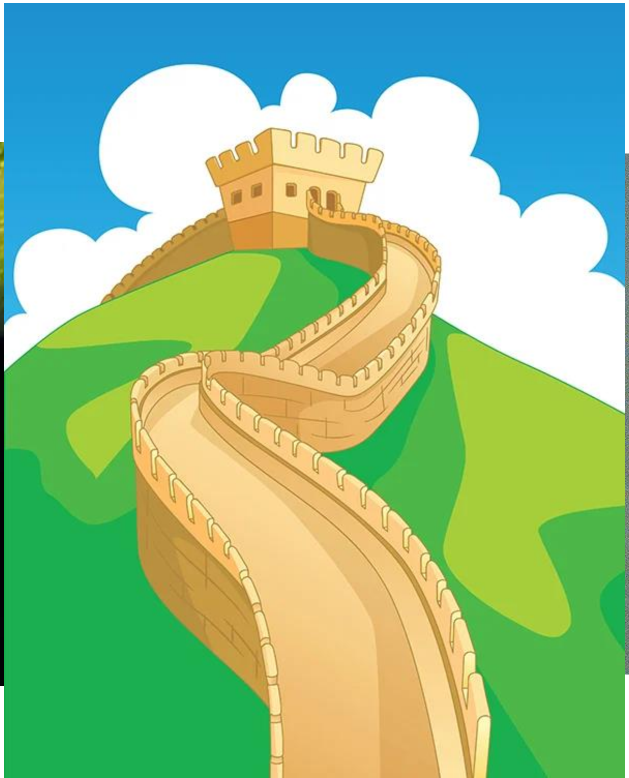
Pilot Studies on Mating Disruption of Grape Mealybugs in WA. State Wine Grape Vineyards

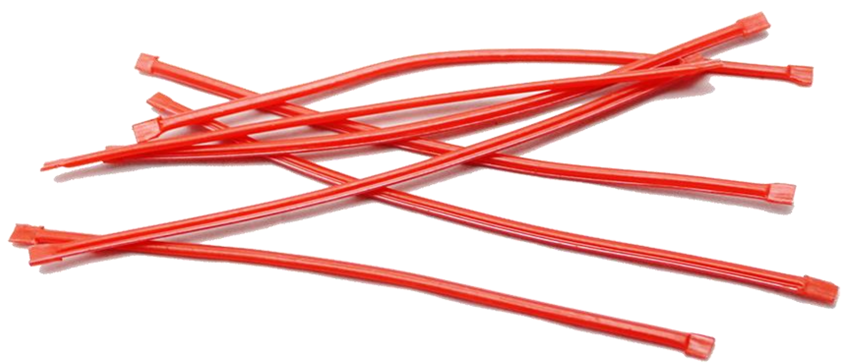
Doug Walsh and Stephan Onayemi

WSU-IAREC, Entomology



Mating Disruption- Background

- Mating disruption involves the use of artificially produced high-pheromone concentrations in a confined area to impede the ability of females and males to communicate with each other.
- This results in fewer males locating and mating with females.
- “Mating Disruption”
- (Carde and Minks, 1995).



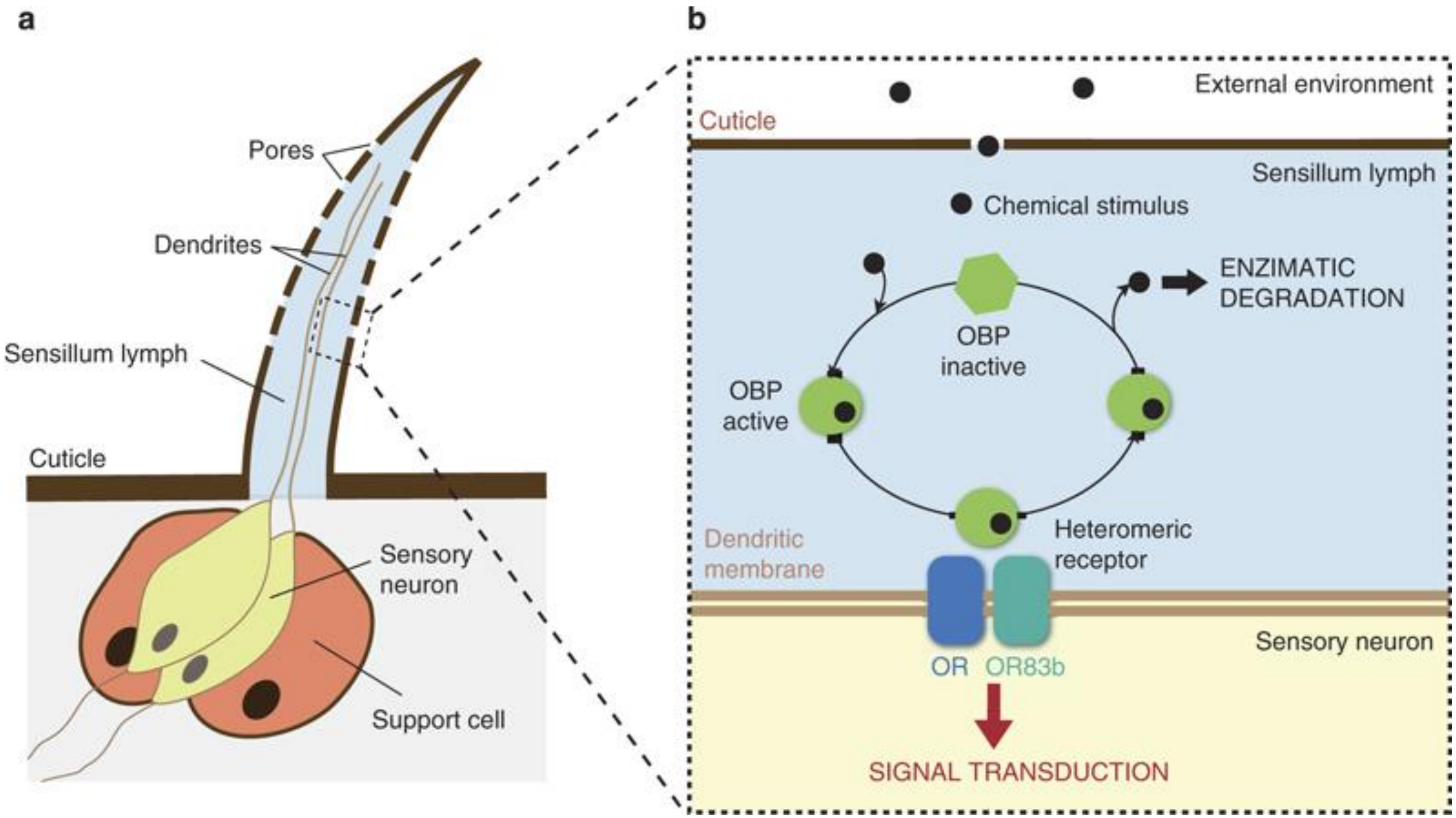
Mating Disruption with Pheromones

- Mating disruption aims to disrupt chemical communication by organisms and interrupt normal mating behavior by dispensing synthetic sex pheromone, thereby affecting the organism's chance of reproduction.
- This can be done by using both attractive and non-attractive pheromone blends.
- Mating disruption with sex pheromones can be effective if the edge effect of mated females flying in from outside the treated area can be avoided.
- This can be done if very large areas are treated or if the area treated is isolated such as in a mountain valley or island.

Mating Disruption with Pheromones

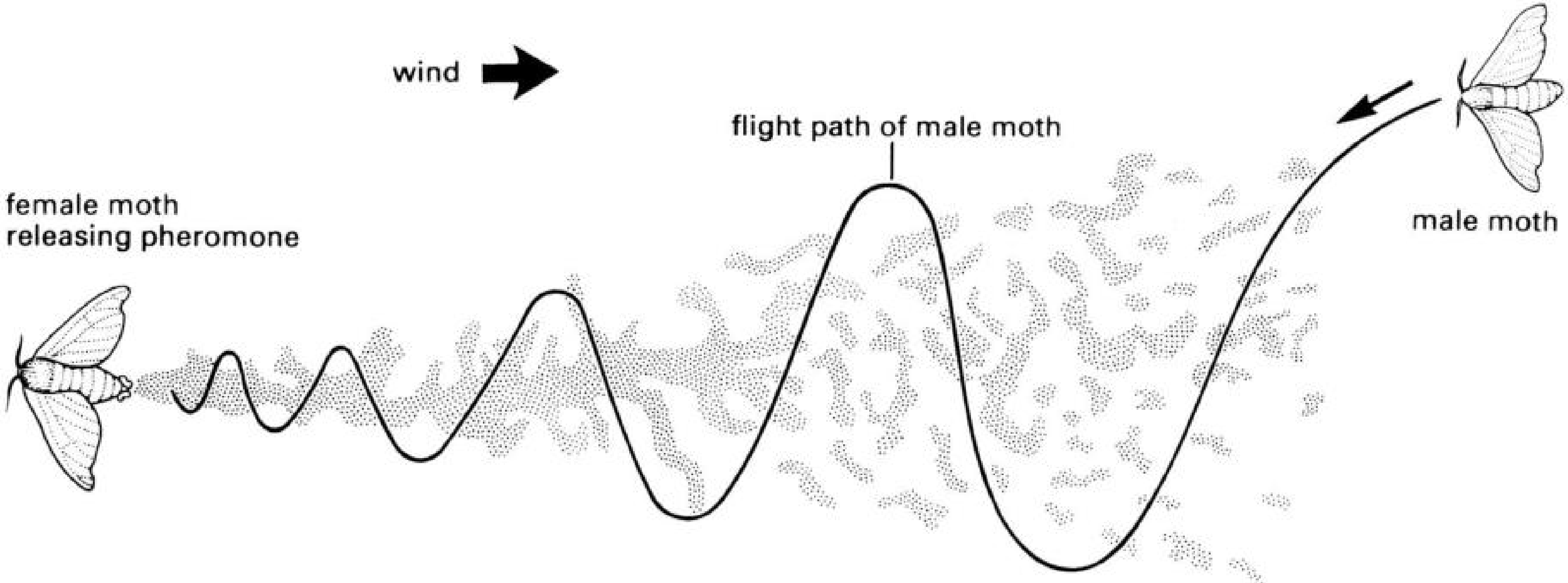
- It is reported that the crop area being managed for specific pests using mating disruption worldwide was 1.9 million acres in 2010.
- The three species with the highest land area under mating disruption are (*Gypsy)* moth (*Lymantria dispar*) in North American forests, the codling moth (*Cydia pomonella*) in apple and pear trees worldwide, and the grapevine moth (*Lobesia botrana*) in grape in the EU and Chile.
- All 3 of these are moths (Lepidoptera)

Pheromones are "smelled" by insects in their antenna in organs called sensilla trichodea



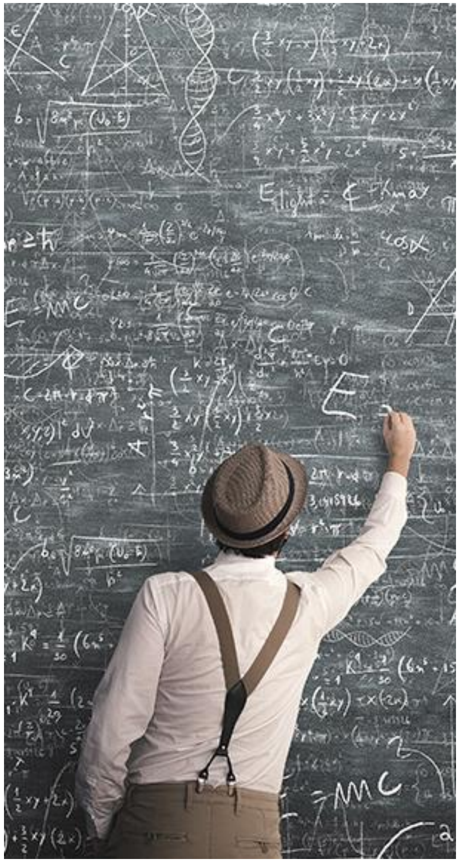
Sex pheromones

- Sex attraction pheromone – often produced by females; volatile, odorous plume

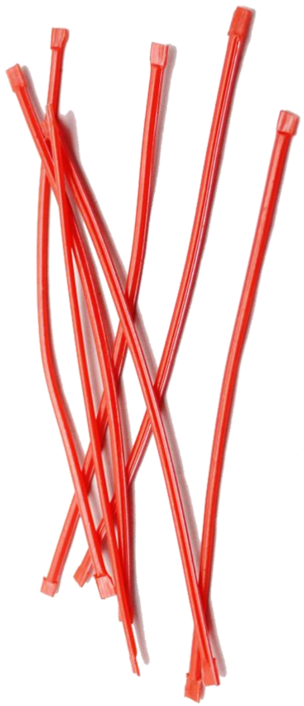


Mating Disruption for the 21st Century: Matching Technology With Mechanism 2015. Miller & Gut, Environ. Entomol. 44(3): 427–453 (2015); DOI: 10.1093/ee/nvv052

- Mating disruption is not a simple phenomenon.
- It involves physics, chemistry, materials science, atmospheric science, biochemistry, physiology, behavior, and sometimes biogeography.
- Moreover, the environmental conditions under which mating disruption operates can shift with geographic region, weather, and growth phase of the crop.



Pheromone Dispensers: Deploying these is labor intensive



Twist Ties from Shen Etsu via Pacific Biocontrol

CIDETRAK Puzzle Piece from Trece

Pheromone sachets

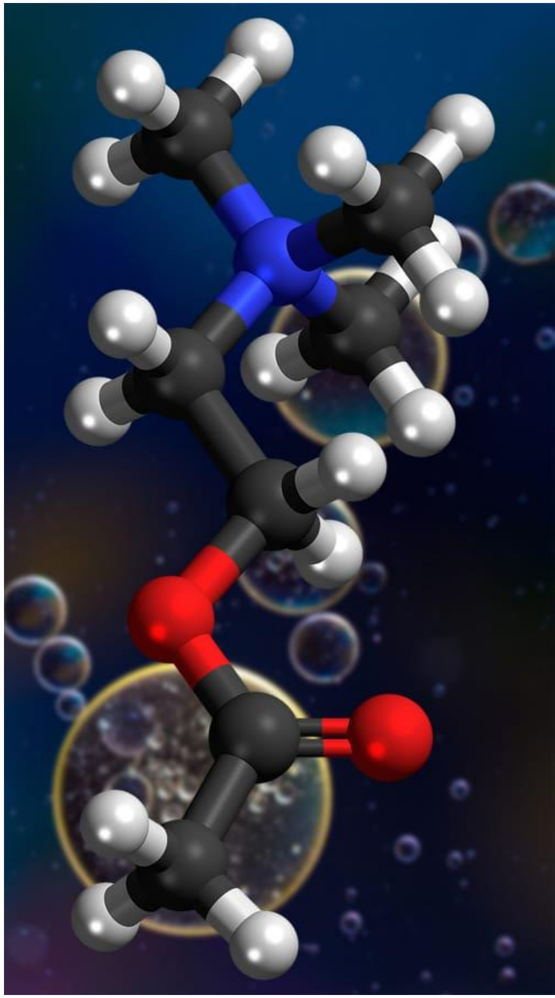
Pheromone Dispensers:
These are far less labor intensive

Pheromone Puffer

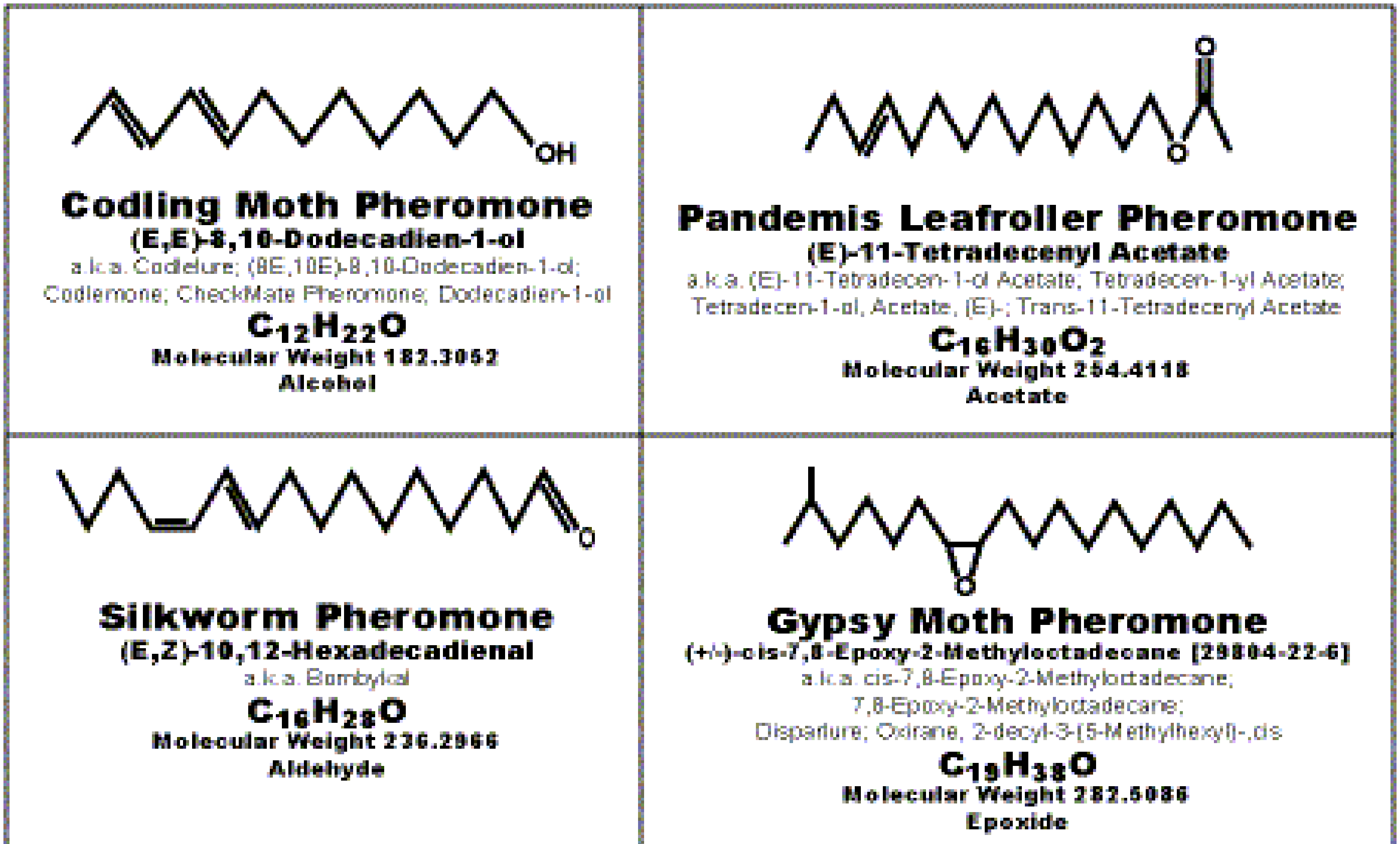
Sprayable Pheromone

Pheromone Facts

- The general size of insect pheromone molecules is limited to about 5 to 20 carbons with molecular weights between 80 and 300.
- This is because below 5 carbons and molecular weights of 80 or less very few kinds of molecules can be bio-synthesized and stored by glandular tissue.
- Above 5 carbons and a molecular weight of 80, the molecular diversity increases rapidly and so does the olfactory efficiency.
- Once a pheromone gets above 20 carbons and a molecular weight of 300, the diversity becomes so great, and the molecules are so big that they no longer are advantageous.
- Most Insect sex pheromones have molecular weights between 200 and 300.



- Most Lepidopteran pheromones are referred to as straight chain pheromones.
- US EPA has eased the regulations for registration of straight chain lepidopteran pheromones for pest control



Mating Disruption for Managing *Prionus californicus* (Coleoptera: Cerambycidae) in Hop and Sweet Cherry

James D Barbour, Diane G Alston, Douglas B Walsh, Michael Pace, Lawrence M Hanks. 2019. https://doi.org/10.1093/jee/toy430

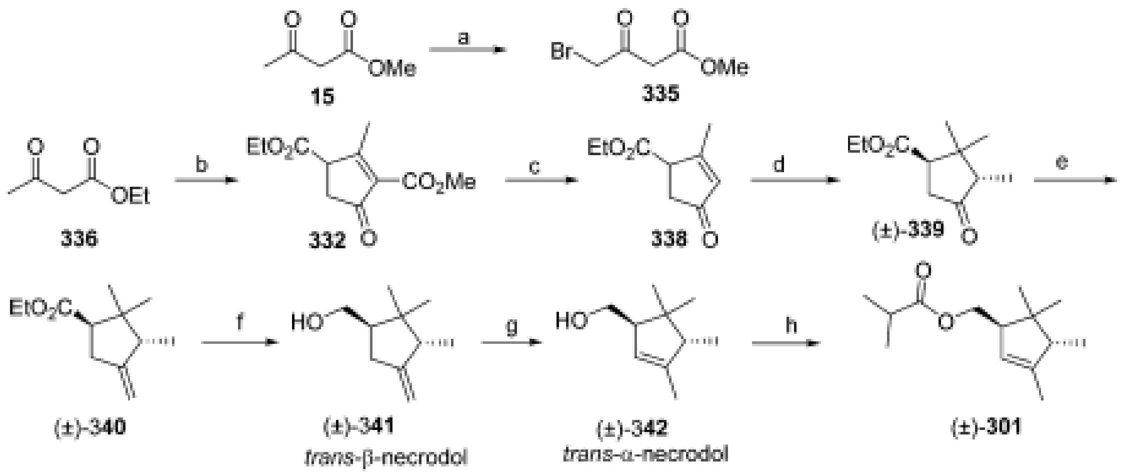
- Adult males are strongly attracted to a volatile sex pheromone, (3R,5S)-3,5-dimethyldodecanoic acid, produced by females.

- This is not a straight chain lepidopteran pheromone.
- EPA is requiring a bunch of studies to register it for mating disruption/ pest control.
- The registrant is not willing to spend the money.

Jocelyn Millar's group at UC Riverside has synthesized the sex pheromones for all the mealybug species in vineyards in California

- This is detailed in this review article:
- Chemistry of the pheromones of mealybug and scale insects. 2015. Yunfan, Z. & J.G. Millar. DOI: 10.1039/c4np00142e
- The sex pheromone of the grape mealybug, *Pseudococcus maritimus*, was identifed by the Millar group as *trans-α-necrodyl isobutyrate* 301.

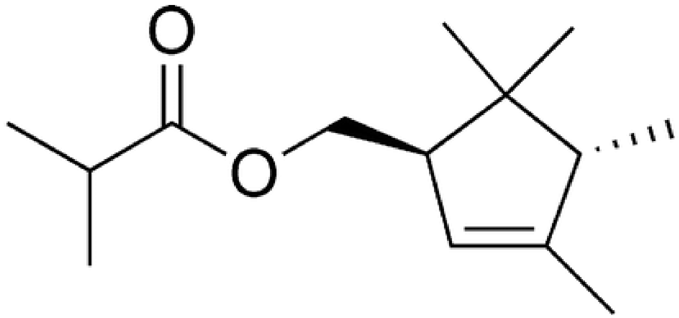
Grape mealybug pheromone synthesis



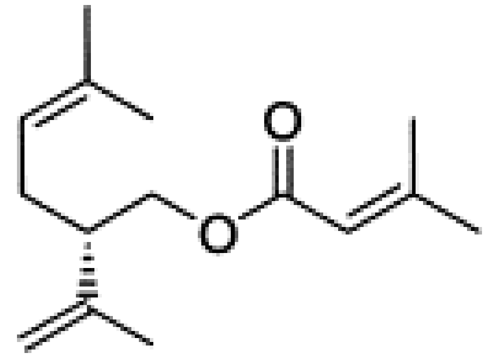
Scheme 52 Synthesis of the racemic grape mealybug pheromone **301**. Reagents and conditions: (a) Br_2, $CHCl_3$; (b) NaH, THF, **335**; (c) NaI, AcOH, diglyme, 28% over 3 steps; (d) Me_2Zn, $Ni(acac)_2$, THF, then HMPA, MeI, 64%; (e) $TiCl_4$, CH_2Br_2, Zn, THF, CH_2Cl_2, 67%; (f) $LiAlH_4$, Et_2O, 97%; (g) Li, ethylenediamine, 65%; (h) isobutyryl chloride, Et_3N, DMAP (cat.), CH_2Cl_2, 93%.

Vine mealybug pheromone has been approved for use in a mating disruption program and is being commercialized.

We'll have to wait and see with grape mealybug.

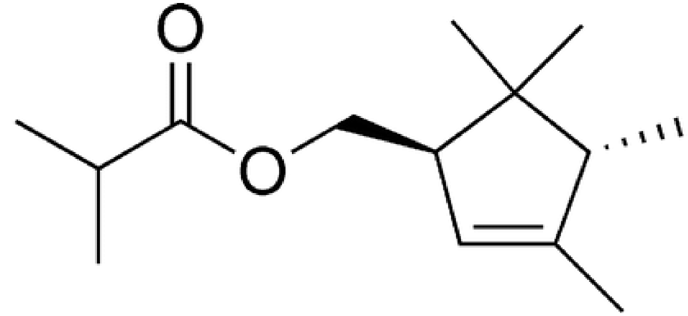


trans-α-necrodyl isobutyrate is the grape mealybug pheromone



(S)-(+)-lavandulyl senecioate is the grape mealybug pheromone

- Jocelyn has told me that the vine mealybug pheromone (S)-(+)-Lavandulyl senecioate, is very simple to synthesize.
- It takes 1 tweak of a commercially available precursor product used in many human scent products like perfumes and hand lotions.



trans-α-necrodyl isobutyrate
grape mealybug pheromone

- Jocelyn has told me that grape mealybug pheromone trans-ὰ-necrodyl isobutyrate takes 4 very difficult and costly steps to synthesize.
- Calling female mealybugs produce an 85% & 15% blend of the (*R,R*) and (*S,S*) enanatiomers.
- The (*R,R*) enantiomer is the attractive stereoisomer, but the (*S,S*) enantiomer synergizes attractiveness.
- Jocelyn has been cynical of commercialization for grape mealybug pheromone.

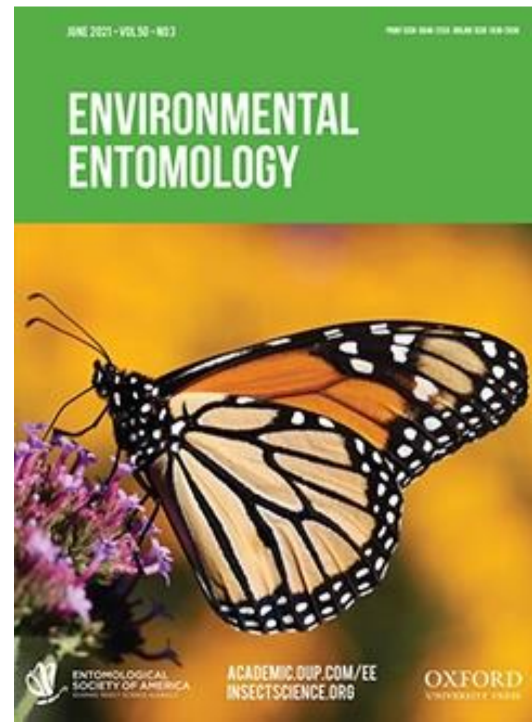
Breakthrough in Israel

- The synthesis method, in two steps, converts the trans-α-necrodyl acetate to trans-α-necrodol and then converts the trans-α-necrodol to the desired trans-α-necrodyl isobutyrate.
- The first step can be achieved by either of a hydrolysis reaction or a reduction reaction.
- In the hydrolysis reaction, the essential oil is mixed in an alc. solvent with a hydroxide base, or by mixing the essential oil in an aq. acid.
- A reduction reaction can instead by performed in an organic solvent in the presence of a reducing agent. The trans-α-necrodol is converted to the desired trans-α-necrodyl isobutyrate by an esterification reaction with a carboxylic acid, an acyl halide, or an acid anhydride.

Lavender (*Lavandula stoechas*) oil has trans-α-necrodyl acetate in it. This will greatly reduce production costs.

Mating Disruption for the 21st Century: Matching Technology With Mechanism

- A review article by:
- James R. Miller and Larry J. Gut from Michigan State University.
- Environ. Entomol. (2015) 44(3): 427–453 (2015); DOI: 10.1093/ee/nvv052



Miller & Gut (2015)

- These authors offer a dichotomous key for eight distinct categories of mating disruption and detail the criteria and methodologies for differentiating among them.
- They conclude that the mechanisms of mating disruption fall into two major categories—**competitive** and **noncompetitive** mating disruption.

Competitive Disruption

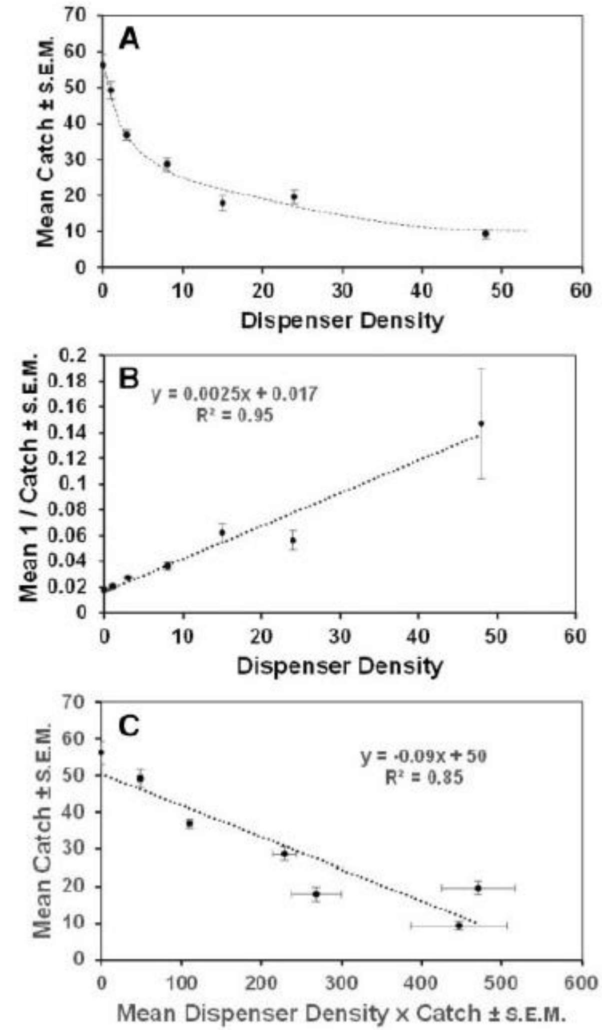
- Under competitive disruption, no impairments are experienced by males, females, or the signal of females.
- Therefore, males can respond to females and traps.
- Competitive disruption is entirely a numbers game where the ratio of dispensers to females and traps is highly consequential.
- This renders the control provided by competitive mating disruption is pest-density-dependent.
- This means that as the abundance of calling females increases, the chance that males can find some of the females and mate increases.

Noncompetitive Disruption

- Males, females, or the signal from females are already impaired when sexual activity commences.
- The pest control achieved noncompetitively offers the notable advantage of being pest-density-independent.
- Males can just go into a narcotic haze, or they can go into hyperactive state in response to pheromone.
- Once the titer of the pheromone is great enough males will not find females.

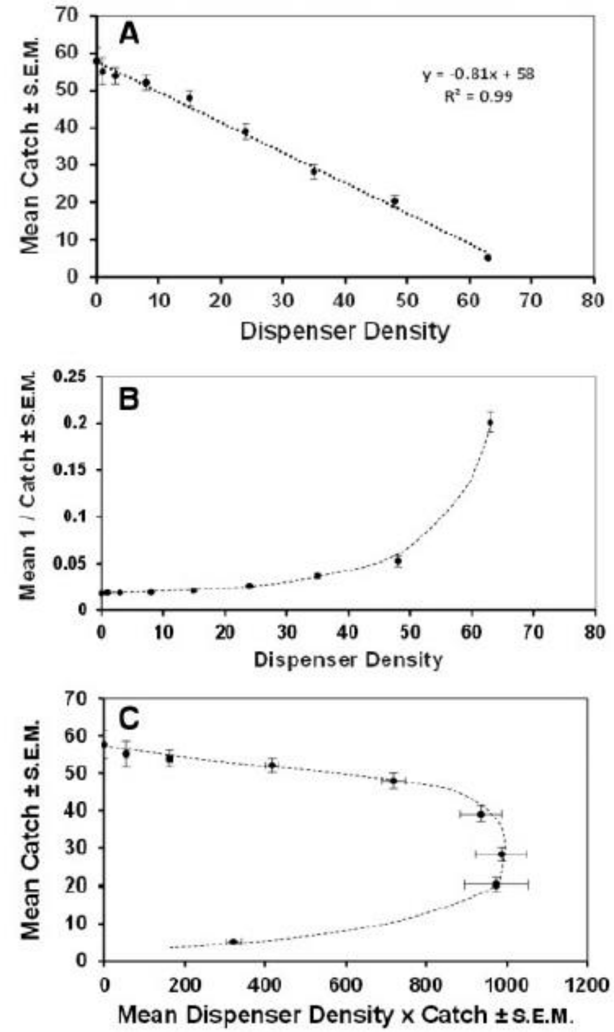
Competitive vs non-competitive mating disruption. How do you determine which you have?

- Dosage–response curves are the best way to distinguish competitive from noncompetitive disruption.
- Purely **competitive disruption** produces a smoothly concave curve when untransformed capture data in sentinel traps are plotted on the y-axis against density of dispensers on the x-axis.
- A straight line with positive slope when the inverse of catch is plotted against density of pheromone dispensers; and a straight line with negative slope when catch is plotted against density of pheromone dispensers X catch.



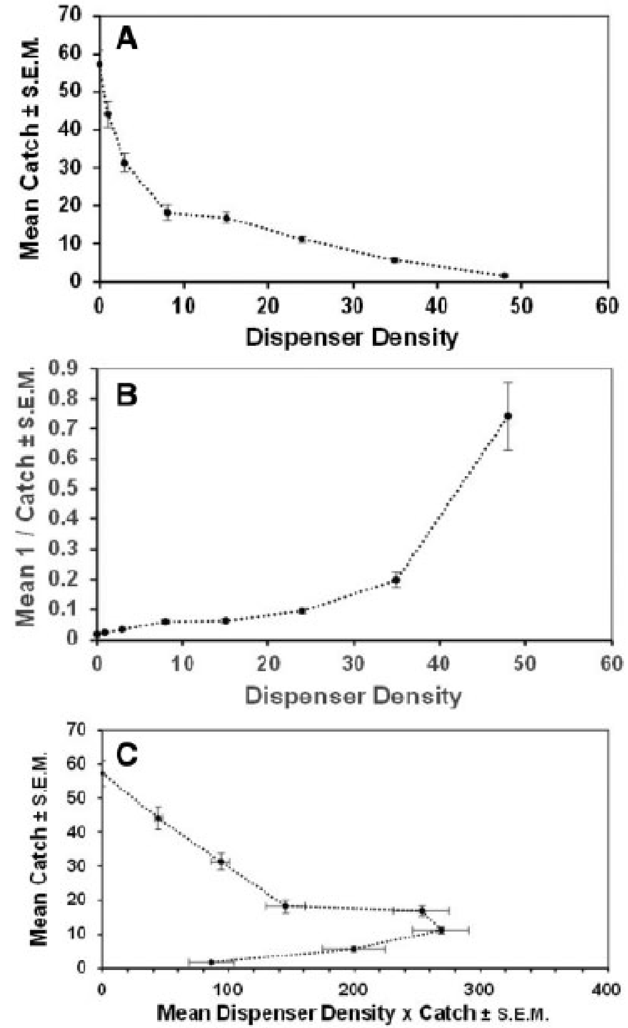
Competitive vs non-competitive mating disruption. How do you determine which you have?

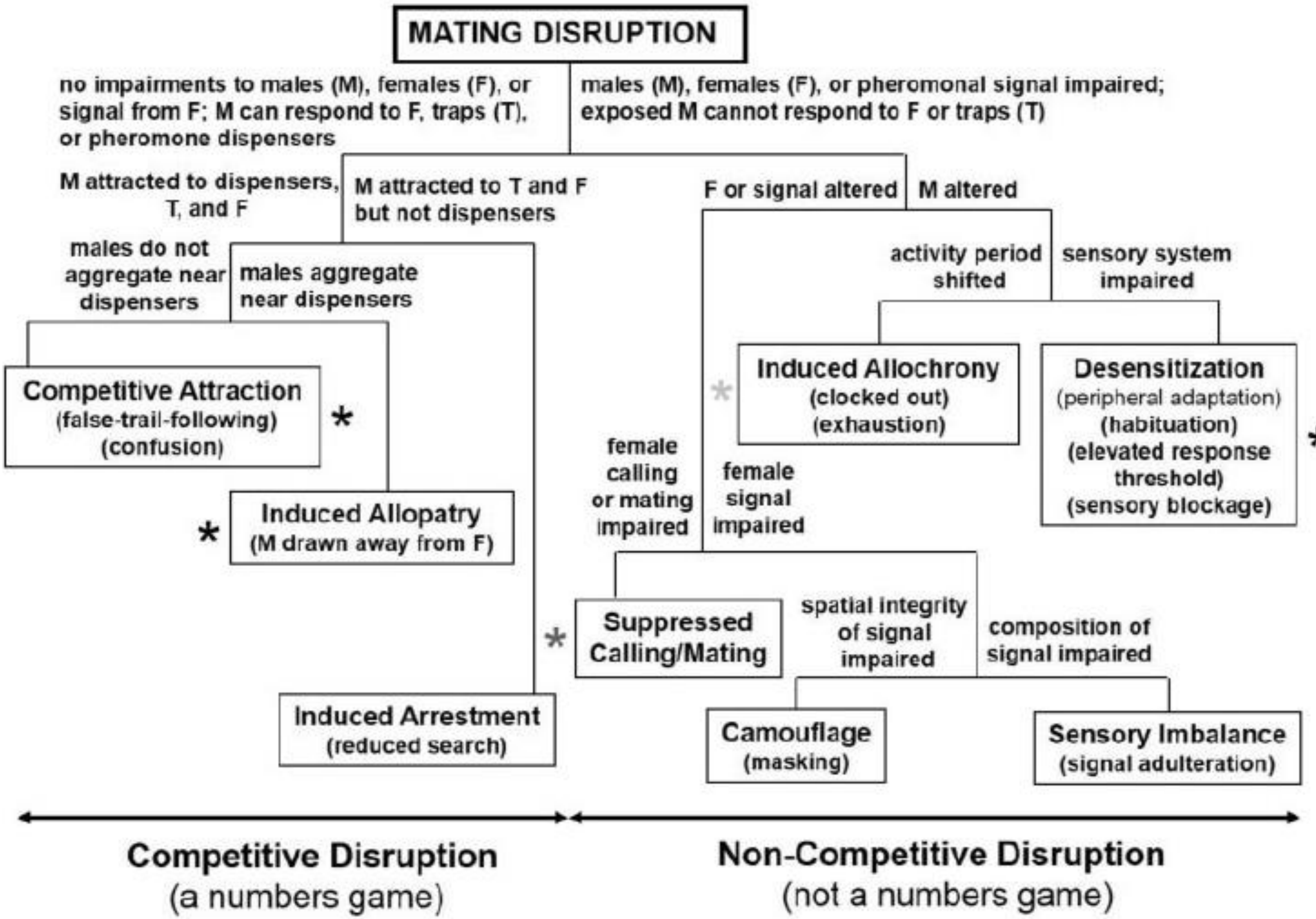
- **<u>Noncompetitive mating disruption</u>** produces: a straight line with negative slope when untransformed capture data are plotted on the y-axis against density of dispensers on the x-axis.
- An upturning curve when the inverse of catch is plotted against density of pheromone dispensers.
- And, a recurving plot when catch is plotted against density of pheromone dispensers X catch.



Competitive vs non-competitive mating disruption. How do you determine which you have?

- Hybrid profiles between competitive and non-competive mating disruption are possible when some males within the population begin the activity period already incapacitated.
- Males not preexposed have the capacity to respond either to traps or pheromone dispensers.
- Competitive mechanisms include competitive attraction, induced allopatry, and induced arrestment.
- Noncompetitive mechanisms include desensitization and inhibition, induced allochrony, suppressed calling and mating, camouflage, and sensory imbalance.

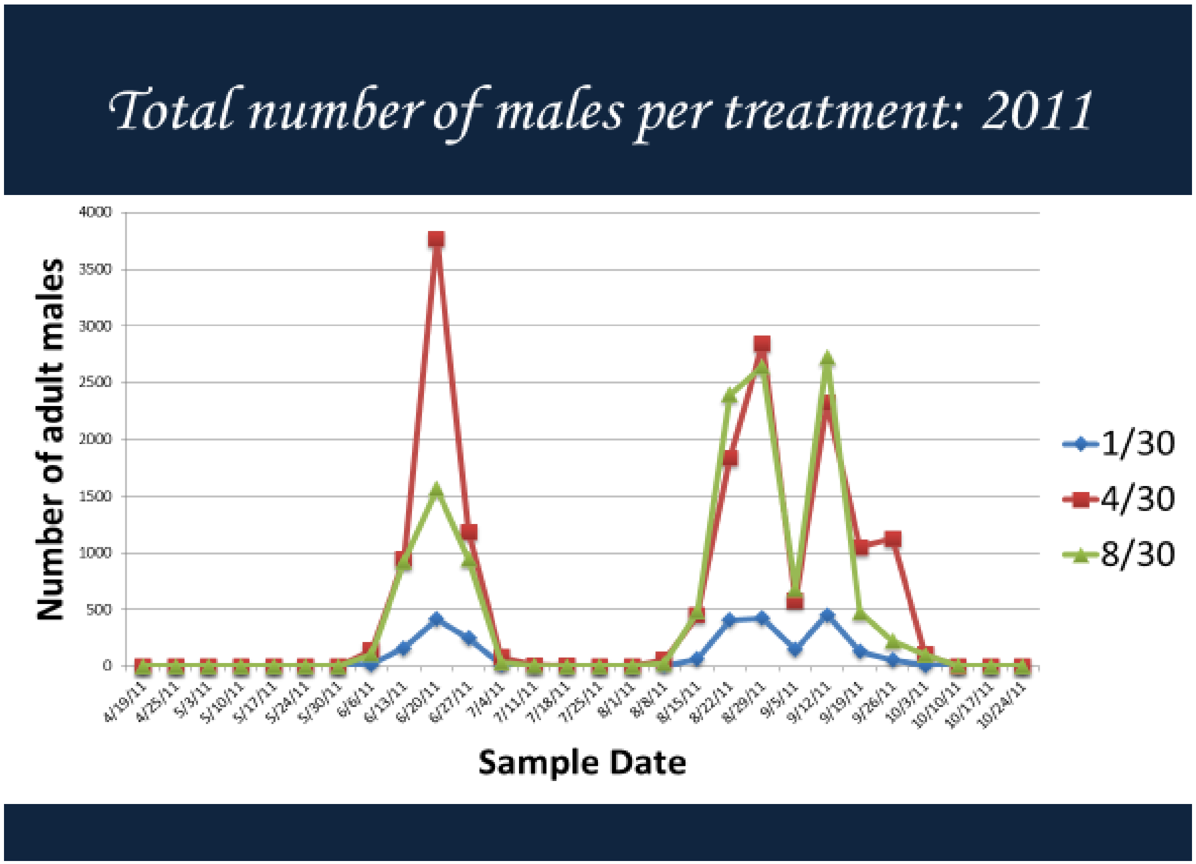




Miller & Gut 2015

Pheromone-Based Monitoring of Pseudococcus maritimus (Hemiptera: Pseudococcidae) Populations in Concord Grape Vineyards

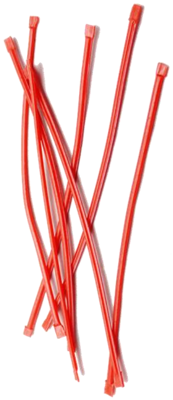
- B. W. Bahder, R. A. Naidu, K. M. Daane, J. G. Millar, D. B. Walsh. 2013. J Econ Entomol. doi.org/10.1603/EC12138



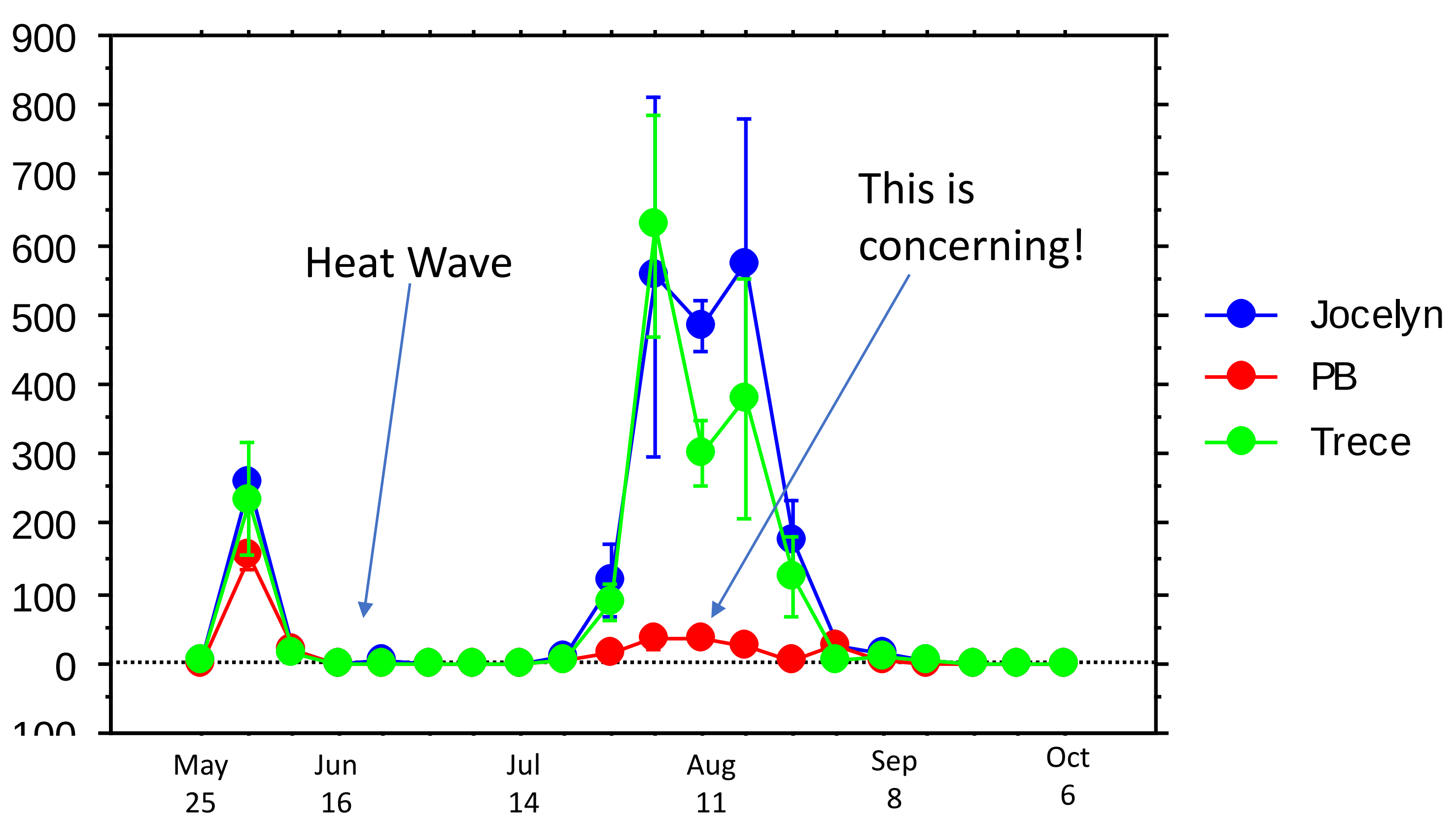
We concluded that at more than 4 traps per 30 acres that the traps were competing with one another at attracting male mealybugs.

Pilot Studies 2021

- We completed two studies with pheromone lures and dispensers in 2021.
- Our 1st study compared trap capture of male mealybugs in Delta traps baited with pheromone lures directly from Jocelyn Millar, lures from Trece Inc, and mating disruption dispensers from Pacific Biocontrol Inc.
- This study was completed in a Concord Vineyard nearby IAREC.



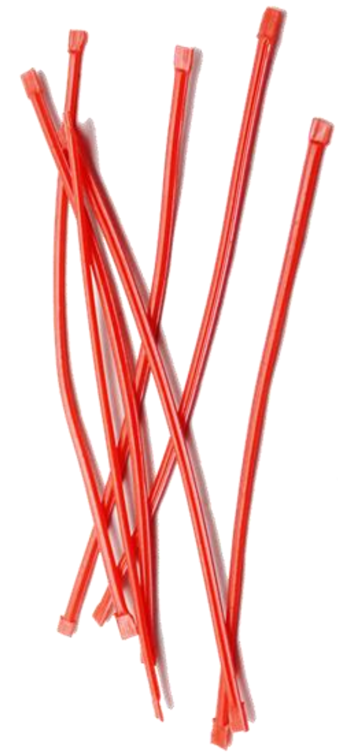
Concord lure studies 2021



Did the PB pheromone dispensers run out of gas?
Or did they release so much pheromone that the male mealybugs could not find the trap?

Mating disruption pilot studies 2021.

- We received 2,000 twist tie dispensers from Pacific biocontrol in spring 2021.
- We deployed these dispensers in 2 blocks at Columbia Crest in Paterson, WA.
- One block was in the WSDA Organic Certification program.
- The second block was conventional.
- Both blocks were older and already riddled with GVLRaV (leafroll) and highly infested with mealybugs.

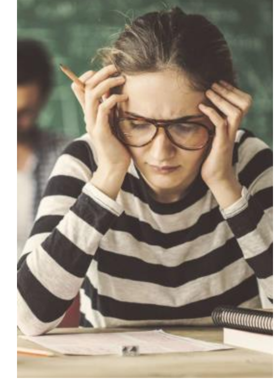


Release rates

- We deployed the twist-tie pheromone imbibed dispensers in the vineyard blocks in mid May.
- We were likely a little late, but Stephan was finishing his MS degree in Pullman.
- We'll get out earlier in 2022.
- Dispensers were deployed at 0, 10, 30, 60 and 100 emitters per acre in 5-acre plots.


10SB36
ZK
10CF90
10CH37
10SB36
10CH95
10CH91
10MR96
10CH92
10CB05
10MR96
10SB20
10SB19
10CB05
10MR96
10CH94
10CH93
10SB20
10SB19
10SB18
10SB18
10SY98
10MR97
10GT02
10GR01-HT
10SB04
10SB04
Organic
10GT02
10GR01-HT
10SB04
10SB04
10GT02
10PN42
10GW22
10CS60
10GT02
10PN42
10SY64
10CS67
10SY70
10CS60
10SY64
PATERSON
Conventional
75
10SY70
10CS67
10CS60
CH75
10MB63
10CS67
0SY77
10SY70
0SY77

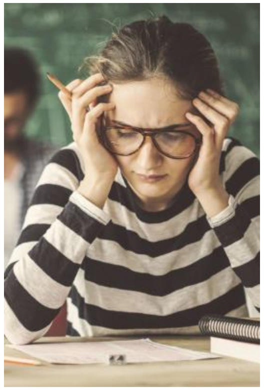
Calculations



- Vineyard layout: vines were spaced every 6 feet and each row is 9 feet apart. Each vine occupies 45 ft^2
- 43560 ft^2 is 1 acre. 217,800 ft^2 is 5 acres.
- 217,800 ft^2/ 45 ft2 per vine is 4,840 vines per 5 acres
- 52 rows is 459 feet; 78 vines in row is 462 feet.
- 459 feet times 462 feet is 212,058 ft^2. This is 500 ft^2 short of 5 acres..
- Close enough for government work.

Dispenser details

- <u>10 emitters per acre</u>- 5 rows with 10 emitters per row is 50 emitters per 5 acres.
- <u>30 emitters per acre</u>- 15 rows with 10 emitters per row is 150 emitters per 5 acres.
- <u>60 emitters per acre</u>- 13 rows with 23 emitters is 299 emitters per 5 acres.
- <u>100 emitters per acre</u>- 25 rows with 20 emitters per row is 500 emitters per 5 acres.

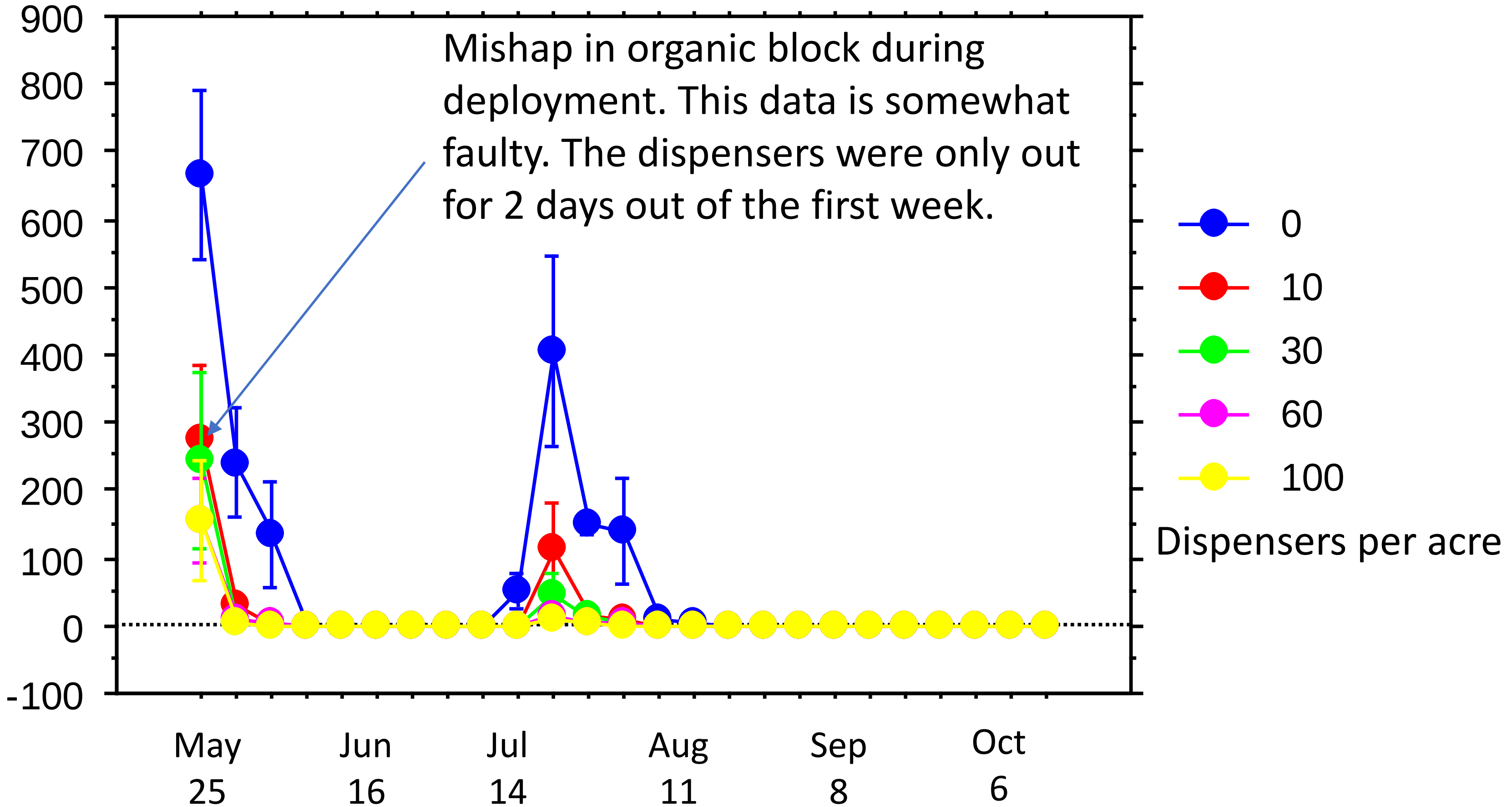


Figuring this out was a headache

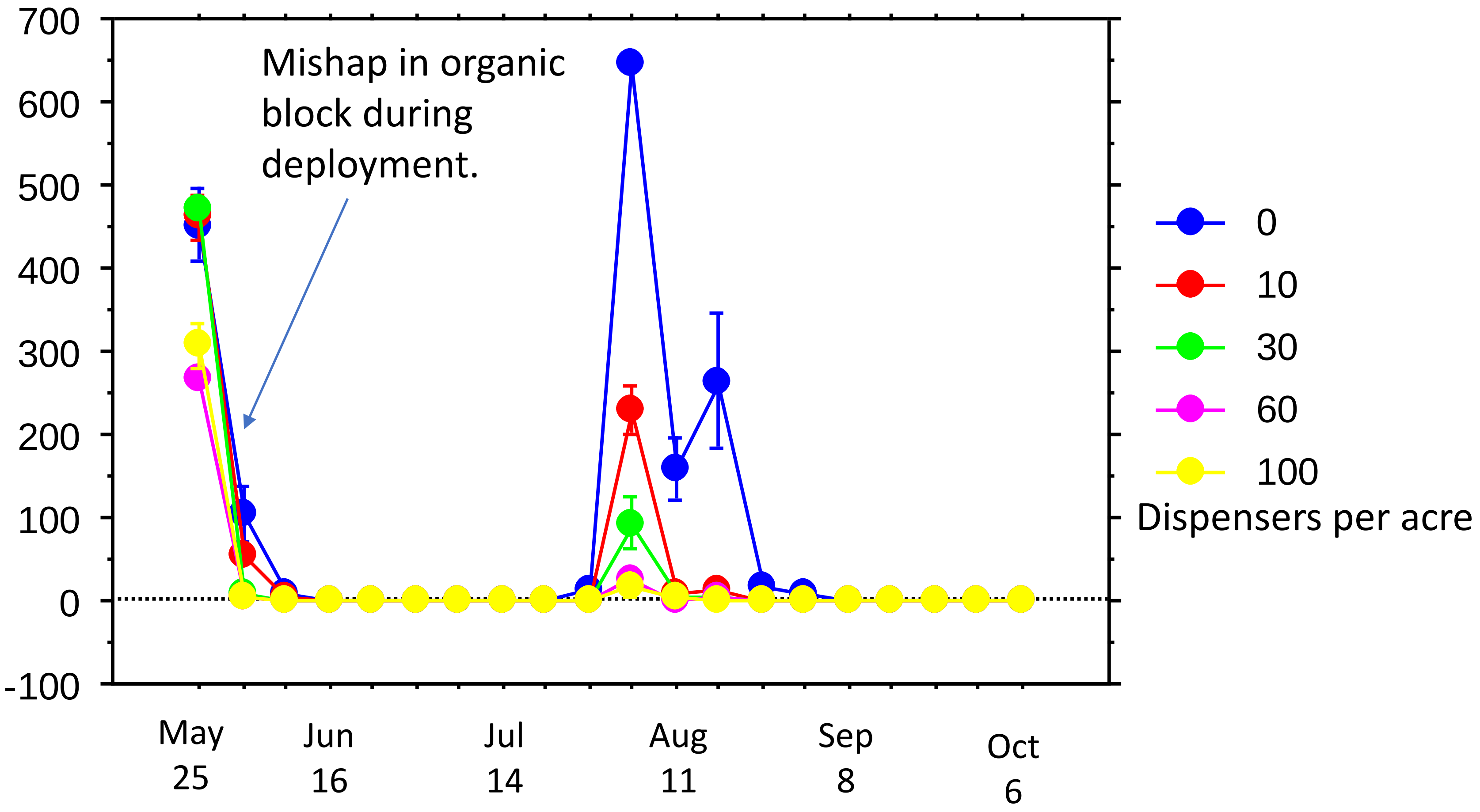
Sentinel traps

- Sentinel traps were placed out in center row (row 26) of each 5-acre plot at vine 33 and 48.
- These were baited with lures from Trece.
- Traps were monitored weekly from May through October 2021

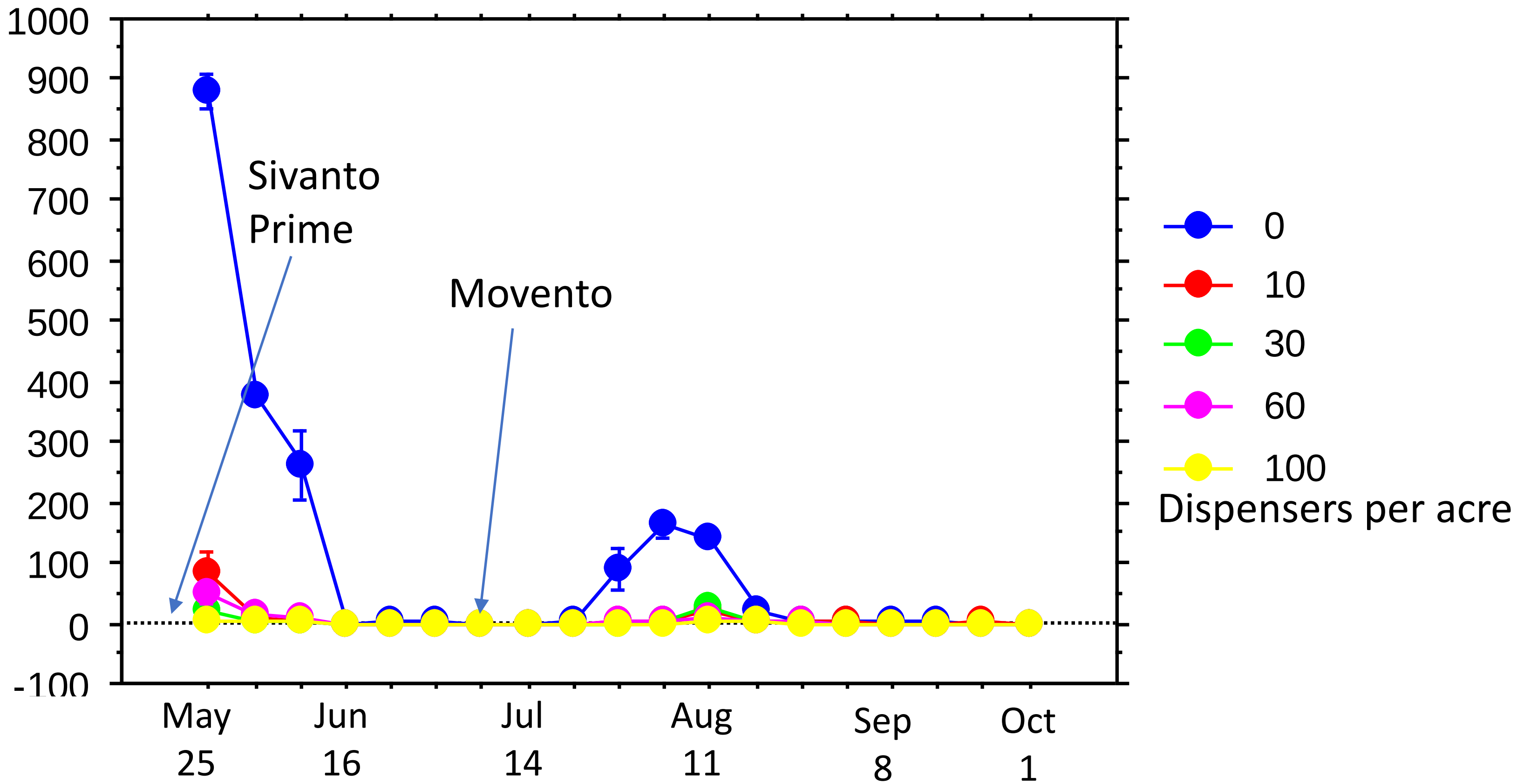
Bulk trap capture of male mealybugs for both blocks combined.



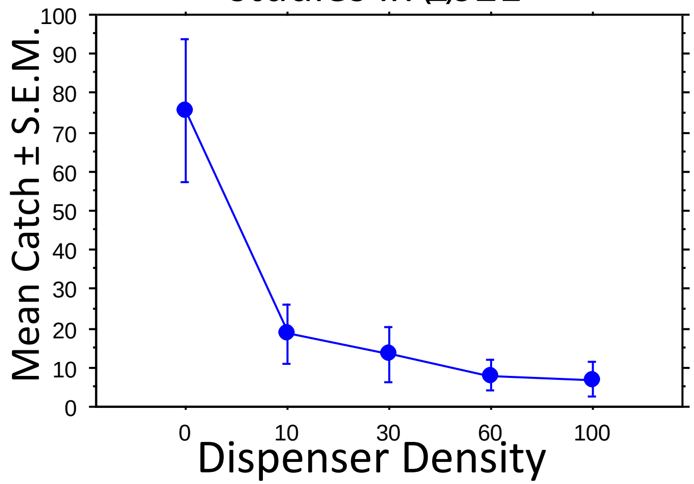
Organic block- Male Mealybugs captured per week.

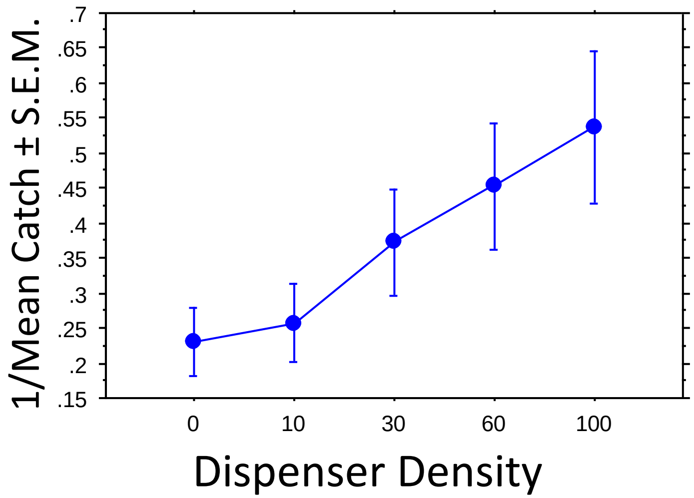


Conventional block- Male Mealybugs captured per week.

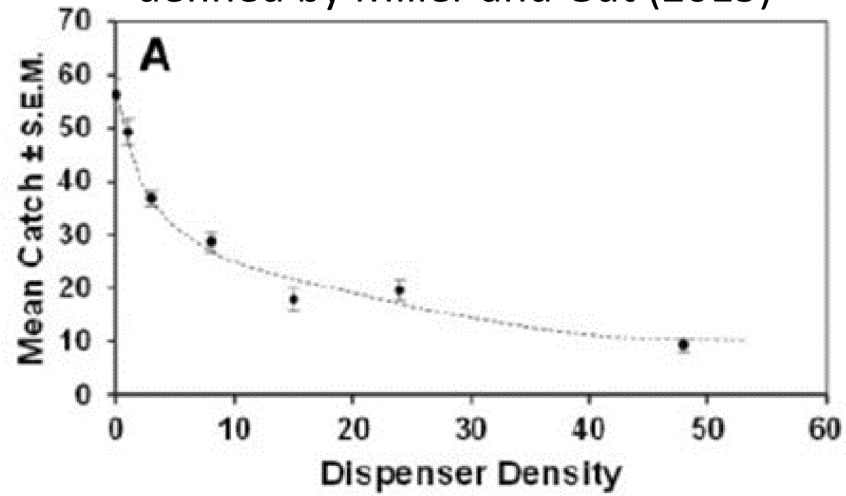


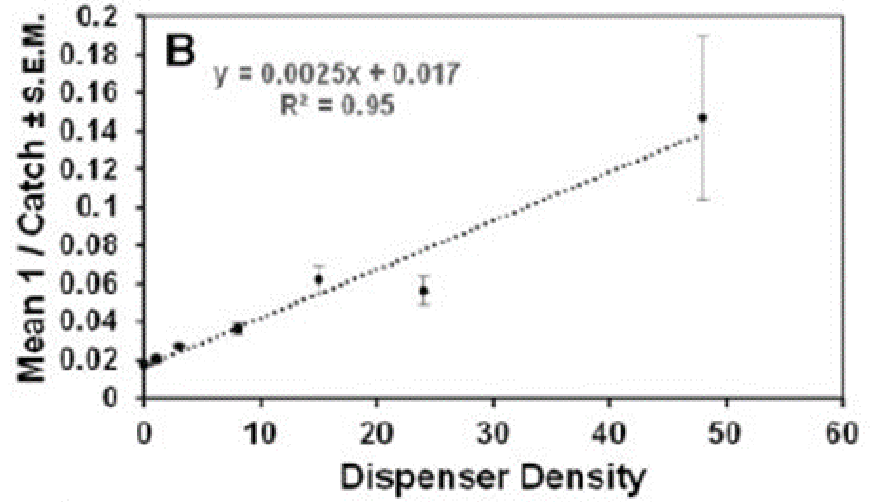
Our actual results from our grape mealybug mating disruption pilot studies in 2021





Competitive mating disruption as defined by Miller and Gut (2015)





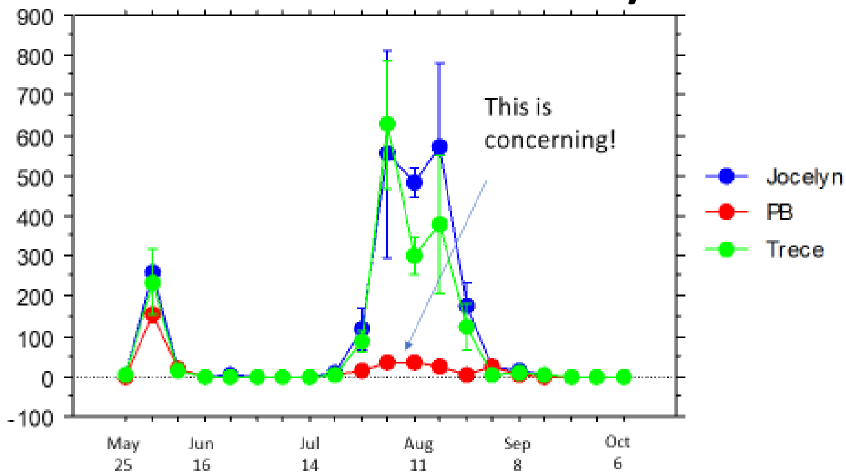
It sure looks like grape mealybug mating disruption is competitive.

Competitive Disruption

- Under competitive disruption, no impairments are experienced by males, females, or the signal of females.
- Therefore, males can respond to females and traps.
- Competitive disruption is entirely a numbers game where the ratio of dispensers to females and traps is highly consequential.
- This renders the control provided is pest-density-dependent.
- Again… This means that as the abundance of females increases there an increased likelihood that some may get mated.
- And it looks like mating disruption is achieved at about 60 pheromone dispersers per acre.

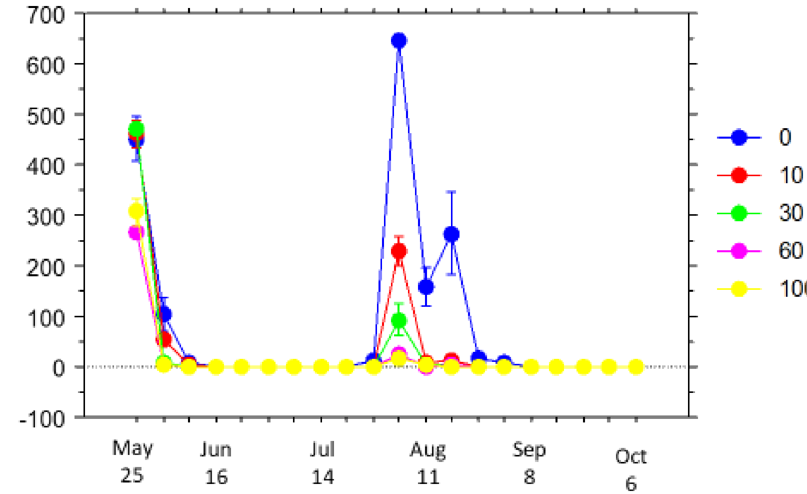
Concerns

Concord lure study



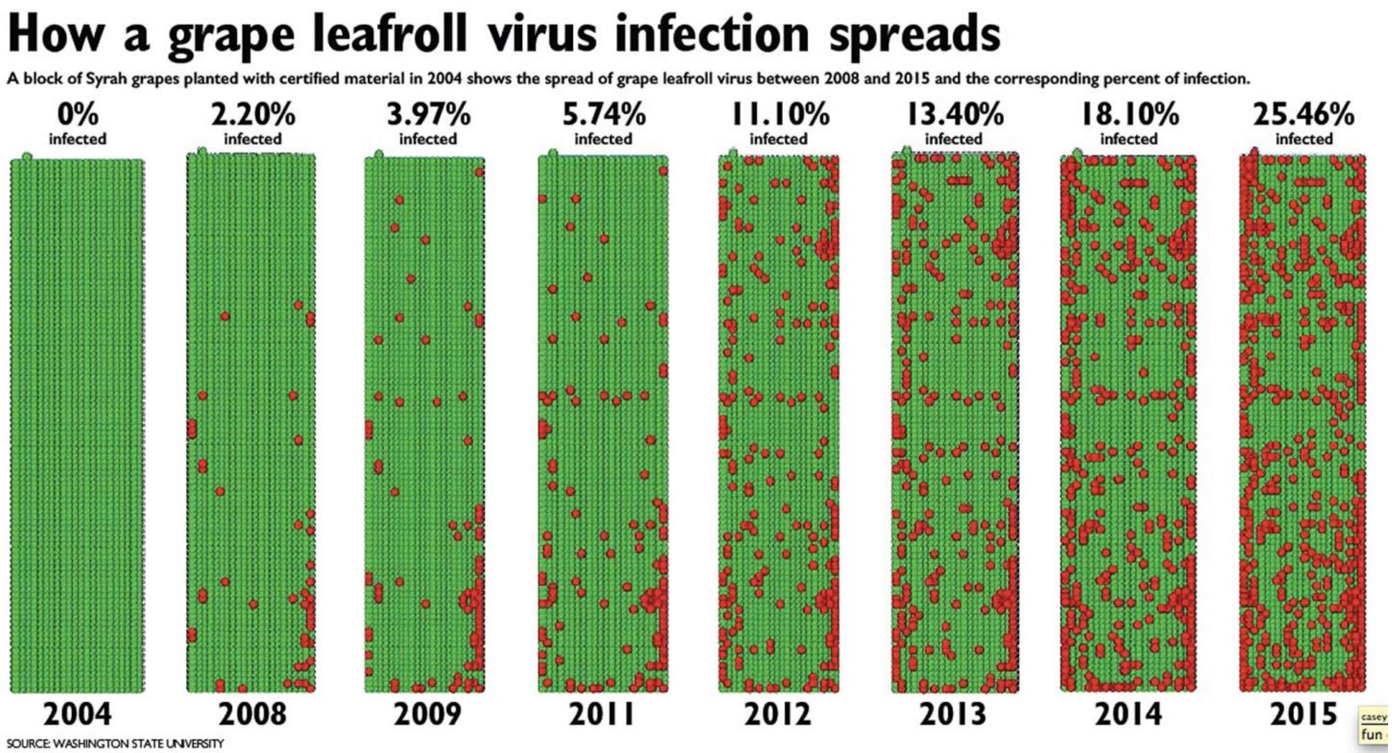
Either the dispensers ran out of gas after the first mealybug flight in our Concord lure studies or the dispensers were releasing so much pheromone that the males could not physically locate the traps the dispensers were in? If it's the later, it may be that mating disruption of grape mealybug may be a hybrid of competitive and noncompetitive?

Organic block- Male Mealybugs captured per week.



Next year we will put out delta traps baited with pheromone dispensers in our mating confusion plots and compare trap counts with traps baited with lures

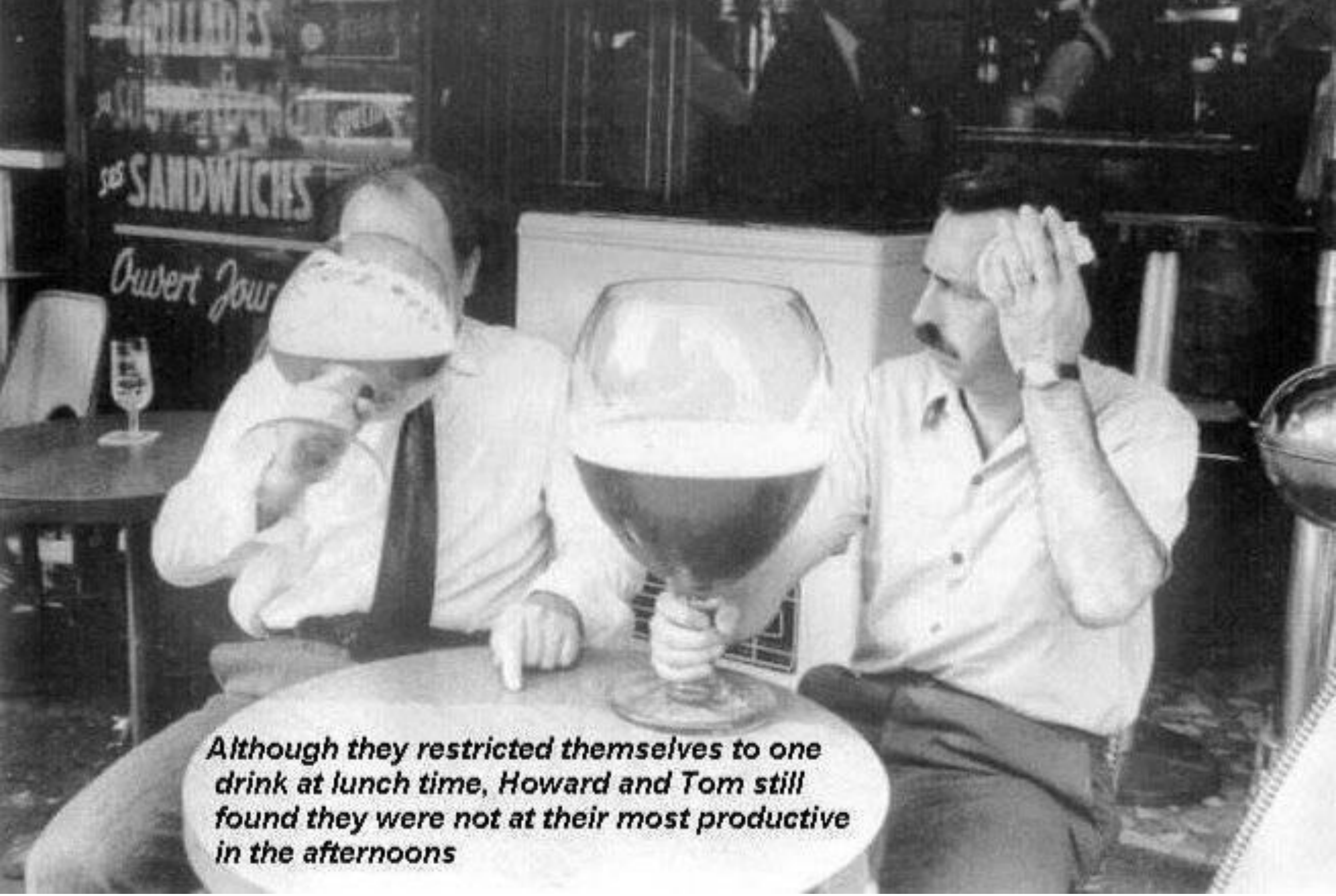
Future Plans- 2022 and beyond



- Hopefully we get enough pheromone dispensers in 2022 to expand our studies in 2022 with increased replication.
- We need to land a larger grant and include Naidu.
- We need Naidu's group to prove we can slow the spread of virus with mating disruption of grape mealybugs.

Acknowledgements- 2021

- Laura Lavine, Dept of Entomology, PhD assistantship.
- Peter McGhee, Pacific Biocontrol, for the pheromone dispensers and advice.
- Brad Higbee, Trece, for pheromone lures.
- Jocelyn Millar, UC Riverside, for pheromone lures, advice, and moral support.
- Kent Daane, UC Berkeley, for advice
- Yun Zhang, Ste Michelle Wine Estates, for the vineyards.
- Melissa Hansen, Washington State Grape and Wine Research Program, for the money.
- Stephan Onayemi, WSU PhD student, for doing the work



Any Questions?