DoE in Horticultural Research

Randall P. Niedz, PhD
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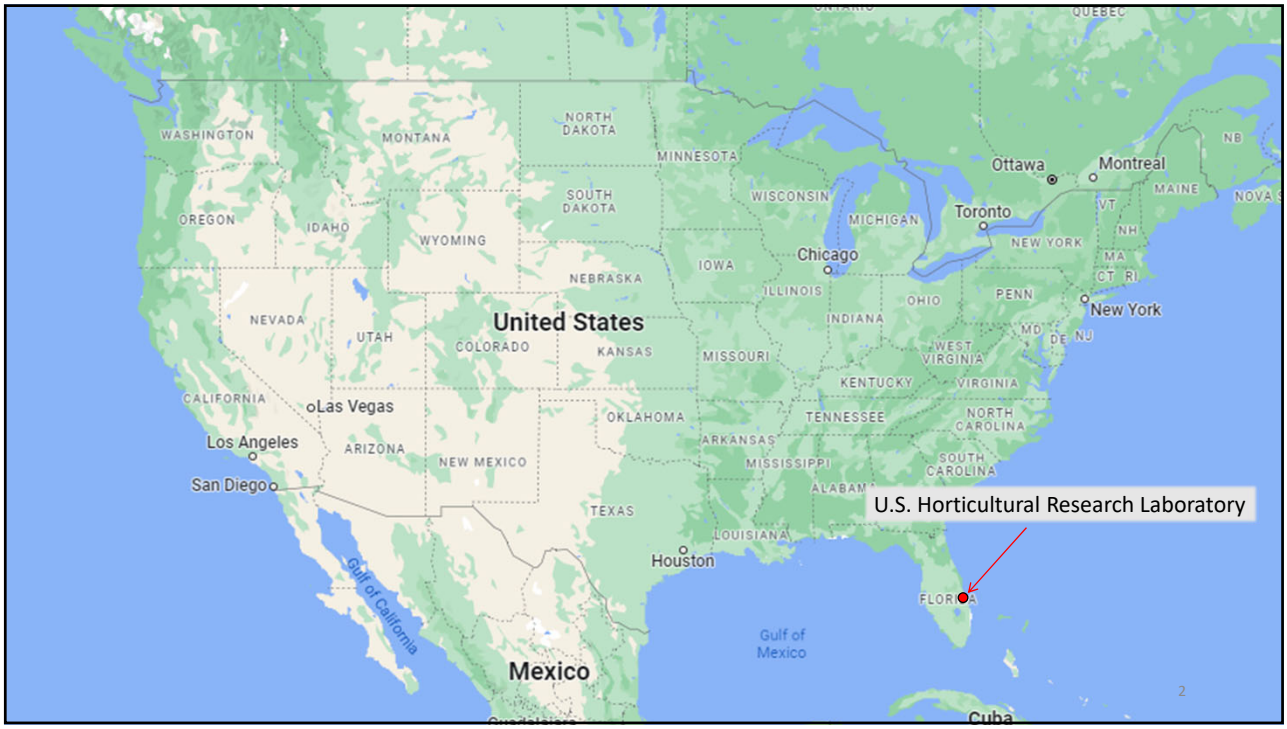
2022 Online DOE Summit
Stat-Ease
Minneapolis, MN




US Department of Agriculture (USDA-ARS)
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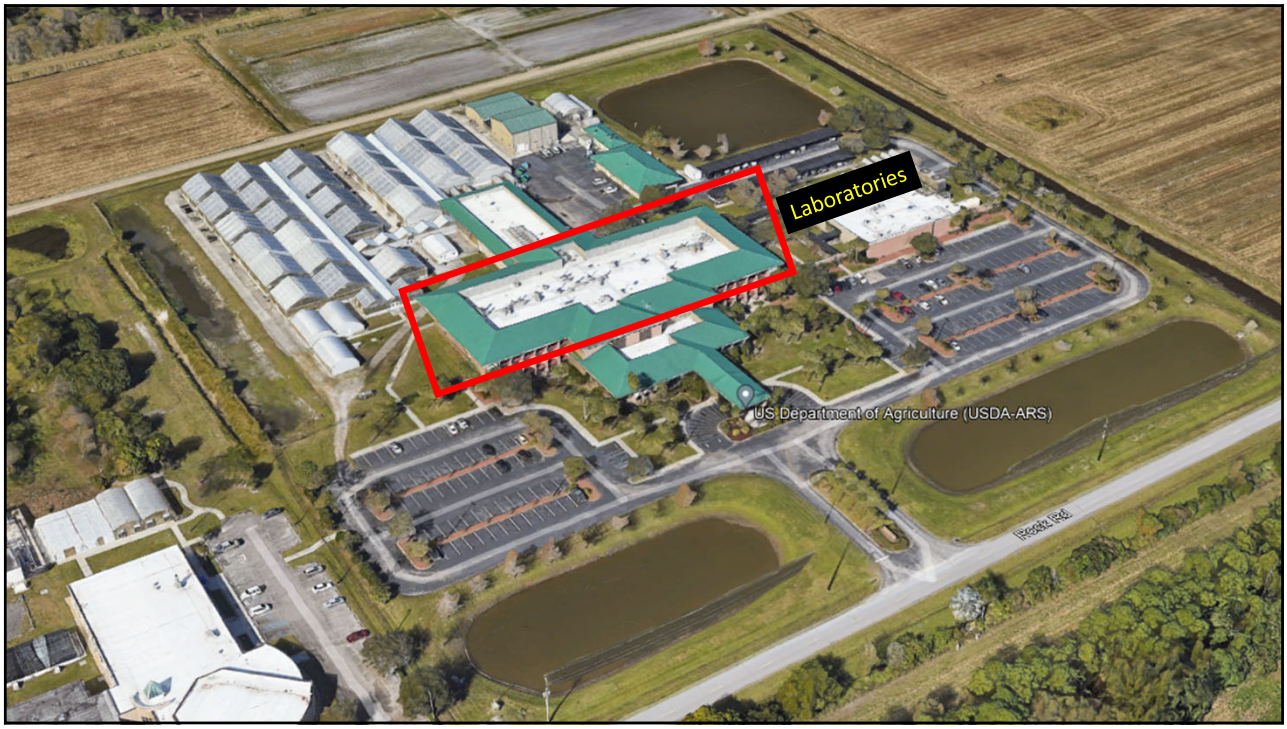
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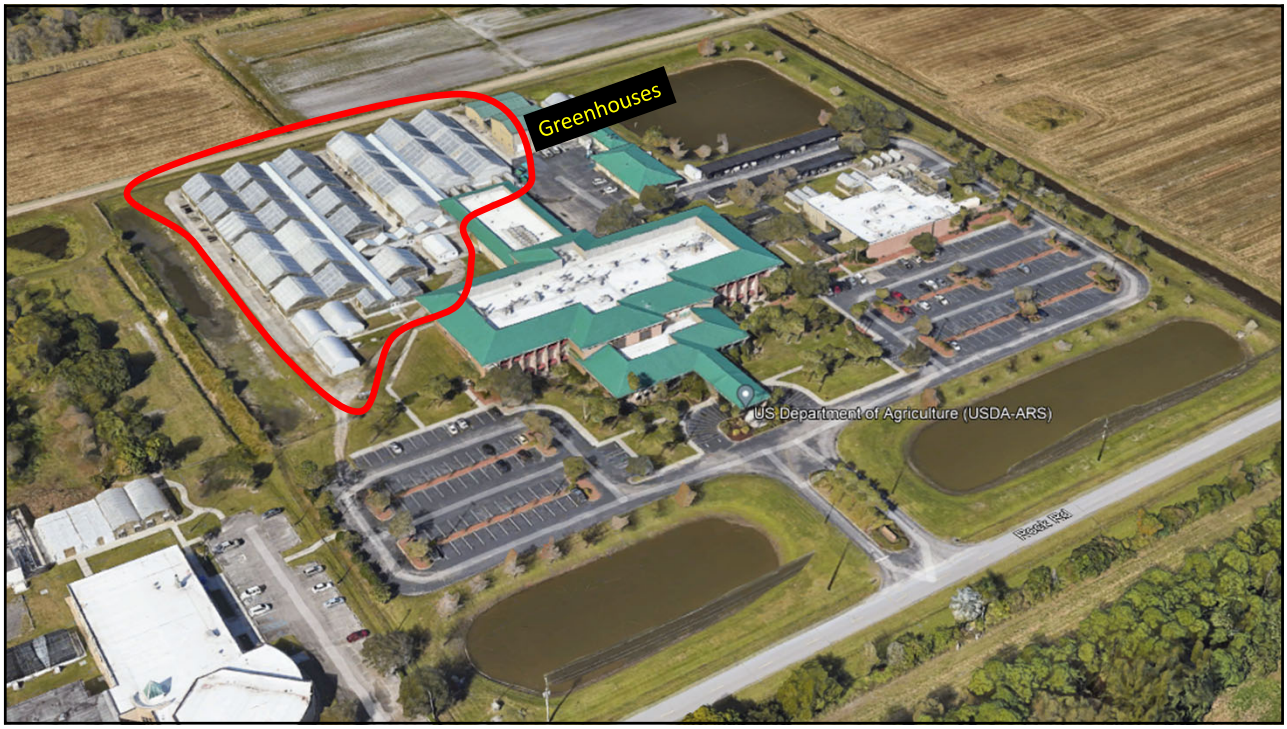
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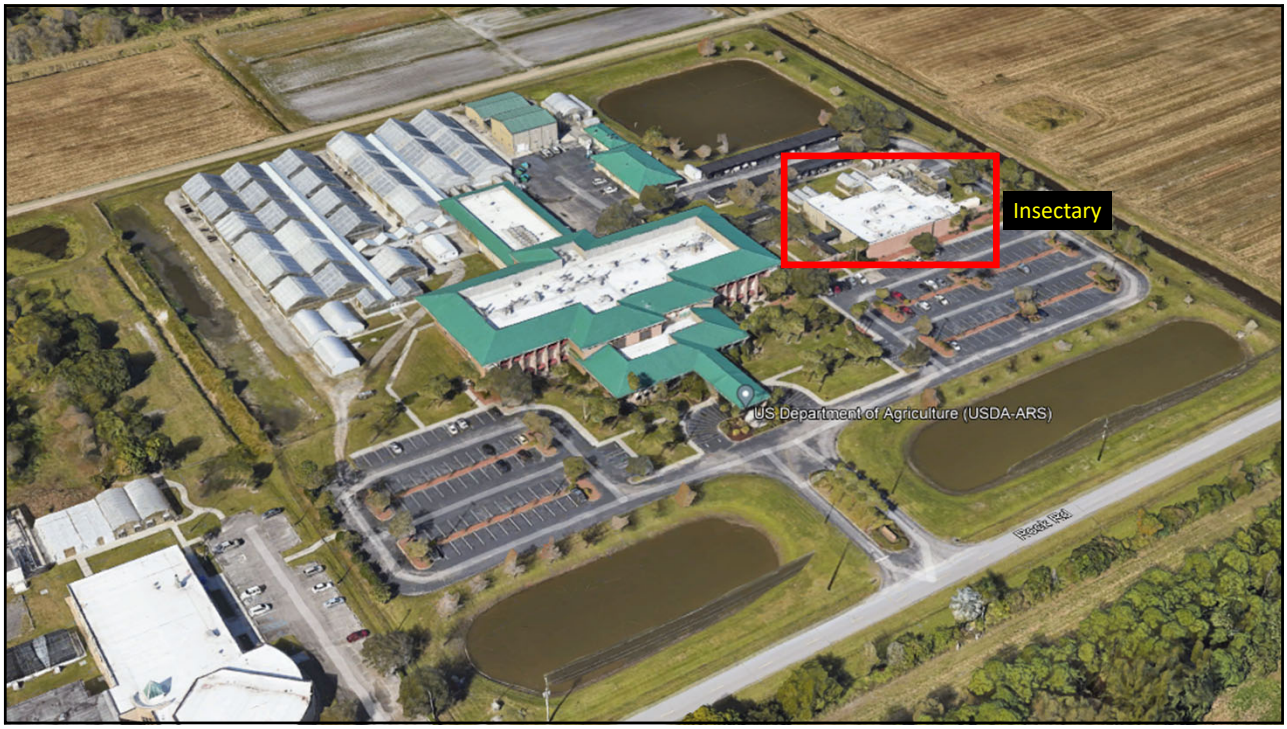
Insectary
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Field
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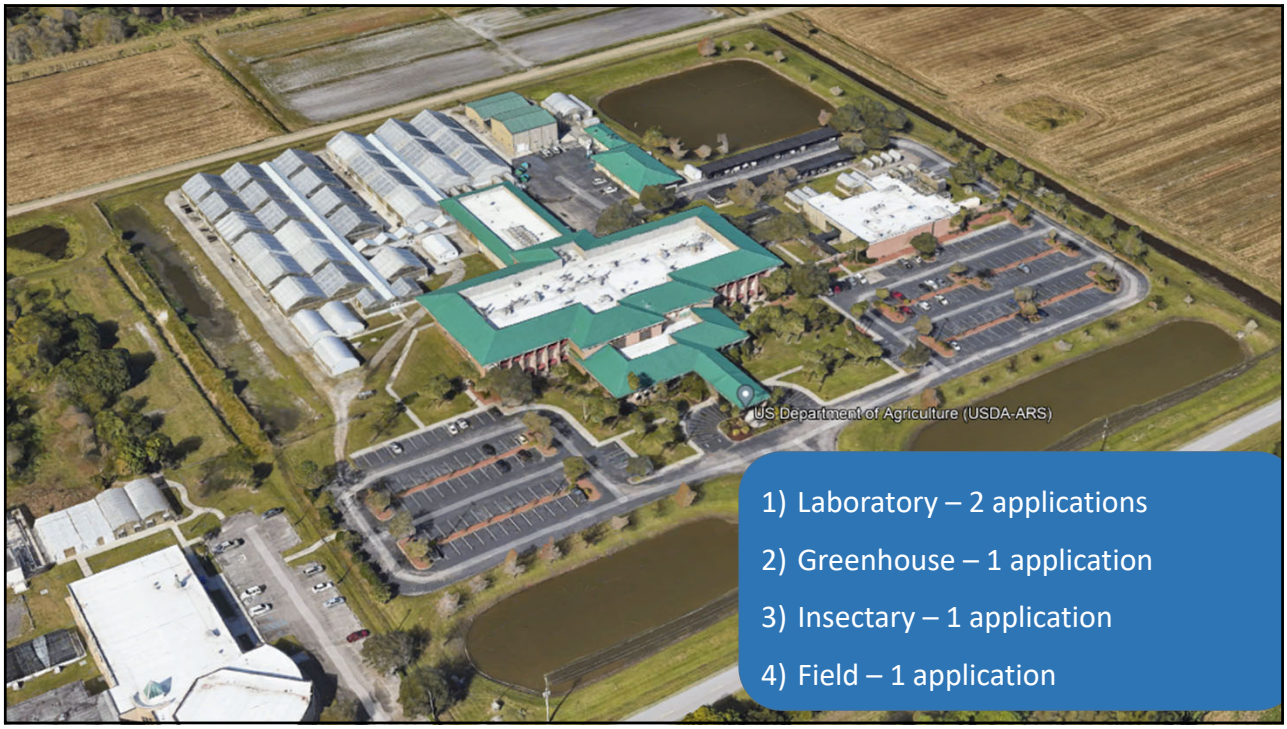
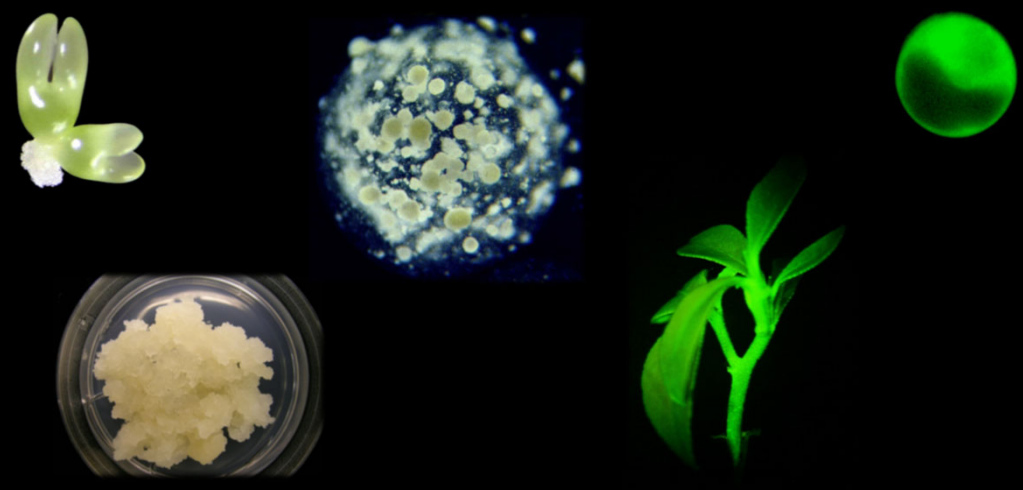

US Department of Agriculture (USDA-ARS)
1) Laboratory – 2 applications
2) Greenhouse – 1 application
3) Insectary – 1 application
4) Field – 1 application

LABORATORY EXPERIMENTS

In vitro culture



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Plant Cell Reports (1995) 14:403–406

Plant Cell Reports

Green fluorescent protein: an *in vivo* reporter of plant gene expression

Randall P. Niedz [1], Michael R. Sussman [2], and John S. Satterlee [2]

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RANDOM SAMPLES

Ex 1B

edited by JOCELYN KAISER

Finding the Hottest Dwarf

Astronomers from Britain, Germany, and the United States have found a missing link in the evolution of the class of small to medium-sized stars to which our own sun belongs. In November's *Monthly Notices of the Royal Astronomical Society*, a team led by astrophysicist Martin Barstow of the University of Leicester describes a hydrogen-rich white dwarf star with a surface temperature of some 90,000 K—the hottest star of its type yet discovered.

When a sunlike star has burned up its nuclear fuel, it expands to become a bloated red giant. Then it blows away its outer layers, leaving a shrunken star—surrounded by a planetary nebula—that typically has a surface temperature of at least 150,000 K. Over time, the nebula disperses, leaving a gradually cooling star known as a white dwarf. Such stars fall into two classes: those with hydrogen-rich atmospheres and those dominated by helium.

Astronomers have been able to observe a clear progression from hot central nebula stars to cooler white dwarfs for those with helium-rich atmospheres. But until now, the hydrogen-dominated white dwarfs seemed to appear suddenly at a cool 70,000 K or less. "The hydrogen-rich branch really had a gap," says Barstow.

Barstow's team filled that gap using ROSAT, the orbiting Anglo/German/U.S. x-ray and extreme-ultraviolet observatory. White dwarfs radiate strongly at ultraviolet wavelengths, and since its 1990 launch ROSAT has identified almost 70 new examples of the breed. By taking spectra of these stars with ground-based optical telescopes, Barstow's team can determine their temperatures. Stars' spectra contain gaps caused by the absorption of radiation by hydrogen, and these "absorption lines" vary with mass and temperature.

The hottest white dwarf in this survey resolves a heated theoretical debate. To explain the lack of such stars, some theorists had speculated that cooler examples evolved from a subset of helium-rich white dwarfs by the sinking of these stars' helium below their surfaces. "It was a major point of contention," says Edward Sion of Villanova University in Pennsylvania. But now, he says, this rather labored theory can be tossed out.

Strength in numbers. Proposed telescope in lower left of drawing and four others form an array that will outperform much larger scopes.

NSF Grant Boosts Telescope Plan

By the year 2000, perhaps from a desert mesa in the southwestern United States, astronomers will probe the heavens with an instrument that can pick out a nickel from 10,000 miles away. That's the promise contained in last month's announcement by the National Science Foundation (NSF) that it has awarded $5.5 million for the construction of an array of telescopes that will produce the highest resolution optical images ever made.

The money went to the Center for High Angular Resolution Astronomy (CHARA) at Georgia State University. The university plans to raise another $5.8 million to build the facility, which will consist of five 1-meter telescopes laid out in a Y shape. Through an emerging technology called optical interferometry, the light from each telescope will travel through evacuated tubes and then be carefully combined, producing images equivalent to those that could be created by a single 400-meter telescope.

In principle, the array could identify the pitcher in a baseball game played on the moon. Instead, its duties will include looking for extrasolar planets and studying binary systems, in which two stars closely orbit each other. Also, says CHARA director Harold McAlister, "it will be the premier tool to image surface features on stars" such as the spots and flares that also mar our sun. The location for the array has yet to be finalized, says McAlister, but the school is negotiating with the University of New Mexico for a site on a mesa 80 miles west of Albuquerque.

Scientific Resource Center Closes

For 14 years, journalists in need of scientific sources have turned to a New York City organization that provided the names of experts in everything from particle physics to environmental catastrophes. But since May, the Scientists' Institute for Public Information (SIPI) has laid off all but four of its 26 staff members and last month president Alan McGowan said it is "phasing out of existence" by the year's end.

Established in 1963, SIPI has worked to bridge the gap between the scientific community and the public by publishing reports and pamphlets, and by sponsoring meetings for journalists on topics as diverse as national security and child development. In 1980, it began a media resource service that last year referred 5000 callers to a database of 30,000 scientists.

McGowan says the nonprofit, which had an operating budget of $2.6 million, is folding because it lost funds for overhead from corporate backers, even though foundation support continued. "It's too bad," says molecular biologist and publisher of *R&D Innovator* Winston Bell, who has served on a SIPI panel. "SIPI was getting science to the public. That's an important service."

Not all is lost, however. Last week SIPI began discussions to transfer its media resource service to the scientific society Sigma Xi. Renee Krever of Sigma Xi says the move will hinge on whether foundations would continue to

Lighting Up New Genes

Researchers have found a green light that gives a "go" sign for gene transfer. Geneticist Randall Niedz of the U.S. Agricultural Research Service and a University of Wisconsin team have inserted the gene coding for green fluorescent protein (GFP), found in the bioluminescent jellyfish *Aequorea victoria*, into orange-tree cells as a "reporter." If its glow is turned on, it indicates that other foreign genes inserted into the plant alongside it have been turned on as well. Scientists can use this signal to determine whether foreign genes they've placed in plants—to improve disease resistance, for example—are working, says Niedz. The work will appear in an upcoming issue of *Plant Cell Reports*.

The GFP gene and a gene of interest are both turned on because they lie in a DNA sequence under control of a single promoter, a genetic "on-off" switch. Other reporter genes have been tried with plants, Niedz says, but the assays often destroy the cell or the signals aren't readily visible.

Bright idea. The glow of a jellyfish protein lights up an orange cell.

SCIENCE • VOL. 266 • 4 NOVEMBER 1994

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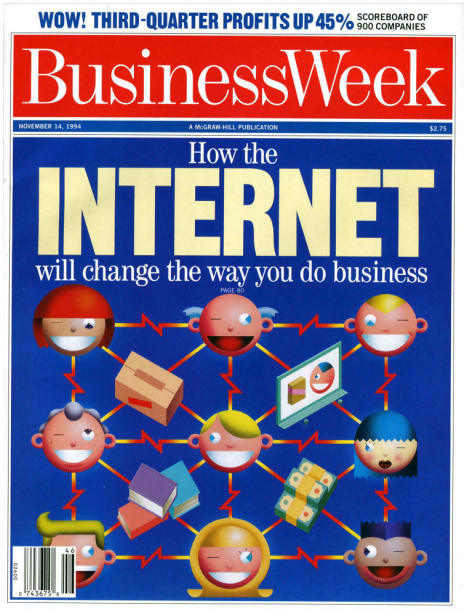

Developments to Watch

EDITED BY RUTH COXETER

SALTING AWAY EXCESS HEAT

PEOPLE USED TO PUT HOT bricks between the covers to warm their beds—and may still do so in some parts of the world. But today, engineers are exploiting other materials that release stored energy: Dow Chemical Co. and Indianapolis-based IPALCO Enterprises Inc. have formed a joint venture called Store Heat & Produce Energy (SHAPE). Their goal is to develop products based on 16 different inorganic salt hydrates that store and release heat at temperatures ranging between 40F and 240F. The salts are nonflammable, don't degrade easily, and are able to store more heat than some alternative materials.

The first product, a truck heater, is due next year. When truckers pull over on cold nights, they run the engine to keep warm, which wastes fuel and increases engine wear. SHAPE's energy storage box can give out enough heat to keep the cab between 65F and 70F for eight hours, say company officials. SHAPE intends to add the material to furnaces, air conditioners, and heat pumps for commercial buyers of electricity, which will allow them to shift more of their demand to cheaper, off-peak hours.

FROM A PIG'S INTESTINE TO YOUR ACHING KNEE

STEPHEN F. BADYLAK FEELS like a used-parts salesman. From discarded pig parts, his team at Purdue University has developed a material to help reconstruct damaged human arteries, veins, ligaments, and tendons.

The process starts with the middle layer of a pig's small intestine, the submucosa, or sis, which is sterilized and molded into sheets and tubes. Animal studies showed that within weeks a tube of sis remodeled itself into a brand-new ligament. And within a year, all traces of the material disappeared.

The material—a composite of proteins, collagen, and bioactive molecules—signals cells to migrate and differentiate into new tissue that takes its place. Badylak speculates that the similarity of pig and human collagen may help prevent an immune system response, since the two differ by just a few amino acids. Two collaborations are under way: DePuy Inc. of Warsaw, Ind., is developing ligaments, and Cincinnati-based Sentron Medical Inc. is working on hernia patches. Badylak estimates human testing will begin within a year.

A GENE THAT SIGNALS SUCCESS

GREEN LIGHT: *Orange cells and fluorescent protein*

A GREEN FLUORESCENT PROTEIN (GFP) GENE GLEANED from jellyfish may shed some light on genetic research. Randall P. Niedz, a plant geneticist with the U.S. Agriculture Dept.'s research service, borrowed the gene from Columbia University scientists, had it modified at the University of Wisconsin, and transferred it into sweet orange citrus cells. When illuminated with blue light and viewed under a microscope, the cells glowed an intense green.

Niedz hopes that the GFP gene can be hooked on to a gene that codes for a desirable trait, such as cold resistance or sweetness, and inserted back into a fruit or vegetable cell. A glowing cell would signal a successful transplant and give away the location of the gene. Paired with a DNA sequence called a promoter, which is sensitive to light or temperature, the GFP could signal when the desirable gene is turned on. Unlike commonly used chemical tests, illumination doesn't destroy the cell. So plants containing the gene could be grown so scientists can observe which parts of the plant express the gene. Anticipating the wrath of biotech nemesis Jeremy Rifkin, Agriculture Dept. scientists at the Gene Expression Laboratory in Berkeley, Calif., are trying to figure out how to delete the jellyfish gene or turn it off once its work is done.

THIS FOAM BLANKET MAY DAMPEN A CHERNOBYL THREAT

CHERNOBYL IS A NUCLEAR disaster that won't go away. Intense gamma radiation is weakening the Ukrainian nuclear power plant's damaged containment vessel, which could collapse and raise a giant cloud of radioactive dust.

What to do? Under an agreement with Ukraine's State Committee of Atomic Energy, New York-based Nuclear Shield International Inc. is figuring out how to spread a spongy foam blanket to seal the ruins in place.

Irradiated concrete disintegrates into a dangerous "hot" dust. Nuclear Shield's foam, which isn't damaged by radiation, is made mainly of polysiloxane, a silica compound, plus metal additives. While it won't stop rays, it should stay intact and keep irradiated materials from spreading. The Moscow-based Kurchatov Institute for Nuclear Physics has endorsed the material's use. Nuclear Shield is a unit of Orbit Technologies Inc.

INNOVATIONS

■ To cut wind drag and boost mileage on a bus, try a 12-foot inflatable tail, says Texas Tech University engineer Herbert J. Carper. It is approved for use in Arizona.

■ Soon baking soda may clean uranium-contaminated soil. Los Alamos National Laboratory researcher Don A. York found that a soda solution can leach out 92% of the radioactive material.

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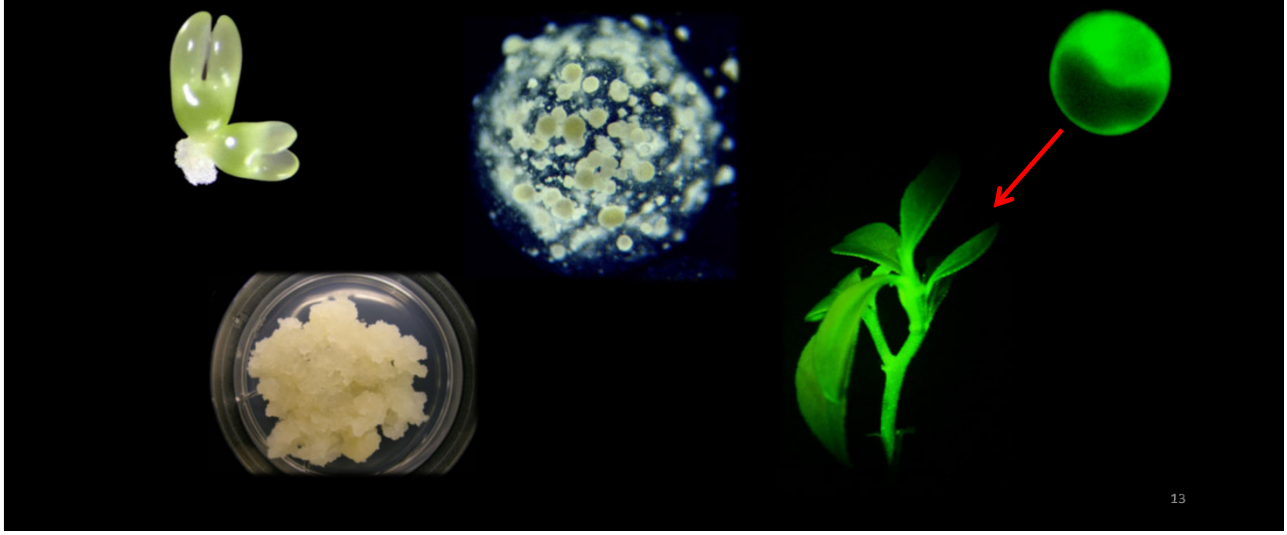
LABORATORY EXPERIMENTS
1) Citrus cell lines.



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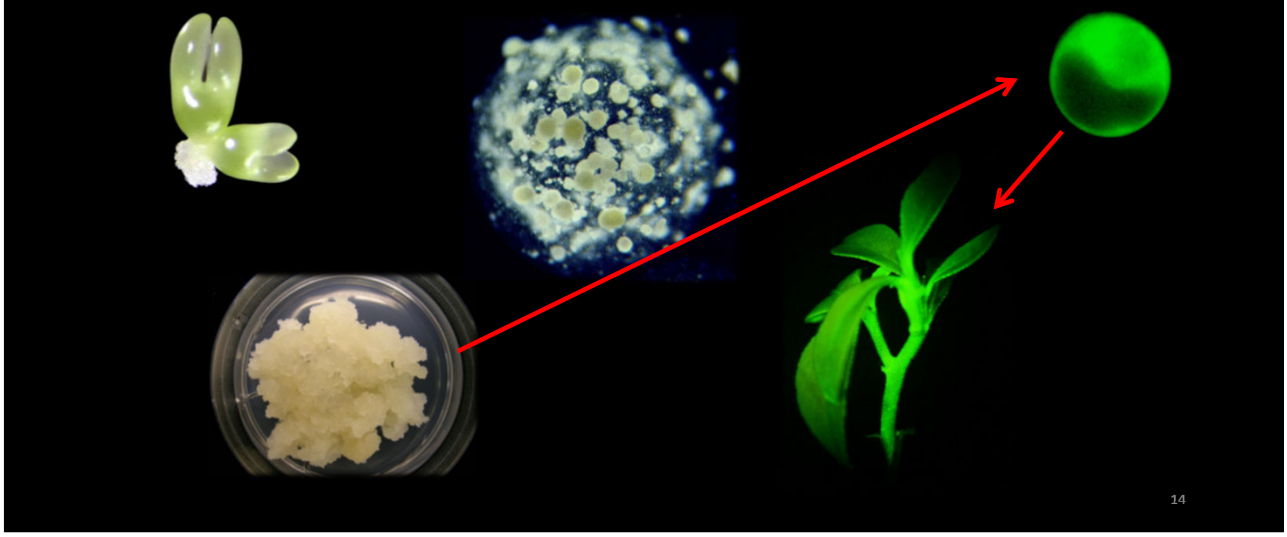
LABORATORY EXPERIMENTS
1) Citrus cell lines.



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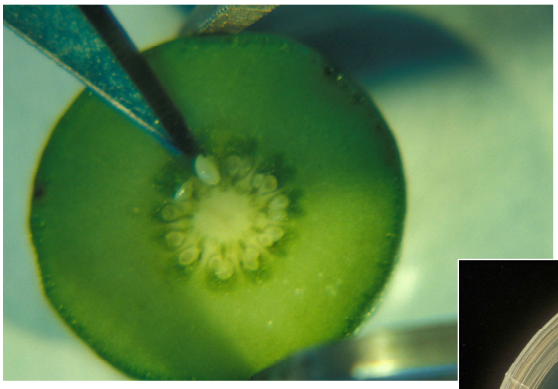

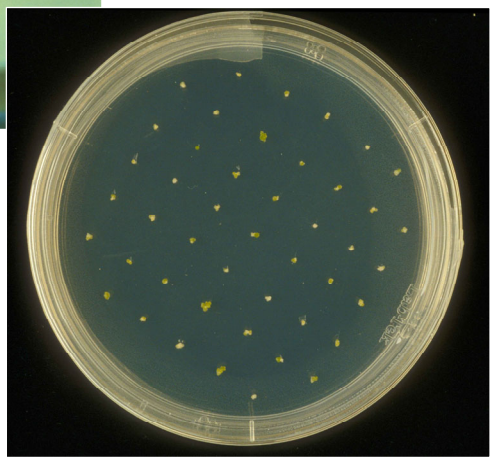

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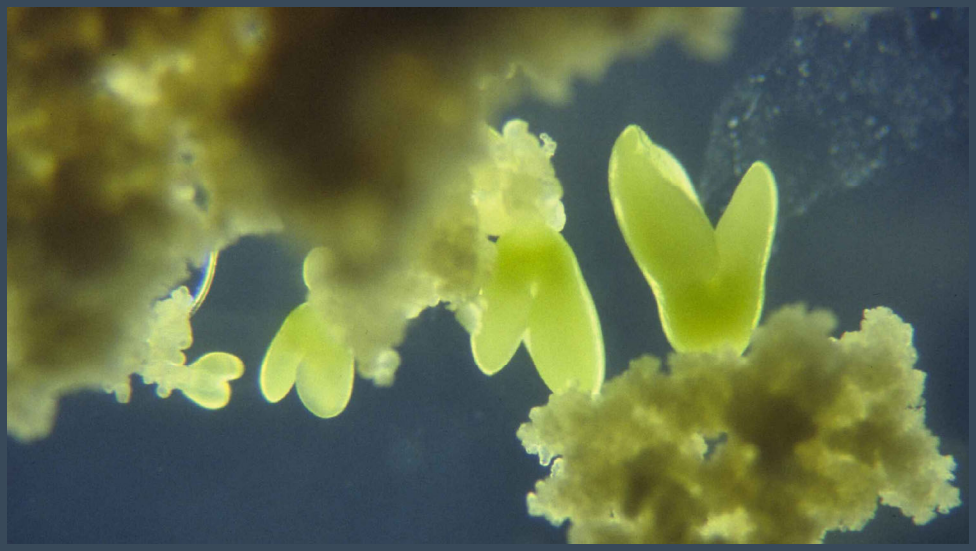

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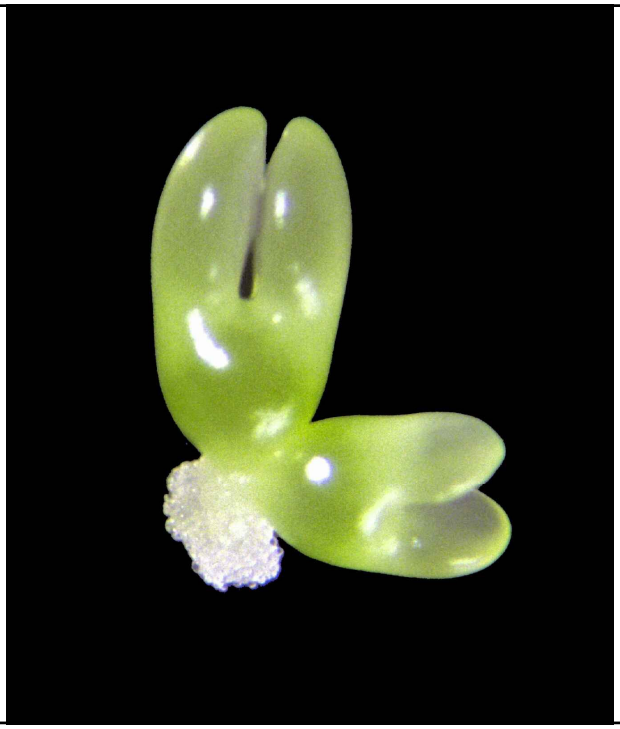

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Murashige and Skoog plant tissue culture medium (MS Medium)

MS Mineral nutrients

1) Ammonium Nitrate (NH_4NO_3)
2) Potassium Nitrate (KNO_3)
3) Calcium Chloride ($CaCl_2$)
4) Magnesium Sulfate ($MgSO_4$)
5) Potassium Phosphate, Monobasic (KH_2PO_4)
6) Boric Acid (H_3BO_3)
7) Cobalt Chloride ($CoCl_2 . 6H_2O$)
8) Cupric Sulfate ($CuSO_4 . 5H_2O$)
9) Manganese Sulfate ($MnSO_4 . H_2O$)
10) Molybdic Acid Sodium Salt ($Na_2MoO_4 . 2H_2O$)
11) Potassium Iodide (KI)
12) Zinc Sulfate ($ZnSO_4 . 7H_2O$)
13) Ferrous Sulfate ($FeSO_4.7H_2O$) + EDTA

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MS Mineral nutrients - Factors

Factor 1	Ammonium nitrate (NH_4NO_3)
Factor 2	Potassium nitrate (KNO_3)
Factor 3	Calcium chloride ($CaCl_2$) Magnesium sulfate ($MgSO_4$) Potassium phosphate (KH_2PO_4)
Factor 4	Boric acid (H_3BO_3) Cobalt chloride ($CoCl_2 . 6H_2O$) Cupric sulfate ($CuSO_4 . 5H_2O$) Manganese sulfate ($MnSO_4 . H_2O$) Molybdic acid ($Na_2MoO_4 . 2H_2O$) Potassium iodide (KI) Zinc sulfate ($ZnSO_4 . 7H_2O$)
Factor 5	Ferrous sulfate ($FeSO_4.7H_2O$) + EDTA

20

MS Mineral nutrients - Factors

Factors – MS salts partitioned into five groups.

1) NH_4NO_3
2) KNO_3
3) Mesos (Ca, Mg, SO_4, Na, Cl, PO_4)
4) Metals (Mn, Zn, Cu, Co, B, I, Mo)
5) Fe ($FeSO_4$ + NaEDTA)

Factor ranges – multiples of MS levels.

1) NH_4NO_3 : 0.5X – 1.5X
2) KNO_3 : 0.5X – 1.5X
3) Mesos : 0.5X – 1.5X
4) Metals : 0.5X – 4X
5) Fe : 0.5X – 4X

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Design

5-factor D-optimal Quadratic Response Surface

Default DX design – 31 runs

21 model, 5 error, 5 LOF

22

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Factor ranges – multiples of MS levels.

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2) KNO_3 : 0.5X – 1.5X
3) Mesos : 0.5X – 1.5X
4) Metals : 0.5X – 4X
5) Fe : 0.5X – 4X

Design

5-factor D-optimal Quadratic Response Surface

Default DX design – 31 runs

21 model, 5 error, 5 LOF

Augmented DX design – 43 runs

21 model, 10 error, 10 LOF, 2 blocks

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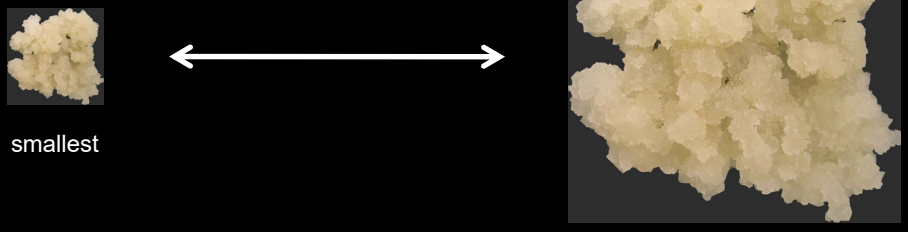
Large range in final fresh weight.

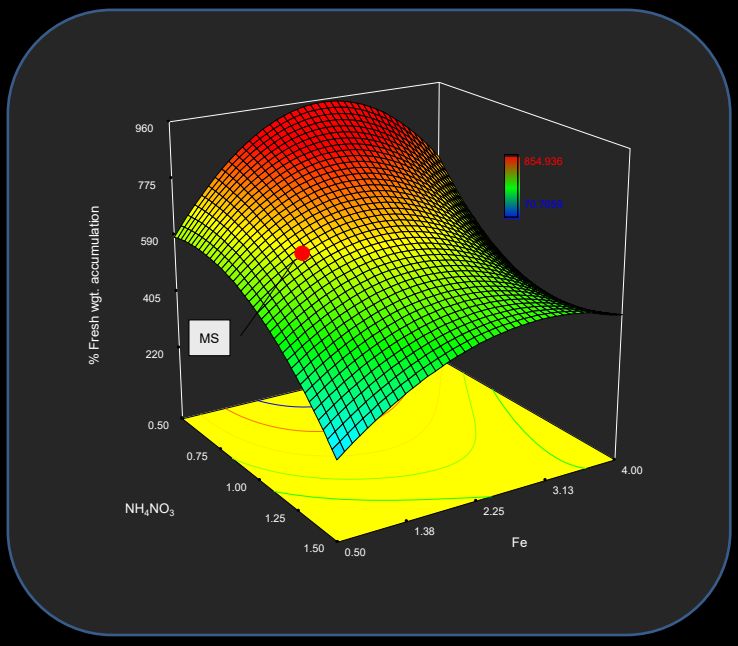


smallest

largest

MS
+690%

Predicted High Growth Point
+928%



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PHYSIOLOGY

Regulating plant tissue growth by mineral nutrition

Randall P. Niedz · Terrence J. Evens

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Abstract The objective of this study was to determine if the growth of sweet orange (*Citrus sinensis* (L.) Osbeck cv. 'Valencia') nonembryogenic callus could be regulated and controlled via the mineral nutrient components of the medium. The 14 salts comprising Murashige and Skoog (MS) basal medium were subdivided into five component groups. These five groups constituted the independent factors in the design. A five-dimensional hypervolume constituted the experimental design space. Design points were selected algorithmically by D-optimality criteria to sample of the design space. Growth of the callus at each design point was measured as % increase of fresh weight at 14 d. An analysis of variance was conducted and a response surface polynomial model generated. Model validation was conducted by mining the polynomial for design points to

Keywords Callus · Sweet orange · Citrus · Salts · Response surface

Introduction

The basic components of plant tissue culture media are the mineral nutrients. How rapidly a tissue grows and the extent and quality of morphogenetic responses are strongly influenced by the type and concentration of nutrients supplied. Early research by Gautheret (1939), Heller (1953), White (1942), Hildebrandt et al. (1946), and Nitsch and Nitsch (1956) culminated in the development of Murashige and Skoog (MS) medium by Murashige and Skoog (1962). The potential benefits of optimizing the

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LABORATORY EXPERIMENTS
2) Micropropagation

28

Citrus rootstock X-639

Agri-Starts David Lawson provided the citrus cultures.

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Regular Two-Level Factorial Design

DesignExpert

Design for 2 to 21 factors where each factor is set to 2 levels. Useful for estimating main effects and interactions. Fractional factorials can be used for screening many factors to find the significant few. The color coding represents the design resolution: **Green** (Characterization) = Res V or higher, **Yellow** (Screening)= Res IV, and **Red** (Ruggedness testing) = Res III.

Replicates: 1 Blocks: 1 Center points per block: 0 Show Generators

Number of Factors

Runs	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
4	2^{2}	2^{3-1}_{III}																		
8		2^{3}	2^{4-1}_{IV}	2^{5-2}_{III}	2^{6-3}_{III}	2^{7-4}_{III}														
16			2^{4}	2^{5-1}_{V}	2^{6-2}_{IV}	2^{7-3}_{IV}	2^{8-4}_{IV}	2^{9-5}_{III}	2^{10-6}_{III}	2^{11-7}_{III}	2^{12-8}_{III}	2^{13-9}_{III}	2^{14-10}_{III}	2^{15-11}_{III}						
32				2^{5}	2^{6-1}_{VI}	2^{7-2}_{IV}	2^{8-3}_{IV}	2^{9-4}_{IV}	2^{10-5}_{IV}	2^{11-6}_{IV}	2^{12-7}_{IV}	2^{13-8}_{IV}	2^{14-9}_{IV}	2^{15-10}_{IV}	2^{16-11}_{IV}	2^{17-12}_{III}	2^{18-13}_{III}	2^{19-14}_{III}	2^{20-15}_{III}	2^{21-16}_{III}
64					2^{6}	2^{7-1}_{VII}	2^{8-2}_{V}	2^{9-3}_{IV}	2^{10-4}_{IV}	2^{11-5}_{IV}	2^{12-6}_{IV}	2^{13-7}_{IV}	2^{14-8}_{IV}	2^{15-9}_{IV}	2^{16-10}_{IV}	2^{17-11}_{IV}	2^{18-12}_{IV}	2^{19-13}_{IV}	2^{20-14}_{IV}	2^{21-15}_{IV}
128						2^{7}	2^{8-1}_{VIII}	2^{9-2}_{VI}	2^{10-3}_{V}	2^{11-4}_{V}	2^{12-5}_{IV}	2^{13-6}_{IV}	2^{14-7}_{IV}	2^{15-8}_{IV}	2^{16-9}_{IV}	2^{17-10}_{IV}	2^{18-11}_{IV}	2^{19-12}_{IV}	2^{20-13}_{IV}	2^{21-14}_{IV}
256							2^{8}	2^{9-1}_{IX}	2^{10-2}_{VI}	2^{11-3}_{VI}	2^{12-4}_{VI}	2^{13-5}_{V}	2^{14-6}_{V}	2^{15-7}_{V}	2^{16-8}_{V}	2^{17-9}_{V}	2^{18-10}_{IV}	2^{19-11}_{IV}	2^{20-12}_{IV}	2^{21-13}_{IV}
512								2^{9}	2^{10-1}_{X}	2^{11-2}_{VII}	2^{12-3}_{VI}	2^{13-4}_{VI}	2^{14-5}_{VI}	2^{15-6}_{VI}	2^{16-7}_{VI}	2^{17-8}_{VI}	2^{18-9}_{VI}	2^{19-10}_{V}	2^{20-11}_{V}	2^{21-12}_{V}

30

5-Factor 2-Level Resolution V Fractional Factorial

Run	Block	Factor 1 NH4NO3 xMS	Factor 2 KNO3 xMS	Factor 3 Mesos xMS	Factor 4 Minors xMS	Factor 5 Fe xMS
1	1	1.5	0.5	2	2	1
2	1	0.125	1.5	2	2	1
3	1	0.125	0.5	2	2	3
4	1	1.5	1.5	2	0.5	1
5	1	1.5	1.5	0.5	2	1
6	1	1.5	0.5	0.5	2	3
7	1	0.125	1.5	0.5	2	3
8	1	1.5	1.5	2	2	3
9	2	0.125	1.5	2	0.5	3
10	2	0.125	1.5	0.5	0.5	1
11	2	0.125	0.5	0.5	0.5	3
12	2	1.5	0.5	2	0.5	3
13	2	1.5	0.5	0.5	0.5	1
14	2	0.125	0.5	0.5	2	1
15	2	1.5	1.5	0.5	0.5	3
16	2	0.125	0.5	2	0.5	1

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5-Factor 2-Level Resolution V Fractional Factorial

Run	Block	Factor 1 NH4NO3 xMS	Factor 2 KNO3 xMS	Factor 3 Mesos xMS	Factor 4 Minors xMS	Factor 5 Fe xMS
1	1	1.5	0.5	2	2	1

Amounts of stock solution are simply multiples of MS medium.

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MS

Factor 1 NH4NO3 xMS	Factor 2 KNO3 xMS	Factor 3 Mesos xMS	Factor 4 Minors xMS	Factor 5 Fe xMS
1	1	1	1	1

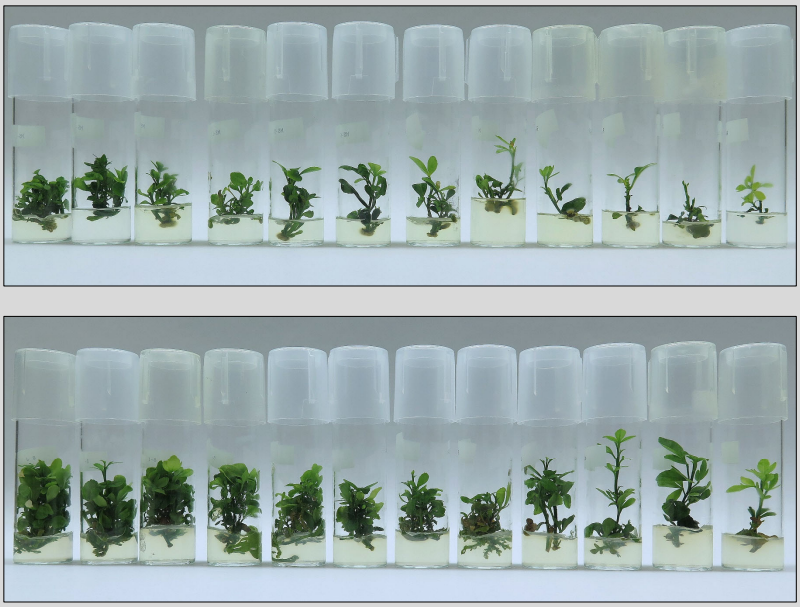
33

MS

34

Shoot number

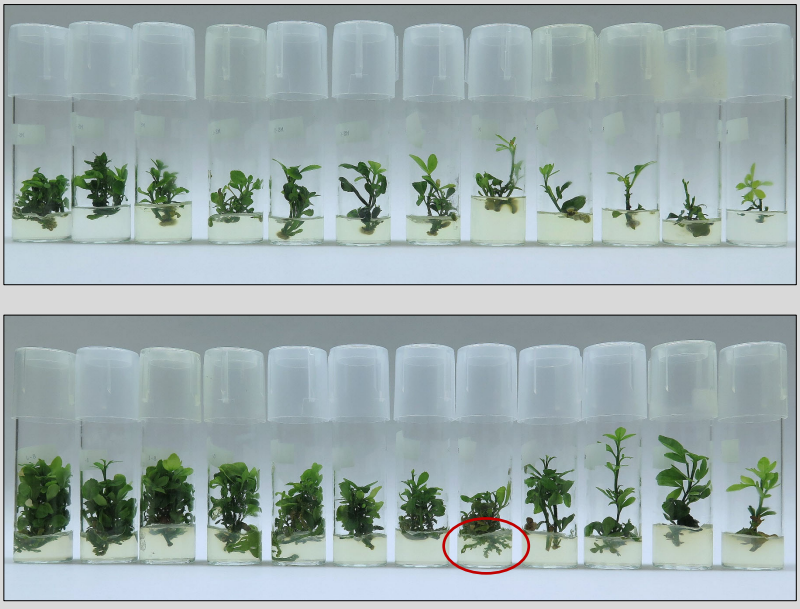
MS



35

Shoot number

MS

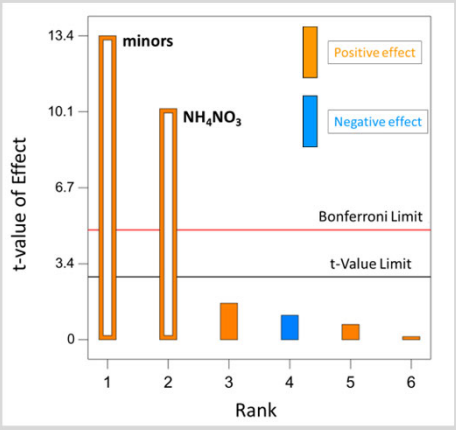


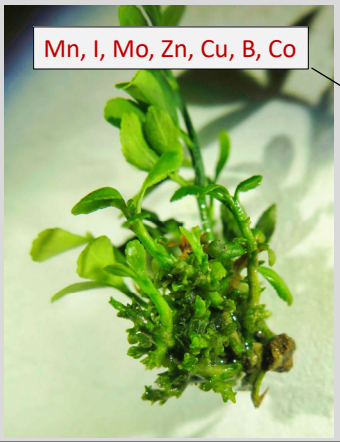
36

38

Shoot number

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	21	2	10	107	0.0003
A-NH_4NO_3	10	1	10	104	0.0005
D-Minors	17	1	17	180	0.0002





$MnSO_4$

KI

Na_2MoO_4

$ZnSO_4$

$CuSO_4$

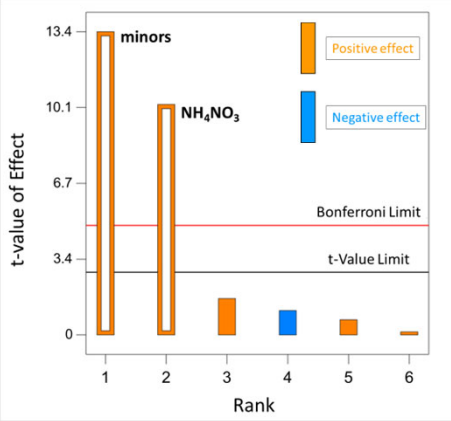
H_3BO_3

$CoCl_2$

40

Shoot number

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	21	2	10	107	0.0003
A-NH_4NO_3	10	1	10	104	0.0005
D-Minors	17	1	17	180	0.0002

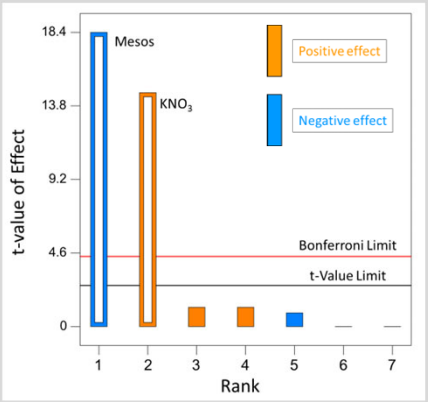


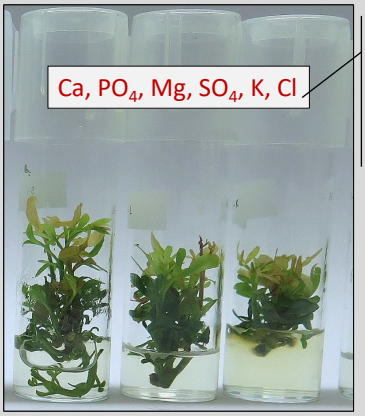
$MnSO_4$
KI
Na_2MoO_4
$ZnSO_4$
$CuSO_4$
H_3BO_3
$CoCl_2$

41

Leaf necrosis

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	101	2	51	295	< 0.0001
B-KNO_3	37	1	37	214	< 0.0001
C-Mesos	58	1	58	338	< 0.0001





$CaCl_2$
KH_2PO_4
$MgSO_4$

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Media Optimization Kit (MS)
Product ID: M5630

- *Pear*
- *Hazelnut*
- *Raspberry*
- *Blueberry*
- *Citrus*

43

Eldridge Wynn
Biological Research Technician

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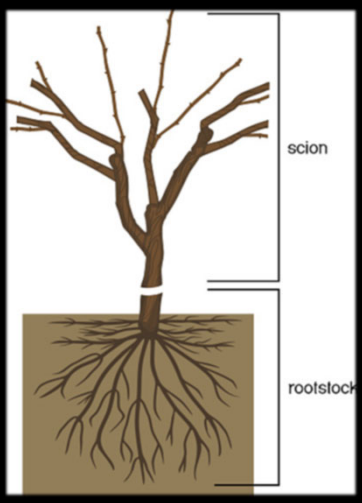
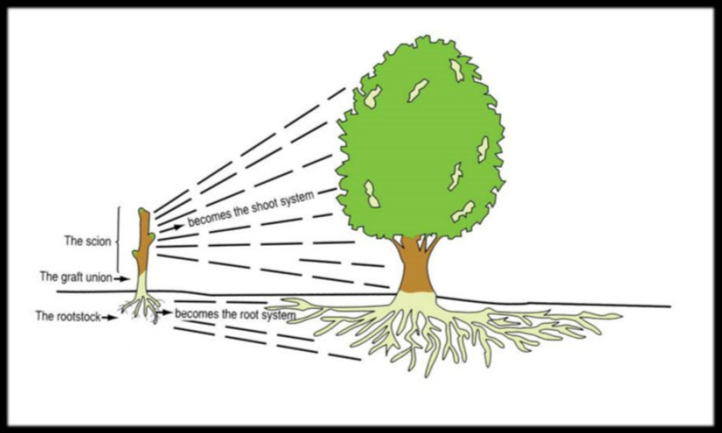
GREENHOUSE EXPERIMENT

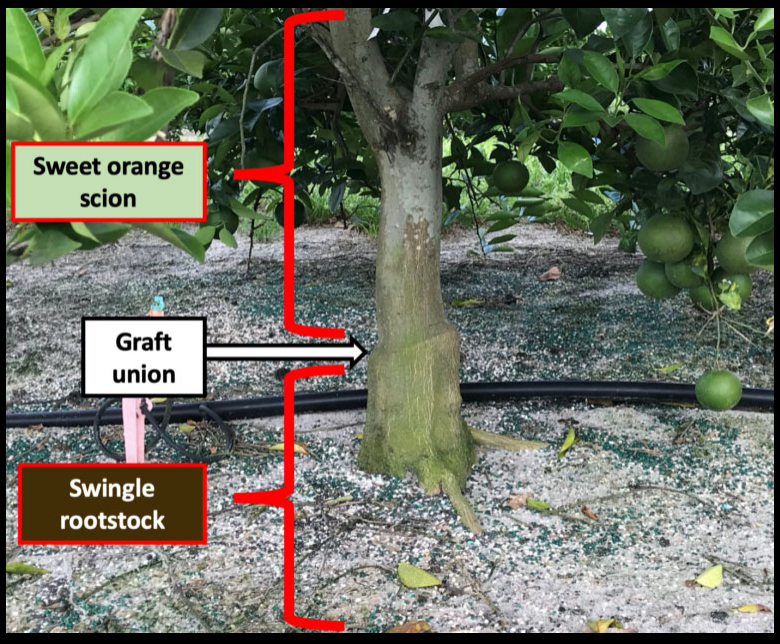
3) Citrus bud grafting.



Fruit trees are composite trees.



45



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Kim Bowman, PhD
Research Geneticist


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Bud grafting experiment

Factors

Solution
1) 6-Benzylaminopurine (BA – a cytokinin)
2) Tween 20 (nonionic surfactant)
3) DMSO (solvent, penetrant of biological membranes)
4) Solvent (water or ethanol)

5) Bud orientation (South or North)
6) Supplemental light type (LED, metal halide, none)

Design - 43 runs in 3 blocks.

6-factor D-optimal Quadratic Response Surface

- 3 numeric factors
- 3 categoric factors

- 26 plants per run
- 43 runs
- 1,118 plants

51

Treatments	Factor 1 BA	Factor 2 Tween 20	Factor 3 DMSO	Factor 4 Carrier	Factor 5 Orientation	Factor 6 Light
1	0	0	5	Etoh	North	MH
2	5	5	0	Aqueous	South	MH
3	0	0	0	Etoh	South	None
4	5	5	0	Etoh	South	LED
5	5	5	0	Etoh	South	LED
6	5	5	5	Etoh	South	MH
7	2.5	2.5	2.5	Aqueous	North	LED
8	5	5	0	Aqueous	South	MH
9	0	5	0	Etoh	North	None
10	0	5	5	Aqueous	South	LED
.	.	.	.	.		
.	.	.	.	.		
.	.	.	.	.		
33	0	0	5	Aqueous	North	None
34	2.5	1.25	1.25	Aqueous	South	None
35	0	5	0	Aqueous	North	LED
36	0	0	0	Aqueous	South	LED
37	0	5	5	Etoh	South	None
38	1.25	2.5	1.25	Etoh	North	MH
39	0	5	5	Aqueous	North	MH
40	5	5	0	Etoh	South	None
42	0	2.5	2.5	Etoh	South	LED
43	2.5	0	2.5	Etoh	North	None

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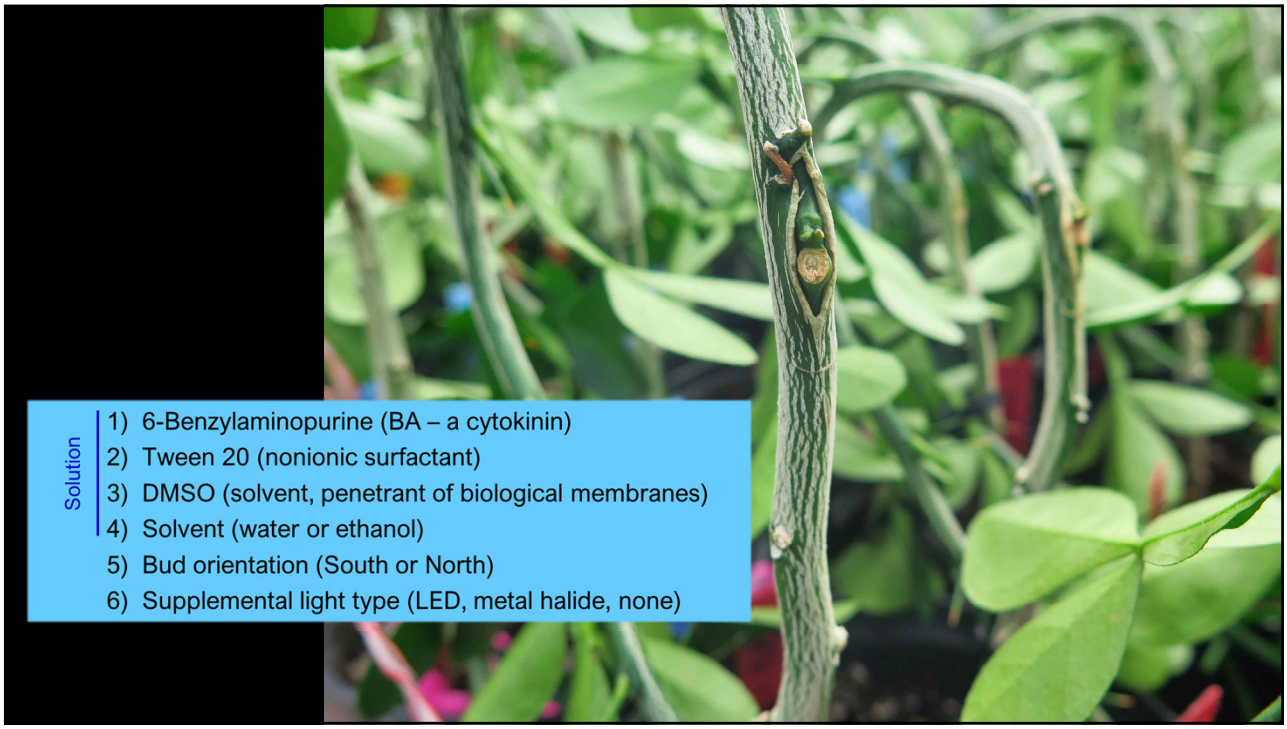
Solution
1) 6-Benzylaminopurine (BA – a cytokinin)
2) Tween 20 (nonionic surfactant)
3) DMSO (solvent, penetrant of biological membranes)
4) Solvent (water or ethanol)
5) Bud orientation (South or North)
6) Supplemental light type (LED, metal halide, none)

% Buds that formed shoots.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Block	0.1756	2	0.0878		
Model	1.38	11	0.1256	13.68	1.63E-08
A – BA	0.1715	1	0.1715	18.68	0.0002
B – Tween-20	0.0041	1	0.0041	0.4499	0.5079
C – DMSO	0.019	1	0.019	2.07	0.1611
D – Carrier	0.0291	1	0.0291	3.17	0.086
E – Orientation	0.6548	1	0.6548	71.35	**3.47E-09**
F – Light	0.0419	2	0.021	2.28	0.1205
BF	0.1379	2	0.069	7.51	0.0024
CD	0.056	1	0.056	6.1	0.0199
DE	0.0238	1	0.0238	2.59	0.1188
Residual	0.257	28	0.0092		
Lack of Fit	0.0919	23	0.004	0.1211	0.9999
Pure Error	0.165	5	0.033		
Cor Total	1.81	41			

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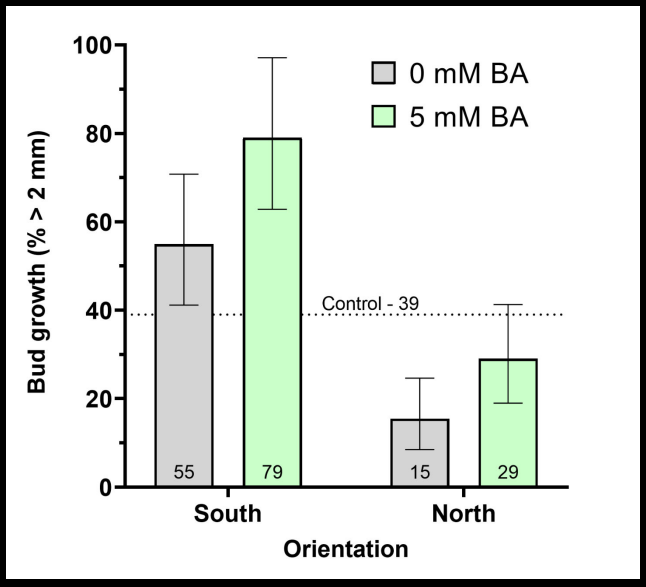
% Buds that formed shoots.



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Leaf area of bud shoots.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Block	4209.33	2	2104.67		
Model	26793.23	10	2679.32	13.49	1.45E-08
A – BA	13929.45	1	13929.45	70.14	**2.39E-09**
B – Tween-20	6.49	1	6.49	0.0327	0.8577
E – Orientation	4680.54	1	4680.54	23.57	**3.51E-05**
F – Light	2918.06	2	1459.03	7.35	0.0025
AF	1332.85	2	666.43	3.36	0.0484
BF	1665.57	2	832.79	4.19	0.0248
A^2	1374.16	1	1374.16	6.92	0.0133
Residual	5957.79	30	198.59		
Lack of Fit	3481.66	25	139.27	0.2812	0.9855
Pure Error	2476.13	5	495.23		
Cor Total	36960.35	42			

58

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INSECTARY EXPERIMENT

4) Root weevil artificial diet.

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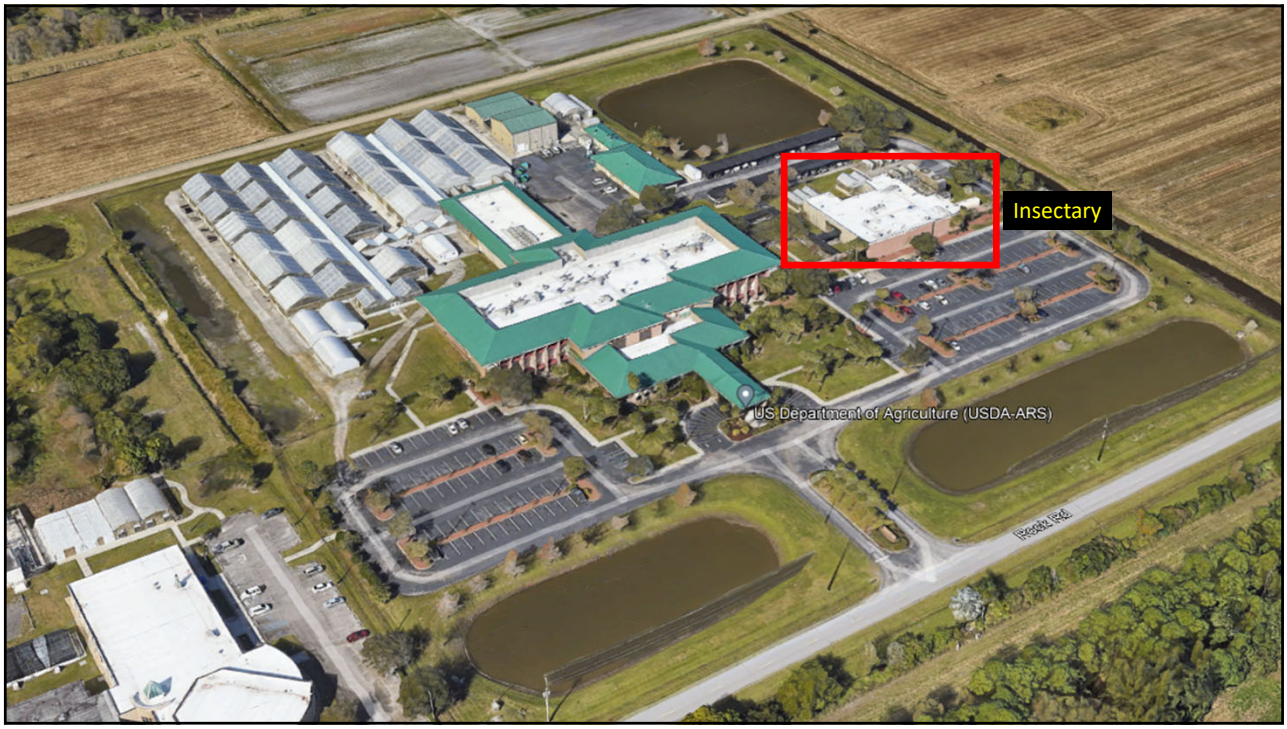

Steve LaPointe, PhD
Research Entomologist

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INSECTARY EXPERIMENT

4) Root weevil artificial diet.

Insectary raised Diaprepes – large and slow.

Wild Diaprepes – smaller and energetic.

Diaprepes Artificial Diet

Ingredient	Kg/batch	Percentage
Cellulose	3.08	31.3
Cottonseed Meal	2.51	25.5
Soy Protein	1.04	10.6
Vitamin mix	0.31	3.2
Casein	0.70	7.1
Wheat Germ	0.60	6.1
Corn starch	0.44	4.5
Methyl paraben	0.15	1.5
Ascorbic acid	0.05	0.5
Sorbic acid	0.05	0.5
Cholesterol	0.03	0.3
Salt mix	0.16	1.6
Sucrose	0.70	7.1
Choline chloride	0.02	0.2
Total	**9.85**	**100**

Vitamin mix

Ingredient	g/batch	Percentage
Vitamin E acetate (50%)	4.981	1.6
Ascorbic acid	84	27
Biotin	0.006	0.002
Calcium pantothenate	0.311	0.1
Cholin dihydrogen citrate	32	10.55
folic acid	0.078	0.025
I-Inositol	6.226	2
Niacin	0.311	0.1
Pyridoxine HCL	0.078	0.025
Riboflavin	0.156	0.05
Thiamin HCL	0.078	0.025
B-12 (0.1%)	0.623	0.2
Sucrose	181	58
Total	**311.300**	**100**

Salt mix

Ingredients	g/batch	Percentage
Calcium carbonate	33.726	21
Copper sulfate	0.063	0.039
Ferric phosphate	2.361	1.470
Manganese sulfate	0.032	0.020
Magnesium sulfate	14.454	9
Potassium aluminum sulfate	0.014	0.009
Potassium chloride	19.272	12
Potassium dihydrogen phosphate	49.786	31
Potassium iodide	0.008	0.005
Sodium choride	16.863	10.5
Sodium floride	0.092	0.057
Tricalcium phosphate	23.929	14.900
Total	**160.600**	**100**

Diaprepes Artificial Diet

36 ingredients

Ingredient	Kg/batch	Percentage
Cellulose	3.08	31.3
Cottonseed Meal	2.51	25.5
Soy Protein	1.04	10.6
Vitamin mix	0.31	3.2
Casein	0.70	7.1
Wheat Germ	0.60	6.1
Corn starch	0.44	4.5
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Salt mix	0.16	1.6
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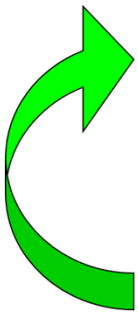
Total	**9.85**	**100**

Diaprepes Artificial Diet

36 ingredients



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Sucrose	0.70	7.1
Choline chloride	0.02	0.2
Total	**9.85**	**100**

Diaprepes Artificial Diet

8 Components (36 ingredients)

	Ingredient	Percentage
	Cellulose	31.3
1	Cottonseed meal	25.5
2	Soy protein	10.6
3	*Vitamin mix*	6.1
4	Casein	7.1
5	Wheat germ	6.1
6	Corn starch	4.5
7	*Salt mix*	1.6
8	Sucrose	7.1
	Total	**100**

Diaprepes Artificial Diet

8 Components (36 ingredients)

	Ingredient	Percentage
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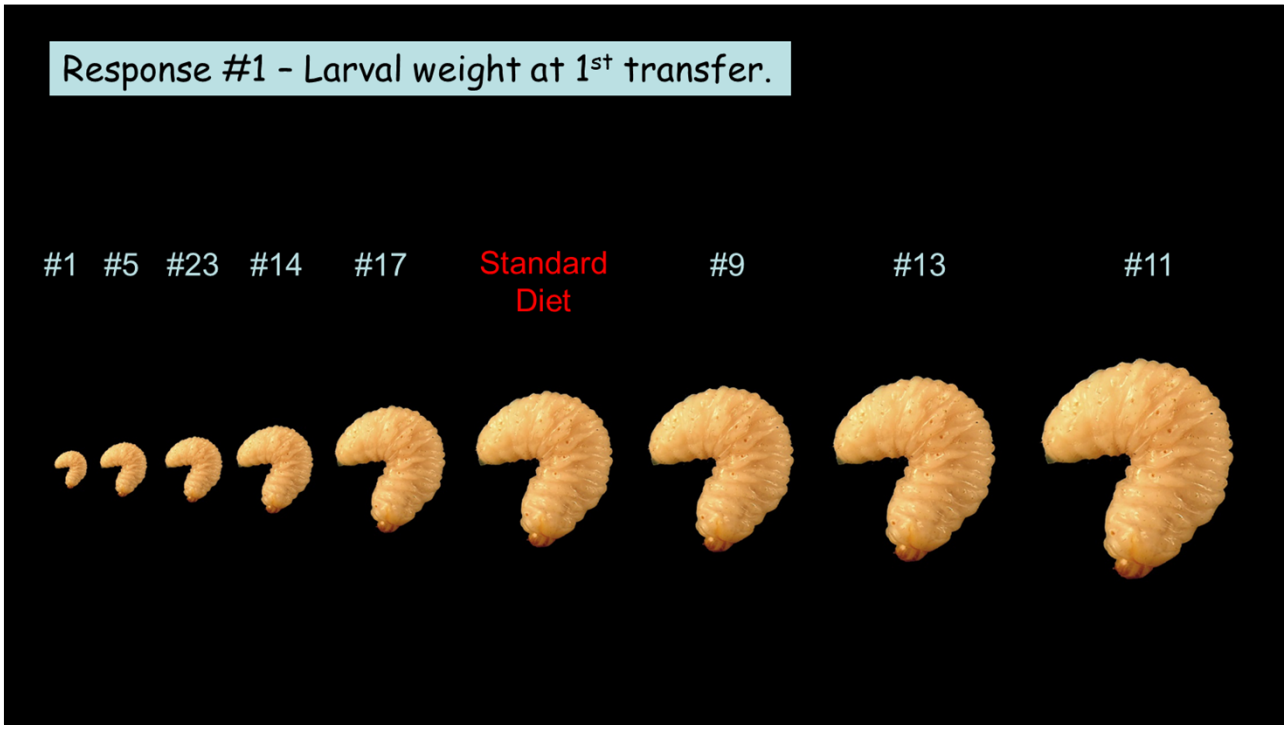
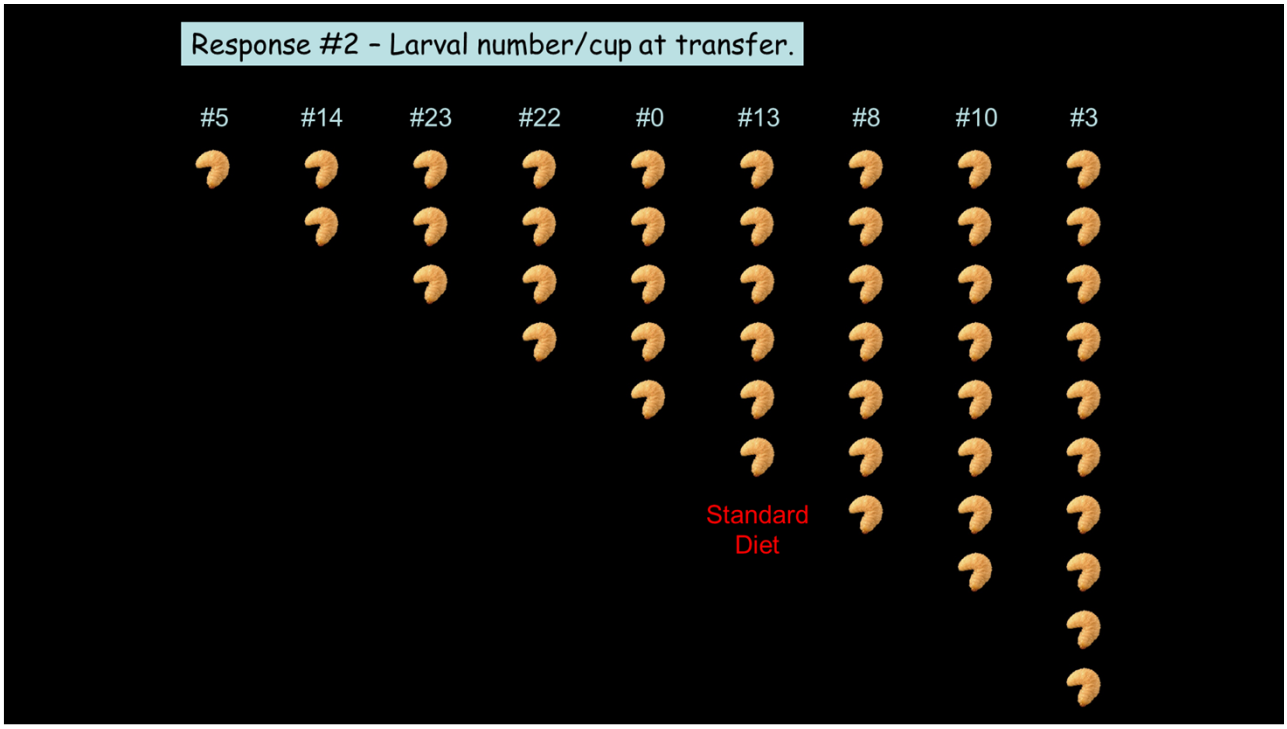
1	Cottonseed meal	25.5
2	Soy protein	10.6
3	*Vitamin mix*	6.1
4	Casein	7.1
5	Wheat germ	6.1
6	Corn starch	4.5
7	*Salt mix*	1.6
8	Sucrose	7.1
	Total	**100**

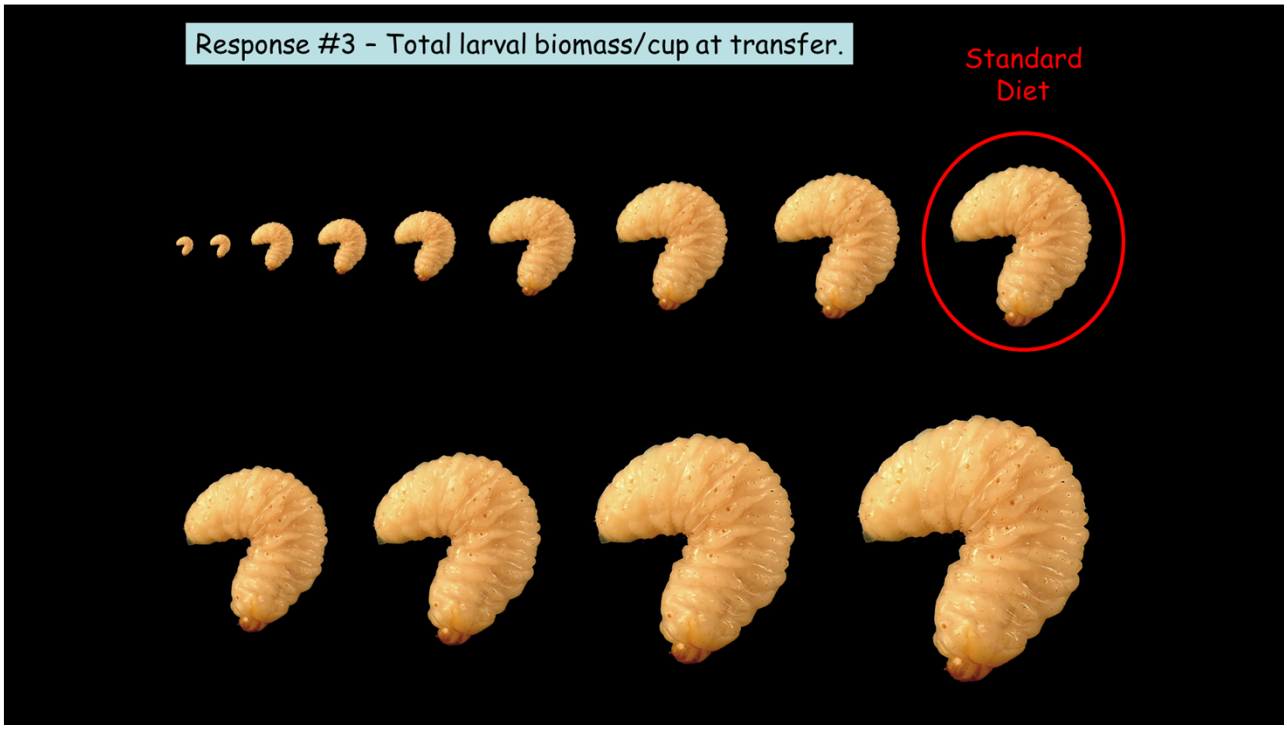
8-component mixture screening design.

Responses measured (7)

1) larval weight and number at 1st transfer
2) time to pupate
3) time to adult
4) adult weight
5) adult sex
6) fecundity


Response #1 – Larval weight at 1st transfer.
#1
#5
#23
#14
#17
Standard Diet
#9
#13
#11


Response #2 – Larval number/cup at transfer.
#5
#14
#23
#22
#0
#13
#8
#10
#3
Standard Diet



	Components
1	Cottonseed meal
2	Soy protein
3	*Vitamin mix*
4	Casein
5	Wheat germ
6	Corn starch
7	*Salt mix*
8	Sucrose
	Total

Primary Driver

Secondary Driver

FIELD EXPERIMENT

5) Citrus leaf miner pheromones.

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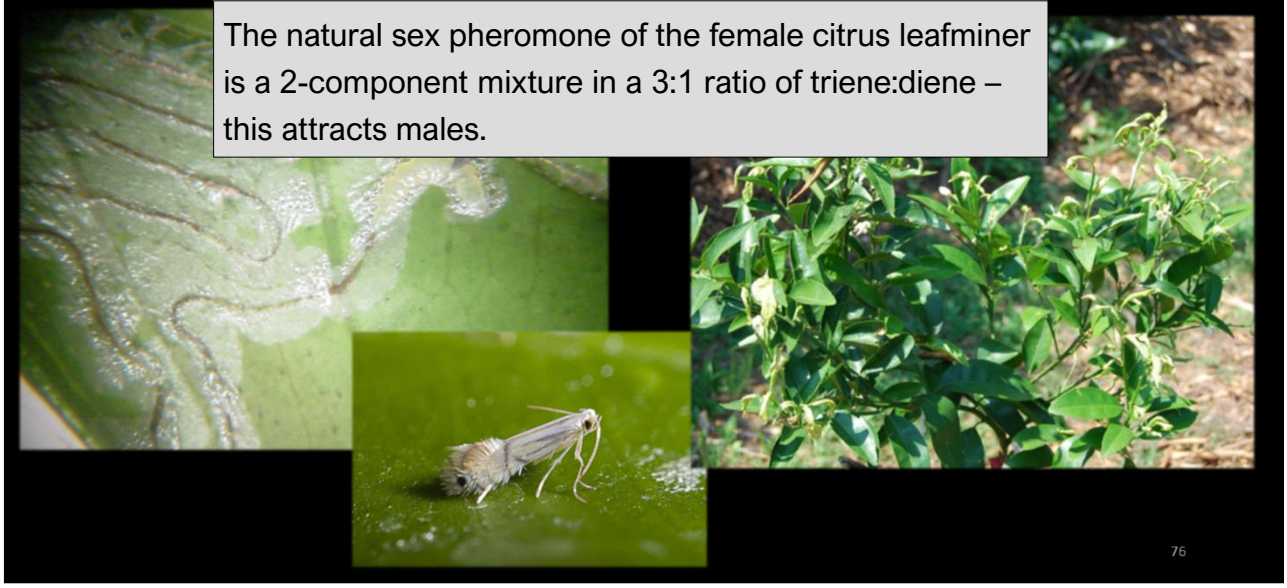
FIELD EXPERIMENT

5) Citrus leaf miner pheromones.

The natural sex pheromone of the female citrus leafminer is a 2-component mixture in a 3:1 ratio of triene:diene – this attracts males.



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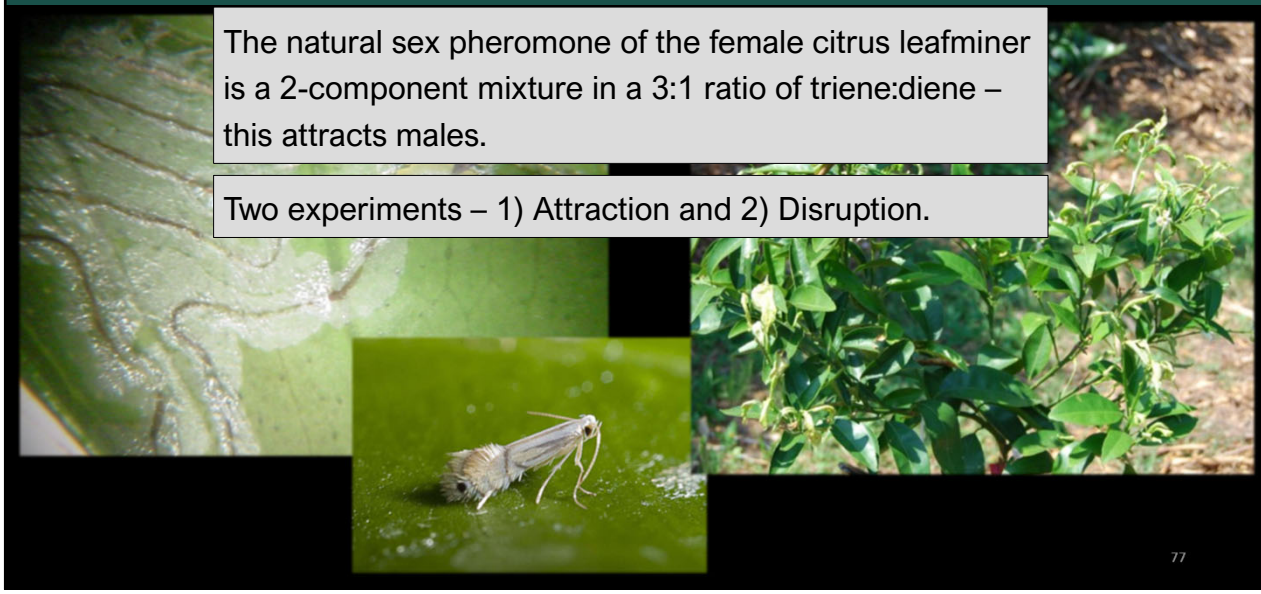
Field Experiment

5) Citrus leaf miner pheromones.

The natural sex pheromone of the female citrus leafminer is a 2-component mixture in a 3:1 ratio of triene:diene – this attracts males.

Two experiments – 1) Attraction and 2) Disruption.



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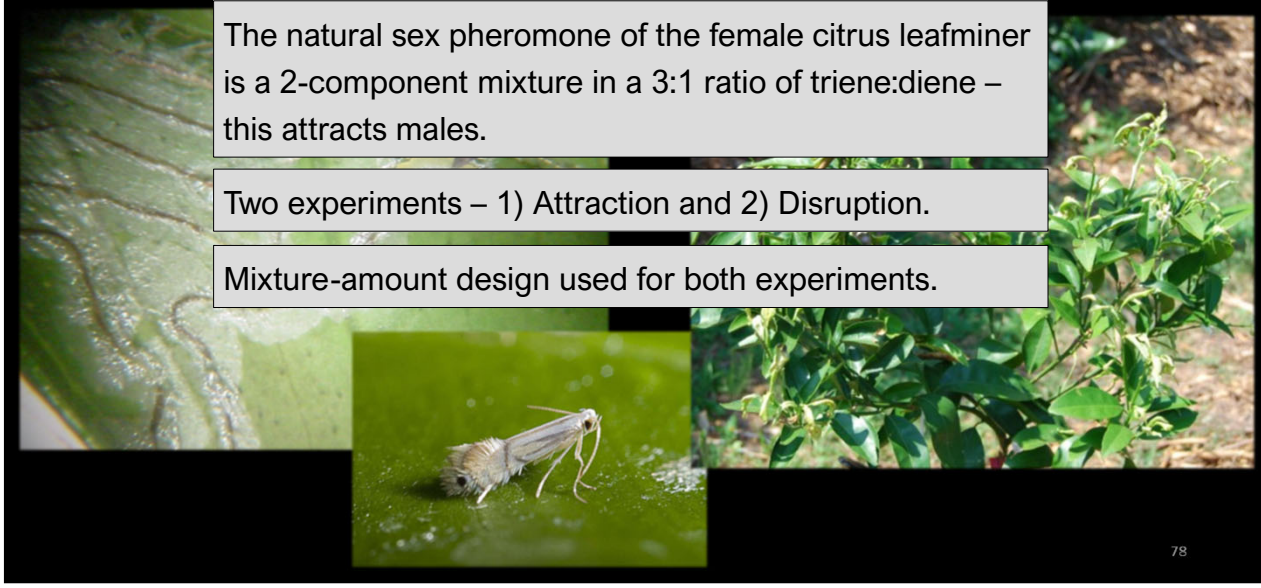
Field Experiment

5) Citrus leaf miner pheromones.

The natural sex pheromone of the female citrus leafminer is a 2-component mixture in a 3:1 ratio of triene:diene – this attracts males.

Two experiments – 1) Attraction and 2) Disruption.

Mixture-amount design used for both experiments.



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Field Experiment

5) Citrus leaf miner pheromones.

The natural sex pheromone of the female citrus leafminer is a 2-component mixture in a 3:1 ratio of triene:diene – this attracts males.

Two experiments – 1) Attraction and 2) Disruption.

Mixture-amount design for both experiments.

Lapointe, S. L. et al. (2009).
Sensory imbalance as mechanism of orientation disruption in the leafminer *Phyllocnistis citrella*: elucidation by multivariate geometric designs and response surface models. *Journal of chemical ecology, 35(8), 896–903.*

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Attraction

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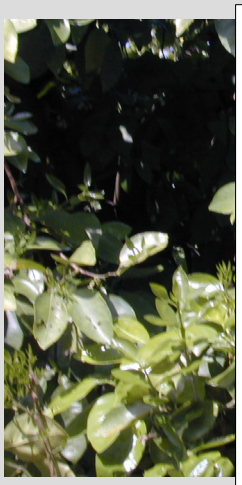
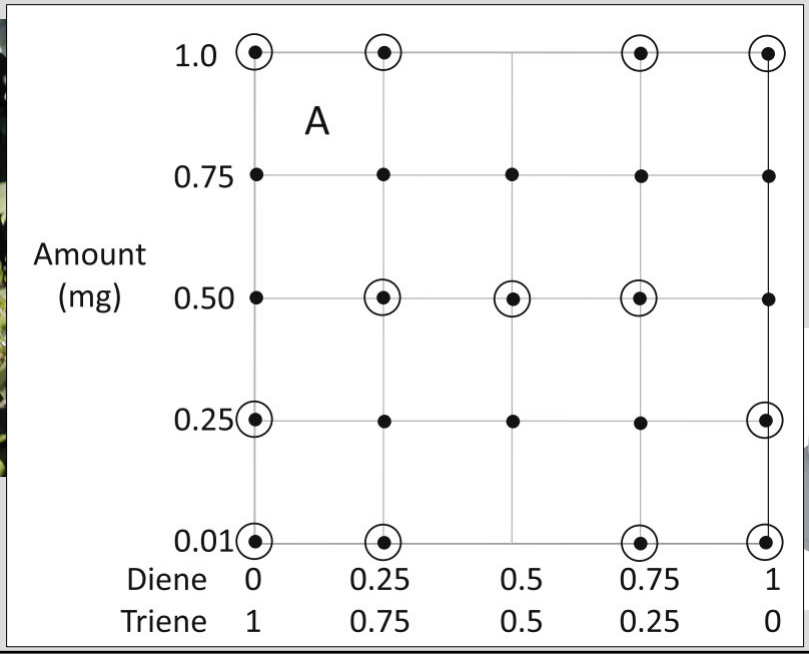
Attraction





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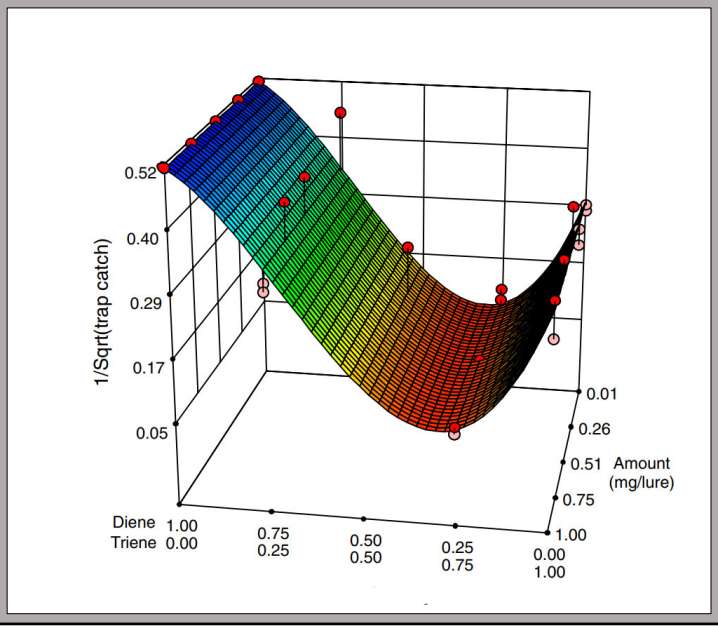
Attraction



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Disruption

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Disruption

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Disruption

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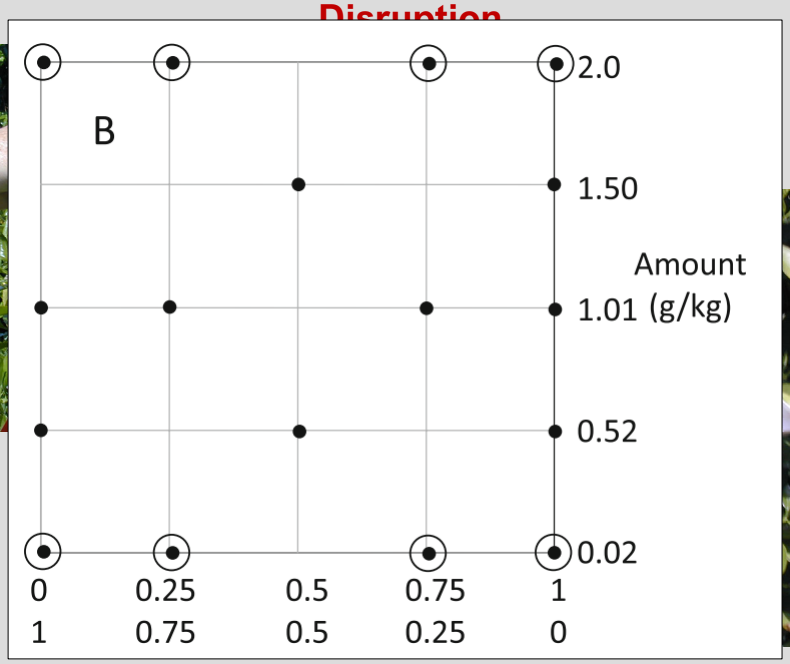
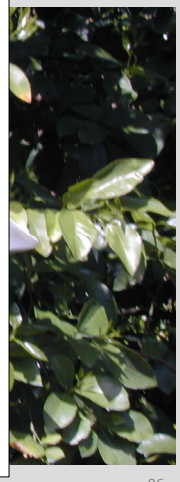
Disruption





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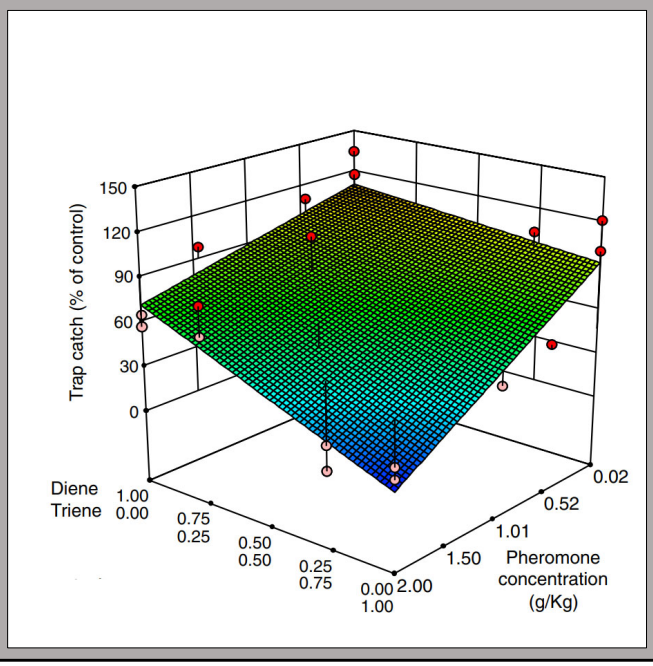
Disruption



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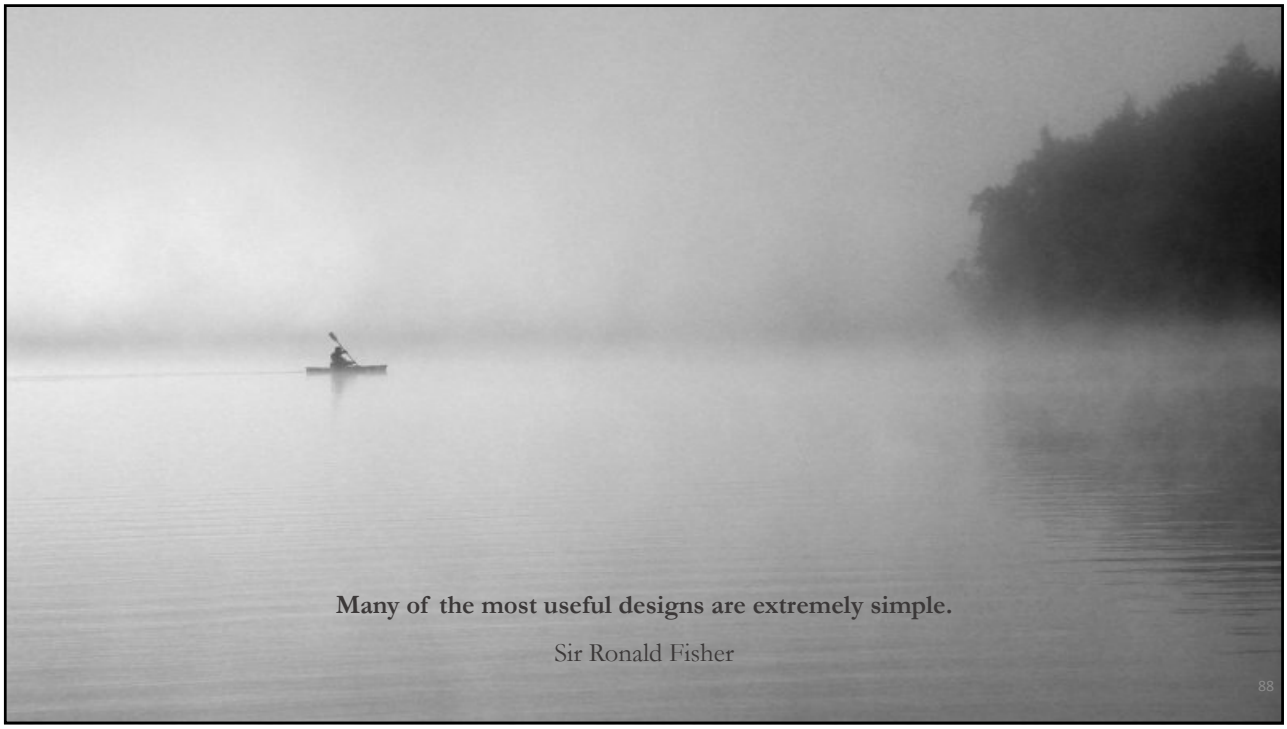

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