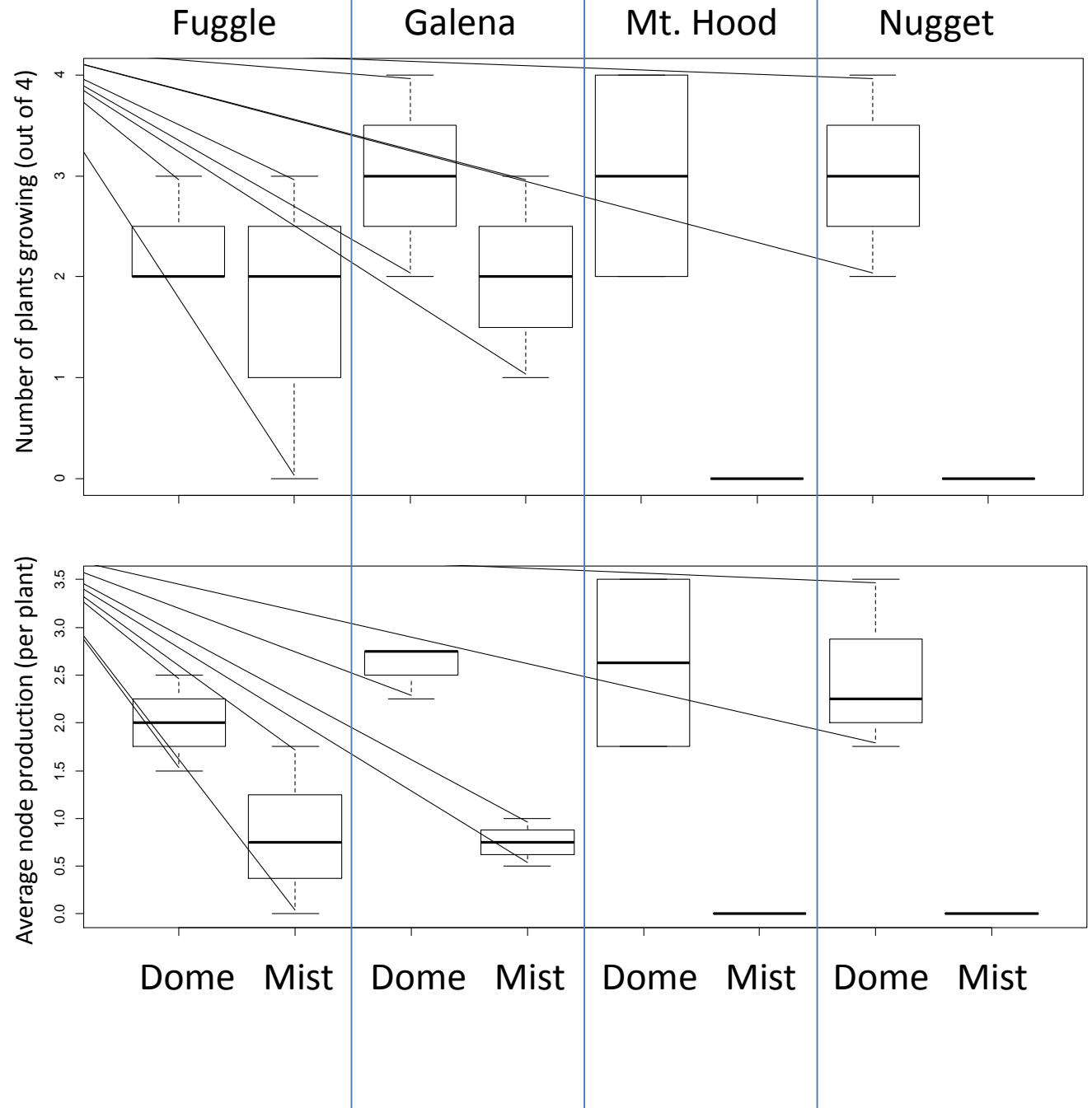
Results

Fuggle and Galena:

- Slightly better success rate under domes than mist
- More rapid bine growth under domes than mist

Mt. Hood and Nugget:

- Misted plants failed completely



Dormancy treatments

- Does putting potted hop plants through a dormancy period improve regrowth?
 - 5 varieties: Nugget, Fuggle, Galena (2 age groups), Willamette, Yakima Gold
 - Dormancy induced by reducing supplemental light in line with natural photoperiod.
 - Plants moved to 4 C cooler at end of October.
 - Set of plants moved to normal greenhouse conditions weekly for 12 weeks (ending Jan 25).
 - Monitoring survival and growth

Future directions for research?

- Virus eradication
 - Useful if there is interest in a breeding program or in making selections from wild or feral stock
- Greenhouse propagation
 - Questions: seasonality of propagation; dormancy and regrowth vigor; varietal differences: ... ?
 - Useful if there are currently issues with supply of rhizomes or plantlets
- Other questions of interest?