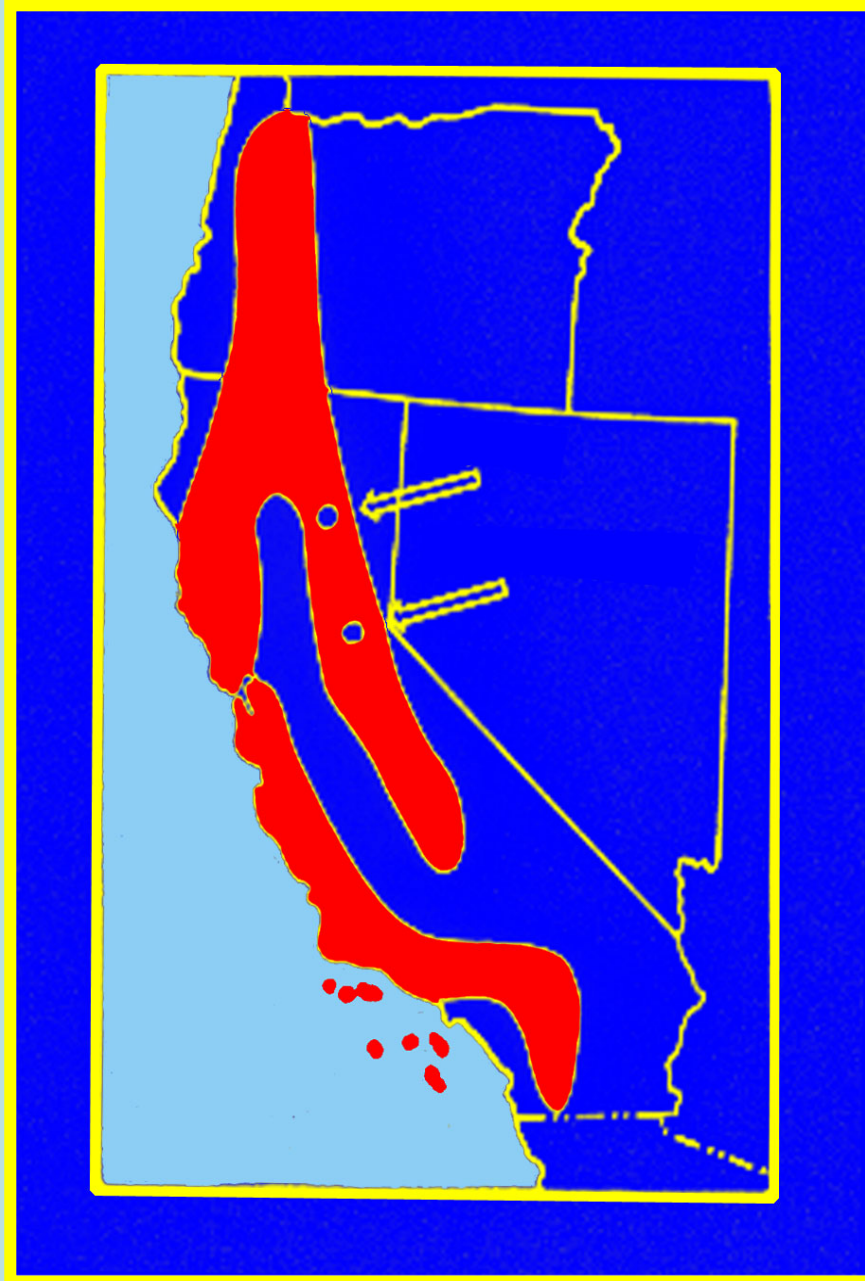


Refining the aggregation pheromone of the California Fivespined Ips, *Ips paraconfusus*, in the Central Sierra Nevada

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Shakeeb Hamud¹, David L. Wood³, Dezene PW.
Huber⁴, and Steven J. Seybold¹

¹USDA Forest Service PSW, ²UC Davis, ³UC Berkeley,
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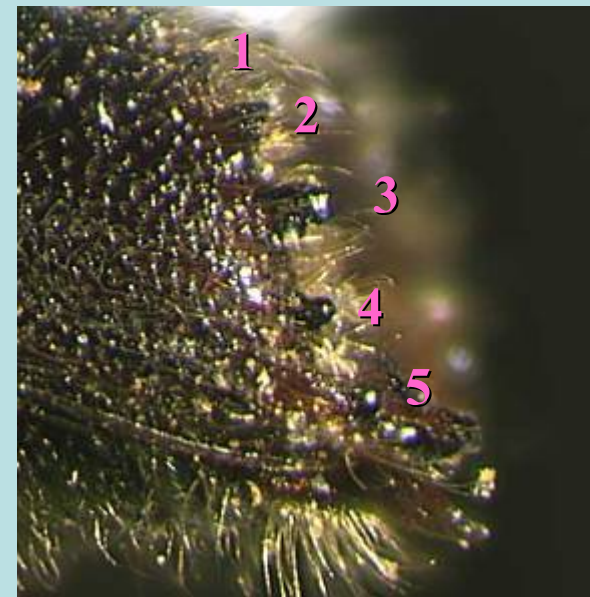
**Distribution of the
the California
Fivespined Ips
*Ips paraconfusus***



Introduction

California Fivespined Ips, *Ips paraconfusus*

| | |
|----------------------|------------------------|
| Ponderosa | <i>Pinus ponderosa</i> |
| Knobcone | <i>P. attenuata</i> |
| Lodgepole | <i>P. contorta</i> |
| Coulter | <i>P. coulteri</i> |
| Sugar | <i>P. lambertiana</i> |
| Western white | <i>P. monticola</i> |
| Bishop | <i>P. muricata</i> |
| Monterey | <i>P. radiata</i> |
| Grey | <i>P. sabiniana</i> |
| Torrey | <i>P. torreyana</i> |





Ips paraconfusus: Impacts

- 1) Outbreaks following poor logging sanitation
- 2) Outbreaks during drought
- 3) Topkilling in large trees; mortality in small trees
- 4) Tree mortality in *Pinus torreyana* stand; *Pinus radiata* street trees

A Fruitful Collaboration

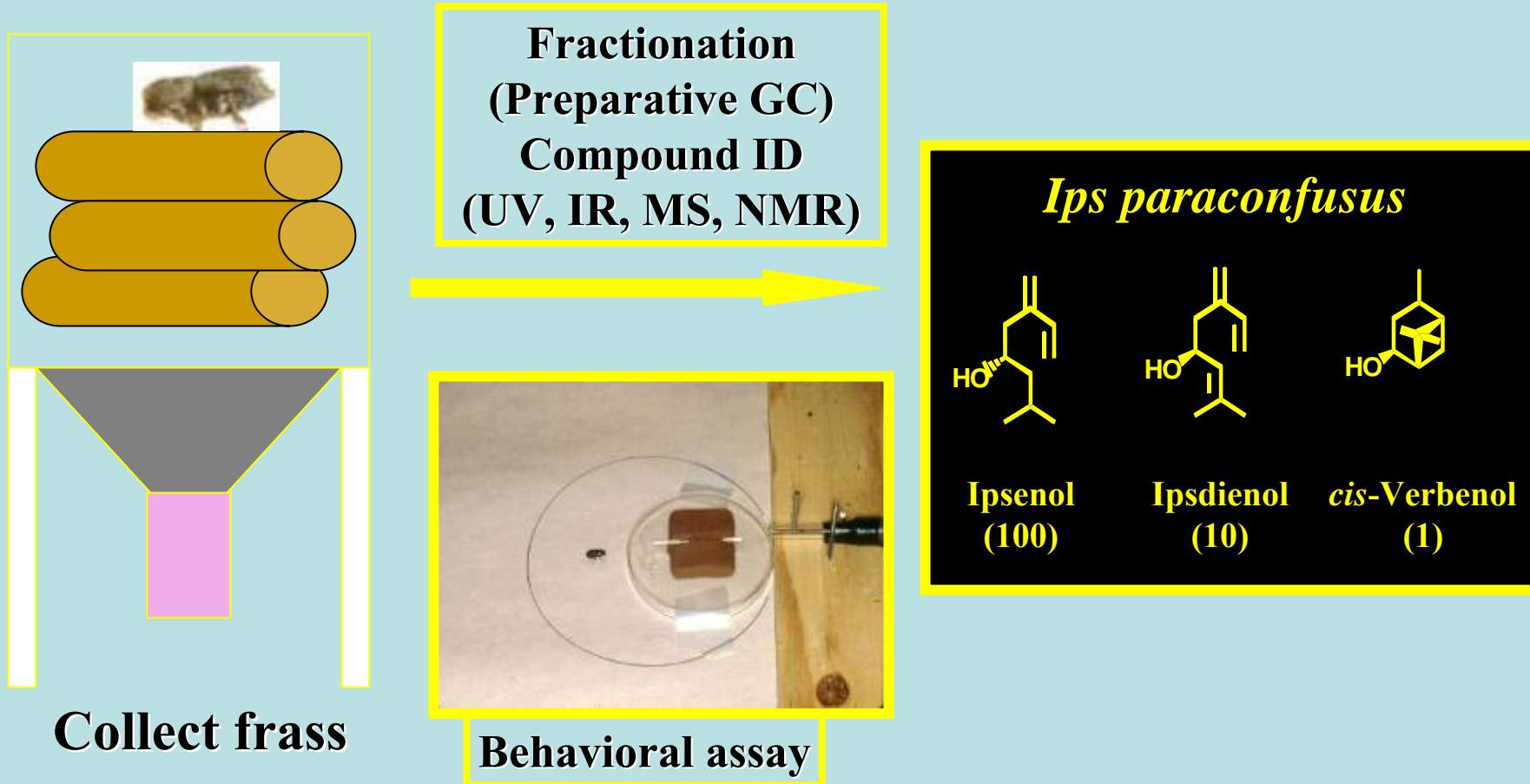


Dave Wood, UC-Berkeley
Insect Behavior



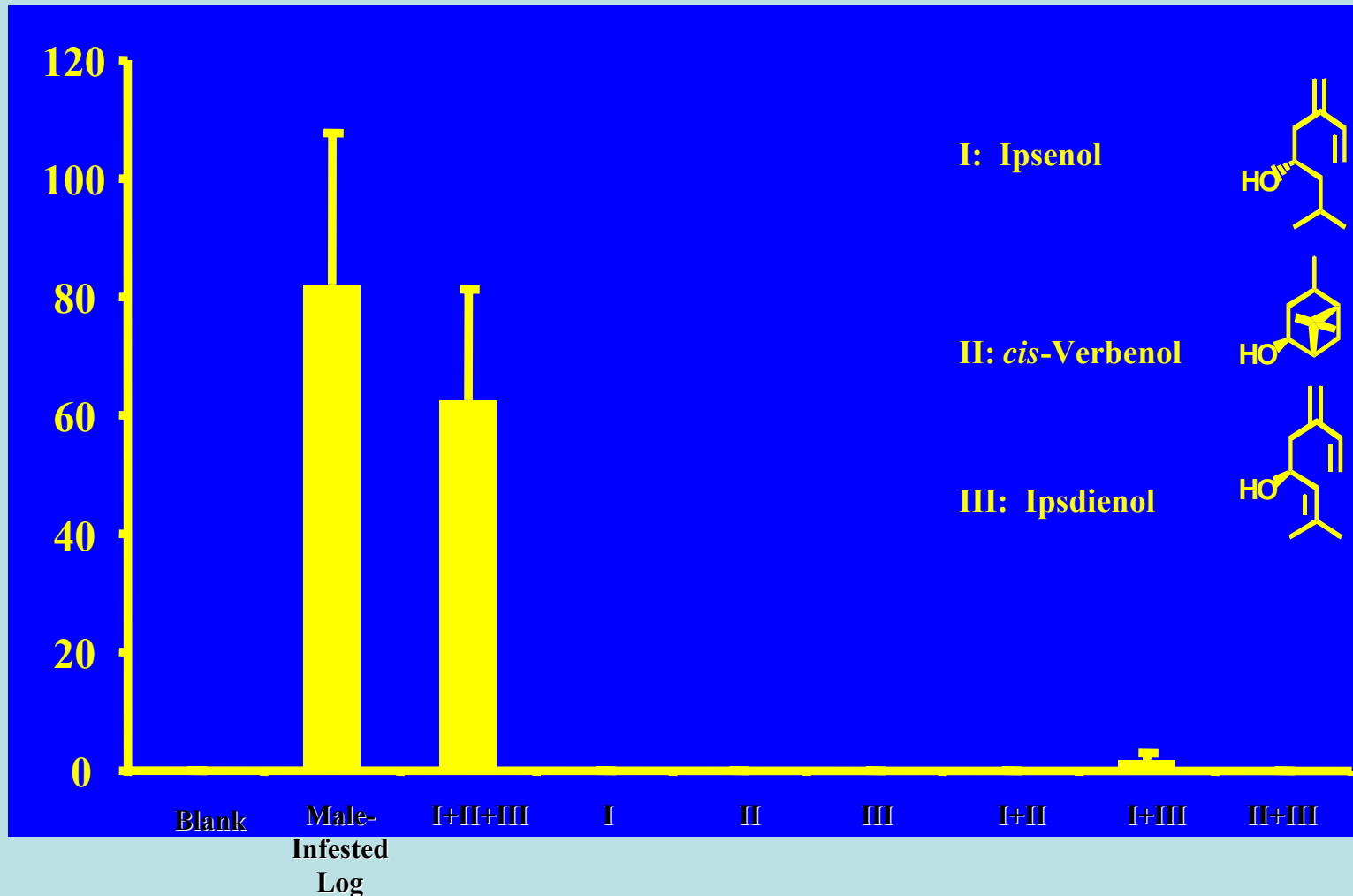
Milt Silverstein, Stanford Research Institute
Bio-organic Chemistry

Isolation of Aggregation Pheromones from Male *Ips paraconfusus*



(Silverstein et al. 1966, *Science*; Wood et al. 1968, *Science*)

Field Test of Individual Pheromone Components



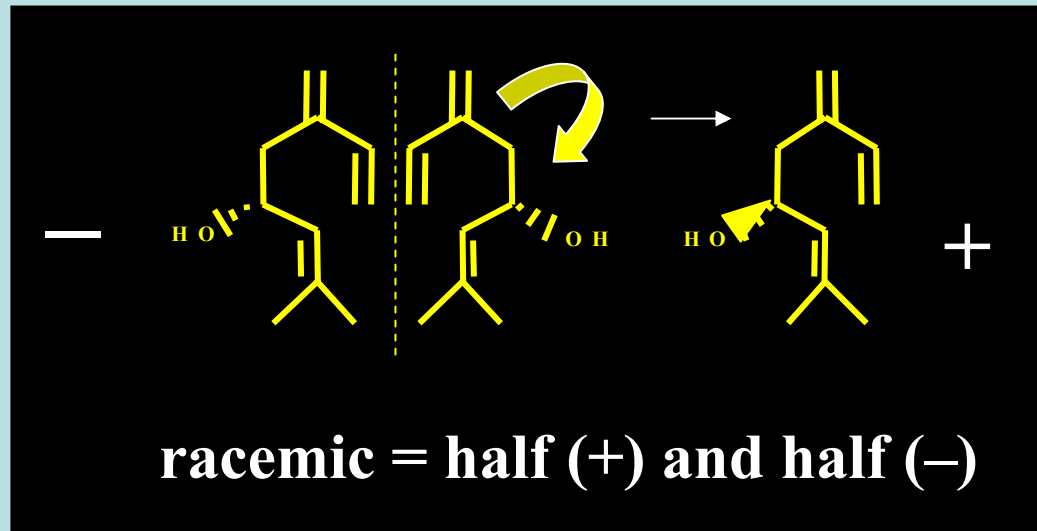
(Wood 1970, "Pheromones of Bark Beetles.")

Conclusions from the original pheromone isolation for *Ips paraconfusus*

- 1) Insects have multi-component pheromones**
- 2) Pheromone components can act synergistically**

Naturally occurring pheromone

| (-)-ipsenol | (+)-ipsdienol | (-)- <i>cis</i> -verbenol (measured in chloroform) |
|-------------|---------------|---|
| 100 | 10 | 1 |



Comparison of naturally occurring and commercial pheromone blends

Natural

| | | |
|---------------|---------------------------|-------------|
| (+)-ipsdienol | (-)- <i>cis</i> -verbenol | (-)-ipsenol |
| 10 | 1 | 100 |

Commercial (Phero Tech)

| | | |
|---------------|--------------------------------------|------------------------------------|
| (+)-ipsdienol | 83%(-), 17%(+)- <i>cis</i> -verbenol | Racemic ipsenol 50%(-), 50% (+) |
| 1 | 3 | 2 |

Refining pheromone

Q: Why not make the commercial blend identical to male produced pheromone?

A: Cost. Racemic blends are less expensive to produce than pure enantiomers

A: The ratio of the components has never been tested before

Cost Comparison

Natural

| (+)-ipsdienol | (-)-<i>cis</i>-verbenol | (-)-ipsenol |
|----------------------|--------------------------------|--------------------|
| \$46 | \$20 | \$38 |

Commercial (Phero Tech)

| (+)-ipsdienol | 83%(-), 17%(+)-<i>cis</i>- verbenol | Racemic ipsenol 50%(-), 50% (+) |
|----------------------|--|--|
| \$46 | \$3 | \$3 |

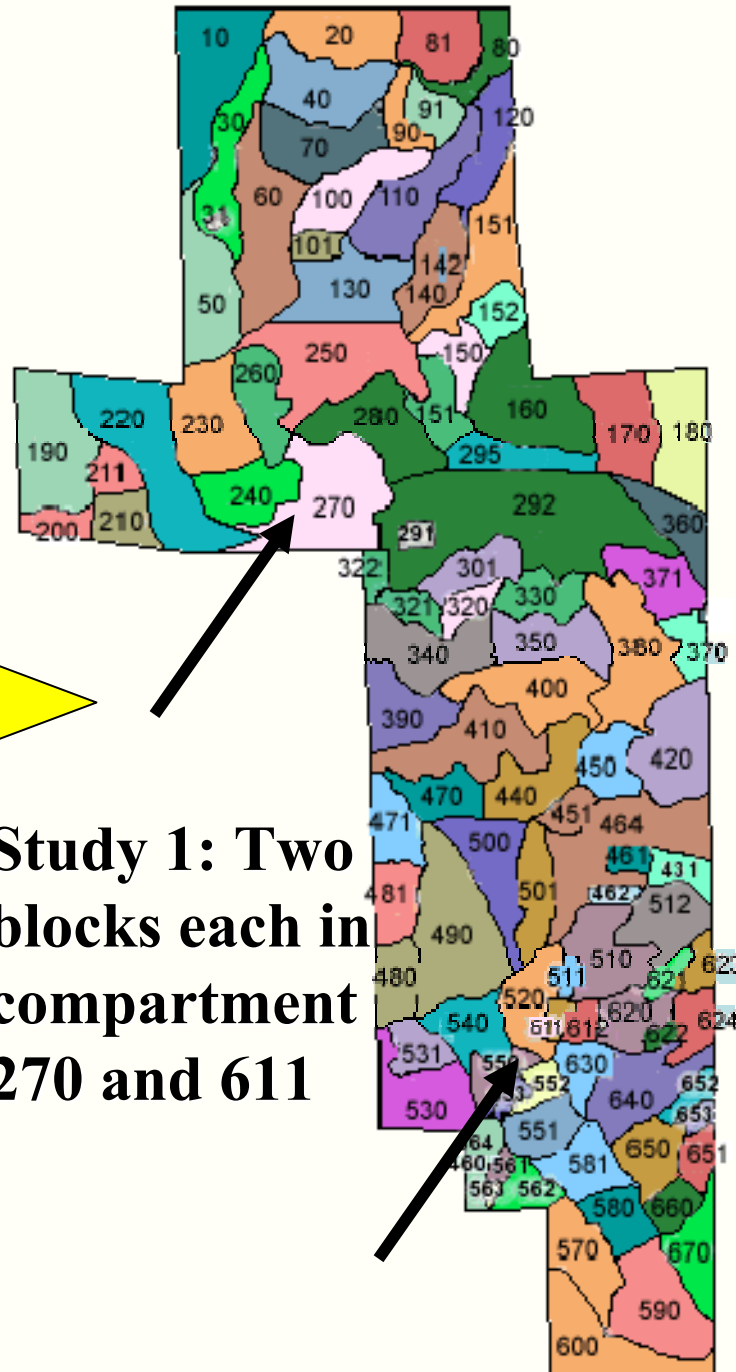
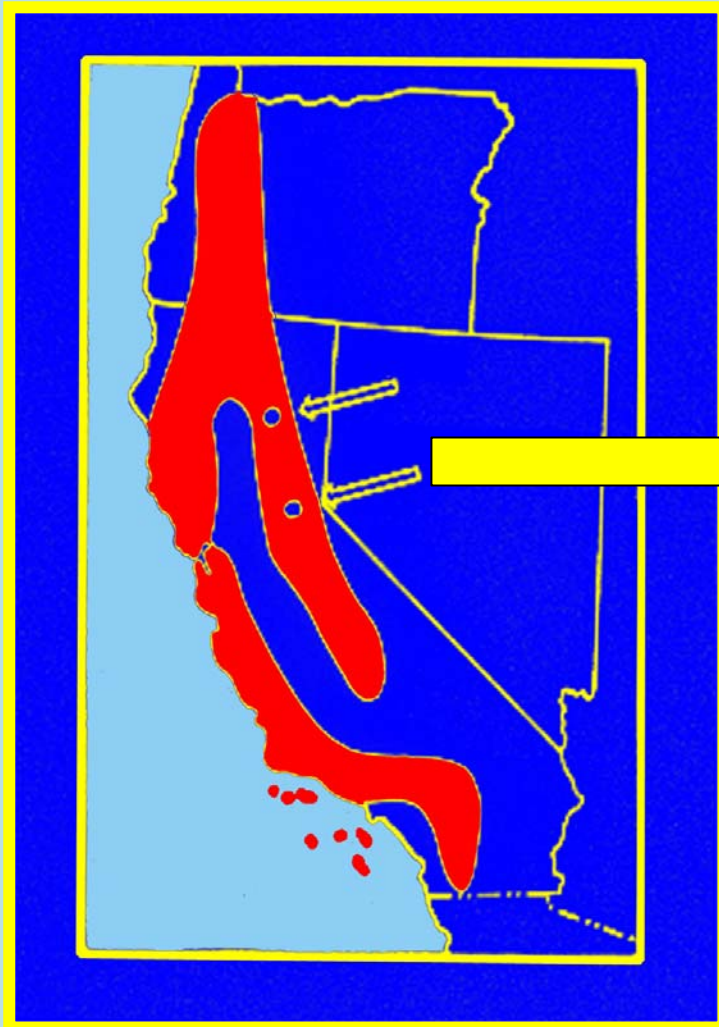
*Racemic ipsdienol: \$4

Objectives

Field trials

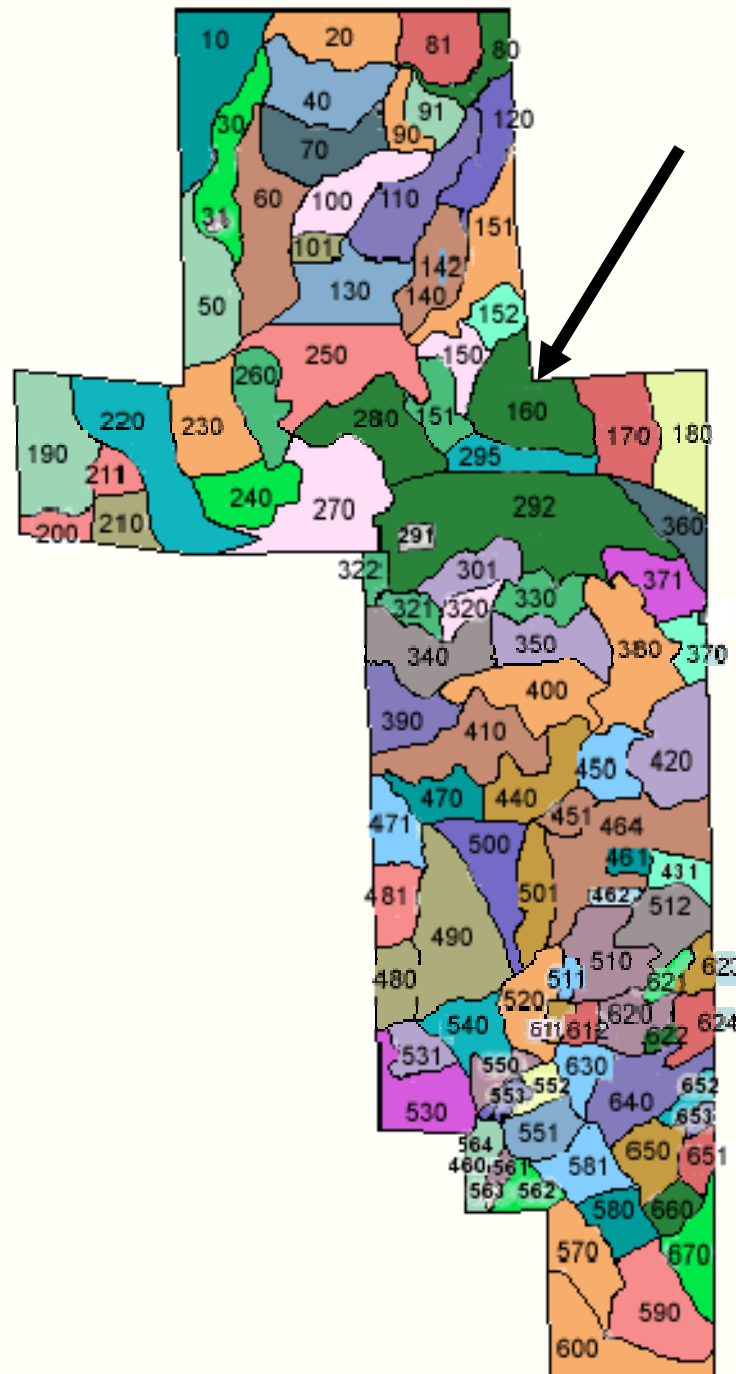
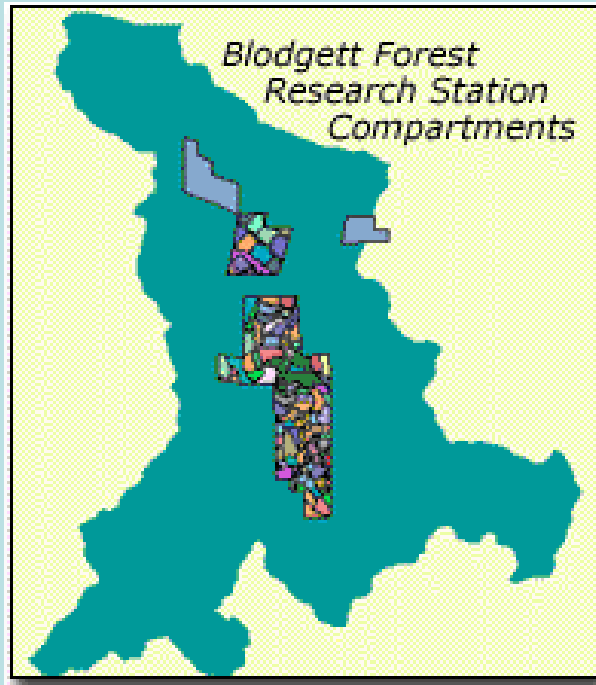
- **Study 1-Optimize ipsdienol component**
 - Summer 2004
- **Study 2-Optimize *cis*-verbenol component**
 - Summer 2005
- **Study 3-Optimize ipsenol component**
 - Summer 2005

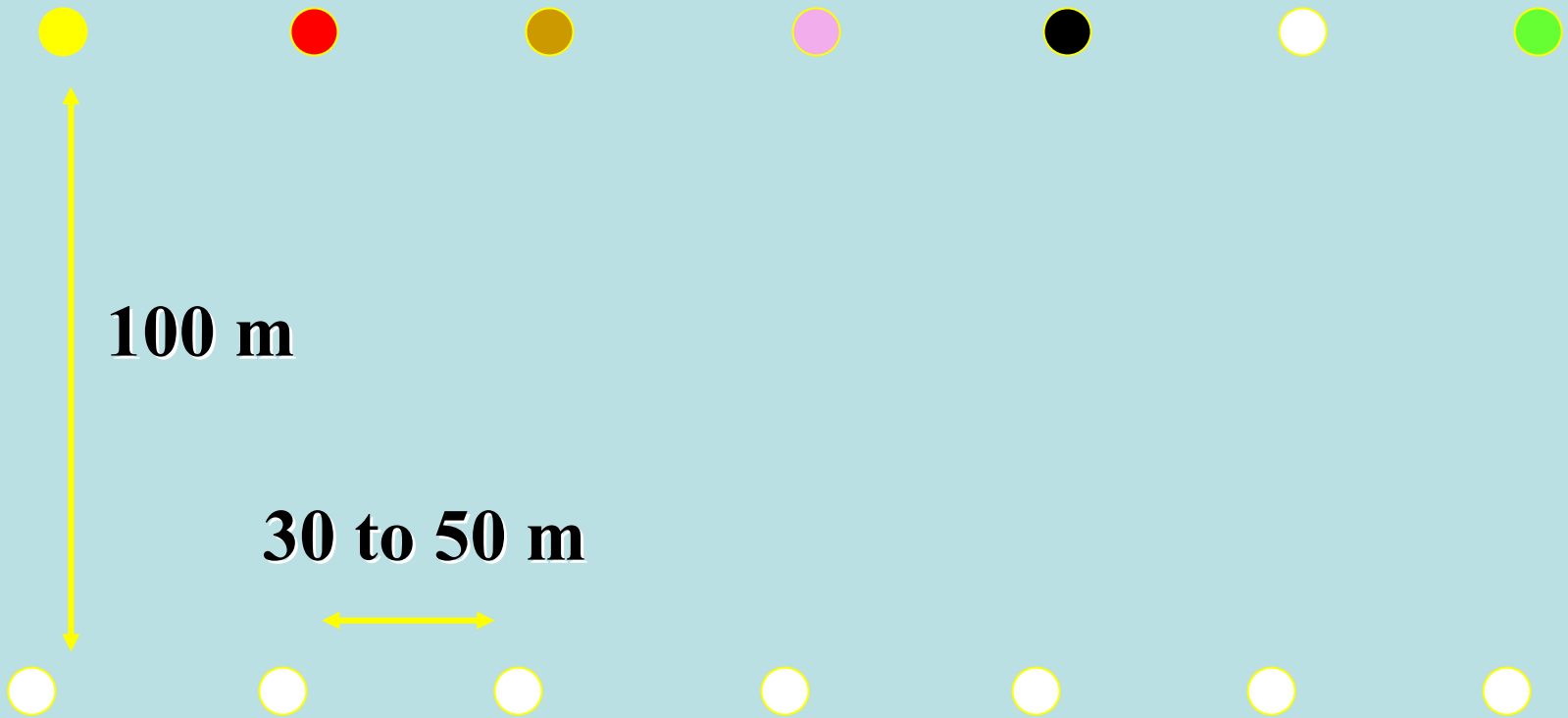
Blodgett Forest (Eldorado Co.)

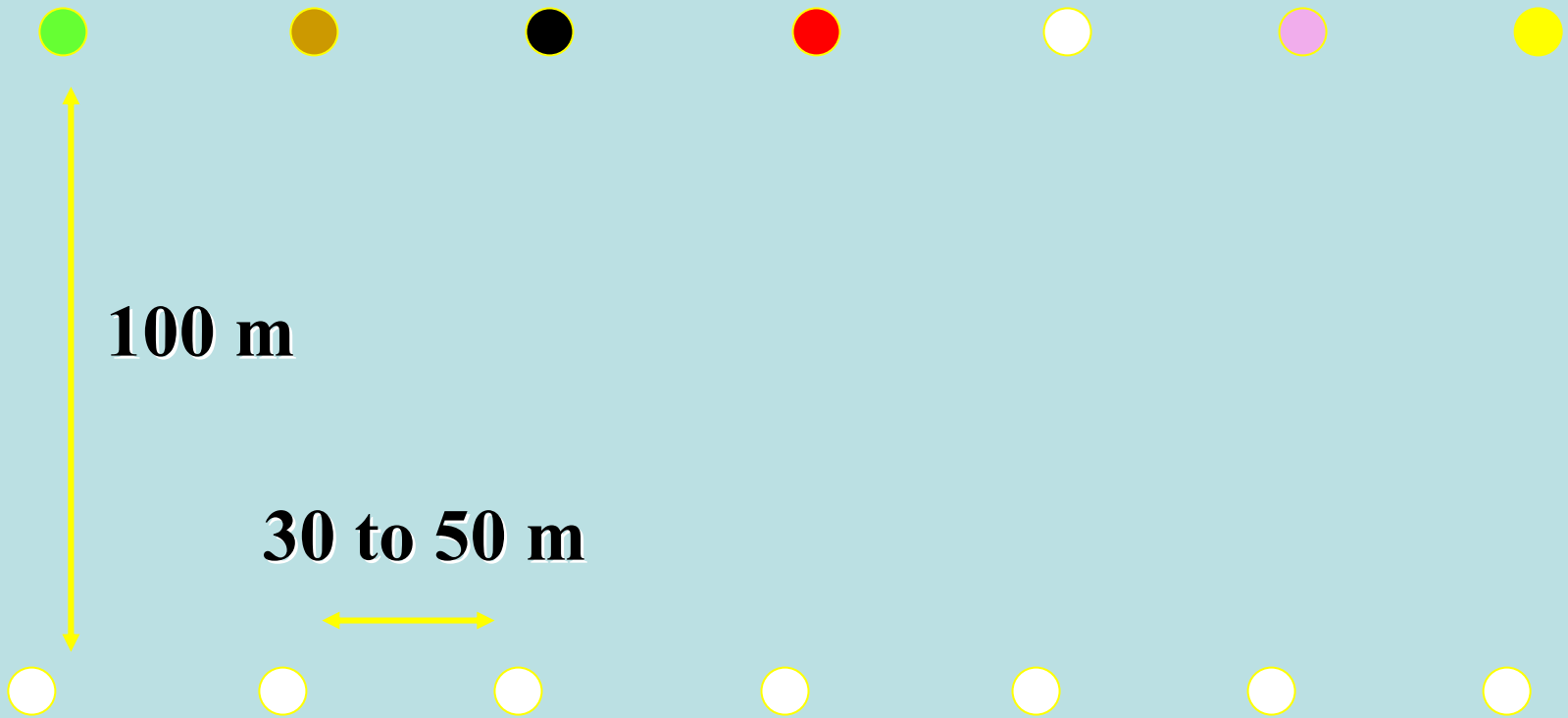


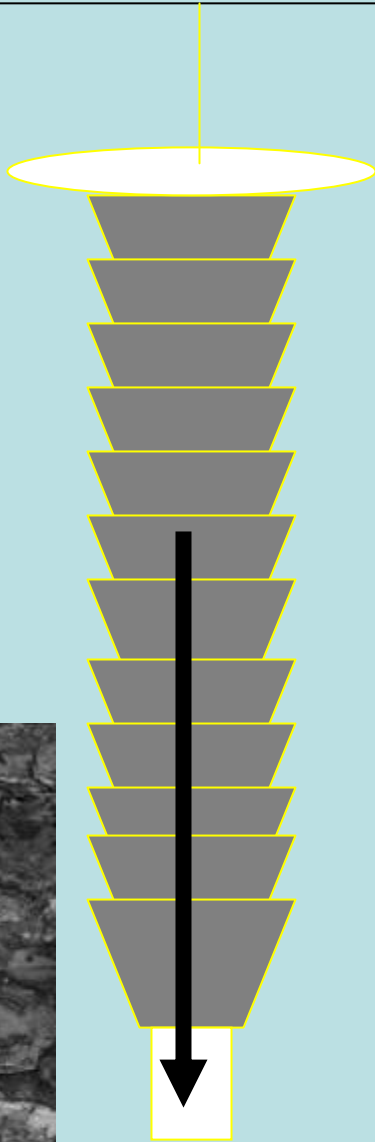
**Study 1: Two
blocks each in
compartment
270 and 611**

Studies 2 and 3: Four blocks in Compartment 160

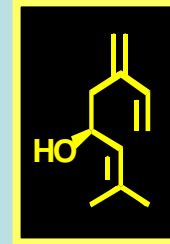








Study 1 – Optimizing Ipsdienol



Treatments

1. **Unbaited control**
2. **Racemic conophthorin**
3. **(+)-Ipsdienol**, racemic ipsenol, 83% **(-)-*cis*-verbenol**, and **racemic conophthorin**
4. **(+)-Ipsdienol**, racemic ipsenol, 83% **(-)-*cis*-verbenol**
5. **(-)-Ipsdienol**, racemic ipsenol, 83% **(-)-*cis*-verbenol**
6. **1x Racemic Ipsdienol**, racemic ipsenol, 83% **(-)-*cis*-verbenol**
7. **2x Racemic Ipsdienol**, racemic ipsenol, 83% **(-)-*cis*-verbenol**

Study 1 – Collected 9 times from August 27, 2004 to September 22, 2004

Study 1 – Optimizing Ipsdienol

Treatments

1. **Unbaited control**
2. **Racemic conophthorin**
3. **(+)-Ipsdienol, racemic ipsenol, 83% (–)-*cis*-verbenol, and racemic conophthorin**
4. **(+)-Ipsdienol, racemic ipsenol, 83% (–)-*cis*-verbenol**
5. **(-)-Ipsdienol, racemic ipsenol, 83% (–)-*cis*-verbenol**
6. **1x Racemic ipsdienol, racemic ipsenol, 83% (–)-*cis*-verbenol**
7. **2x Racemic ipsdienol, racemic ipsenol, 83% (–)-*cis*-verbenol**

Study 1 – Collected 9 times from August 27, 2004 to September 22, 2004

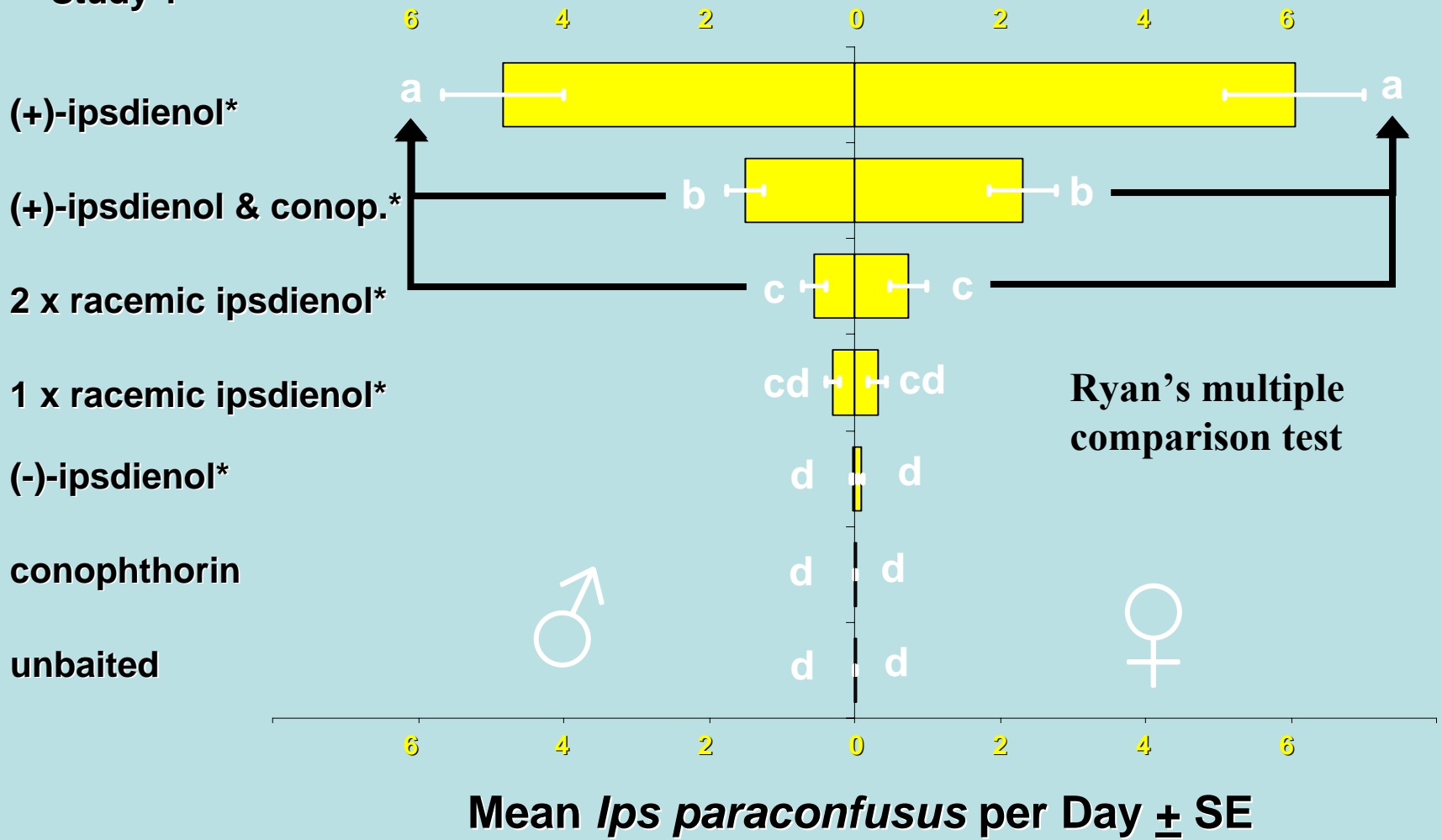
Study 1 – Optimizing Ipsdienol

Treatments

1. Unbaited control
2. **Racemic conophthorin**
3. **(+)-Ipsdienol, racemic ipsenol, 83% (-)-*cis*-verbenol, and racemic conophthorin**
4. **(+)-Ipsdienol, racemic ipsenol, 83% (-)-*cis*-verbenol**
5. **(-)-Ipsdienol, racemic ipsenol, 83% (-)-*cis*-verbenol**
6. **1x Racemic ipsdienol, racemic ipsenol, 83% (-)-*cis*-verbenol**
7. **2x Racemic ipsdienol, racemic ipsenol, 83% (-)-*cis*-verbenol**

Study 1 – Collected 9 times from August 27, 2004 to September 22, 2004

Study 1



ANOVA with important interaction terms

Males: Tx 0.0001, Blk 0.0007, Time 0.0001, Tx*time 0.0001

Females: Tx 0.0001, Blk 0.0001, Time 0.0001, Tx*time 0.0001, Blk*time 0.0924

Study 1 Summary



- ***Ips paraconfusus* is attracted to the blend containing (+)-ipsdienol**
- **It is disrupted by blends containing (-)-ipsdienol or conophthorin**

Study 2 – Optimizing *cis*-Verbenol

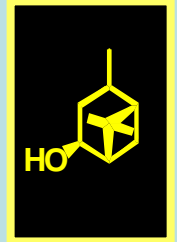


Treatments

1. **Unbaited**
2. **(+)-Ipsdienol and racemic ipsenol** (without *cis*-verbenol)
3. **(-)-*cis*-Verbenol**, (+)-ipsdienol, and racemic ipsenol
4. **(+)-*cis*-Verbenol**, (+)-ipsdienol, and racemic ipsenol
5. **83% (-)-*cis*-Verbenol**, (+)-ipsdienol, and racemic ipsenol

Study 2 – Collected 7 times from July 28, 2005 to August 12, 2005

Study 2 – Optimizing *cis*-Verbenol



Treatments

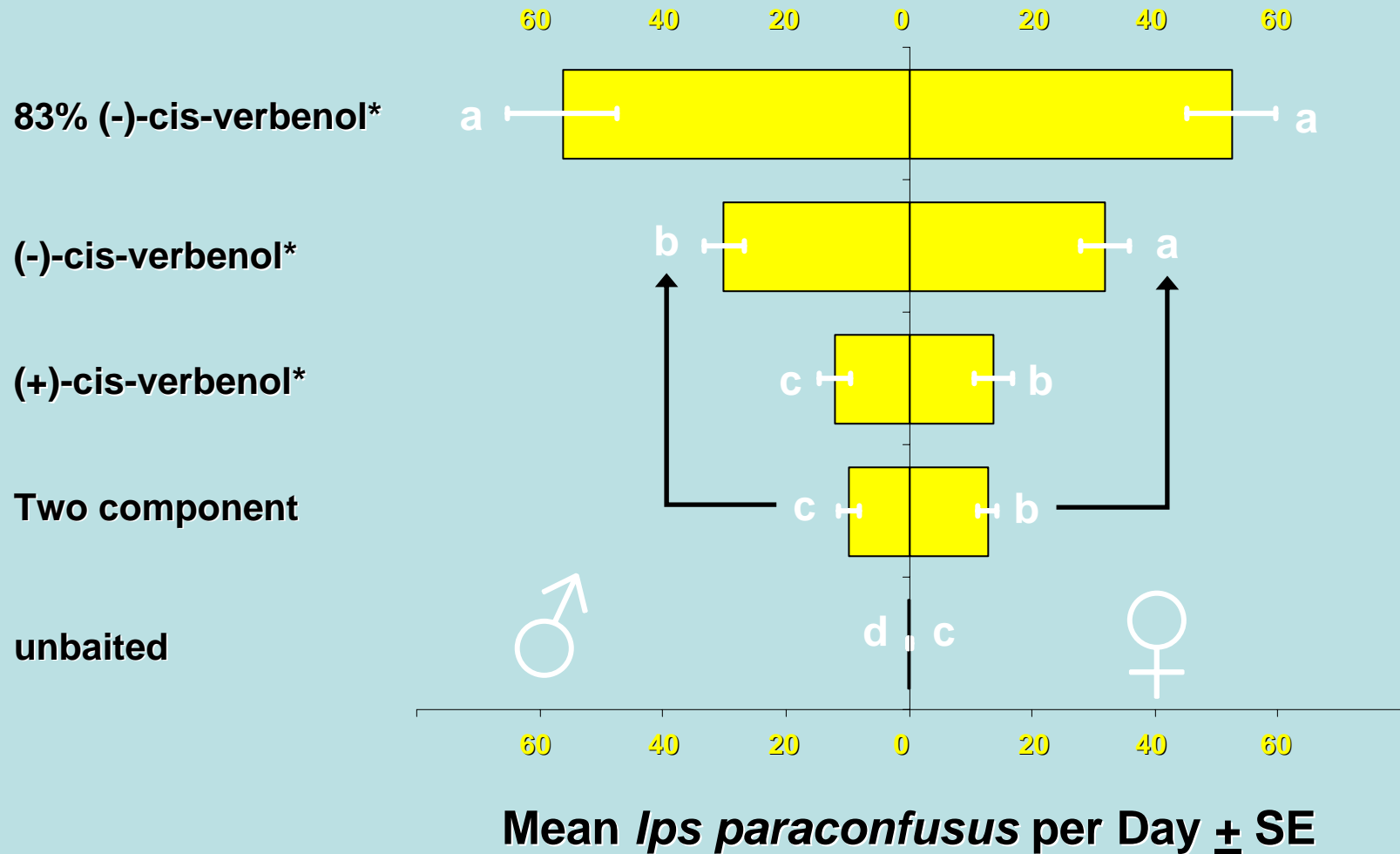
1. **Unbaited**
2. **(+)-Ipsdienol and racemic ipsenol** (without *cis*-verbenol)
3. **(-)-*cis*-Verbenol**, (+)-ipsdienol, and racemic ipsenol
4. **(+)-*cis*-Verbenol**, (+)-ipsdienol, and racemic ipsenol
5. **83% (-)-*cis*-Verbenol**, (+)-ipsdienol, and racemic ipsenol

Pherotech
300 to 600 µg/day
*25° C conditions



Chem Tica
80 µg/day
*20° C conditions

Study 2



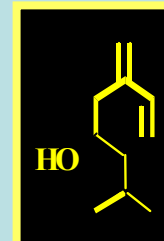
Males: Tx 0.0001, Blk 0.0103, Time 0.1205, Tx*time 0.0001
Females: Tx 0.0001, Blk 0.3730, Time 0.0235, Tx*time 0.0001

Study 2 Summary



- ***Ips paraconfusus* is more attracted to (-)-*cis*-verbenol than (+)-*cis*-verbenol**
- **(+)-*cis*-verbenol may not enhance or reduce the response to ipsenol and ipsdienol**

Study 3 – Optimizing Ipsenol

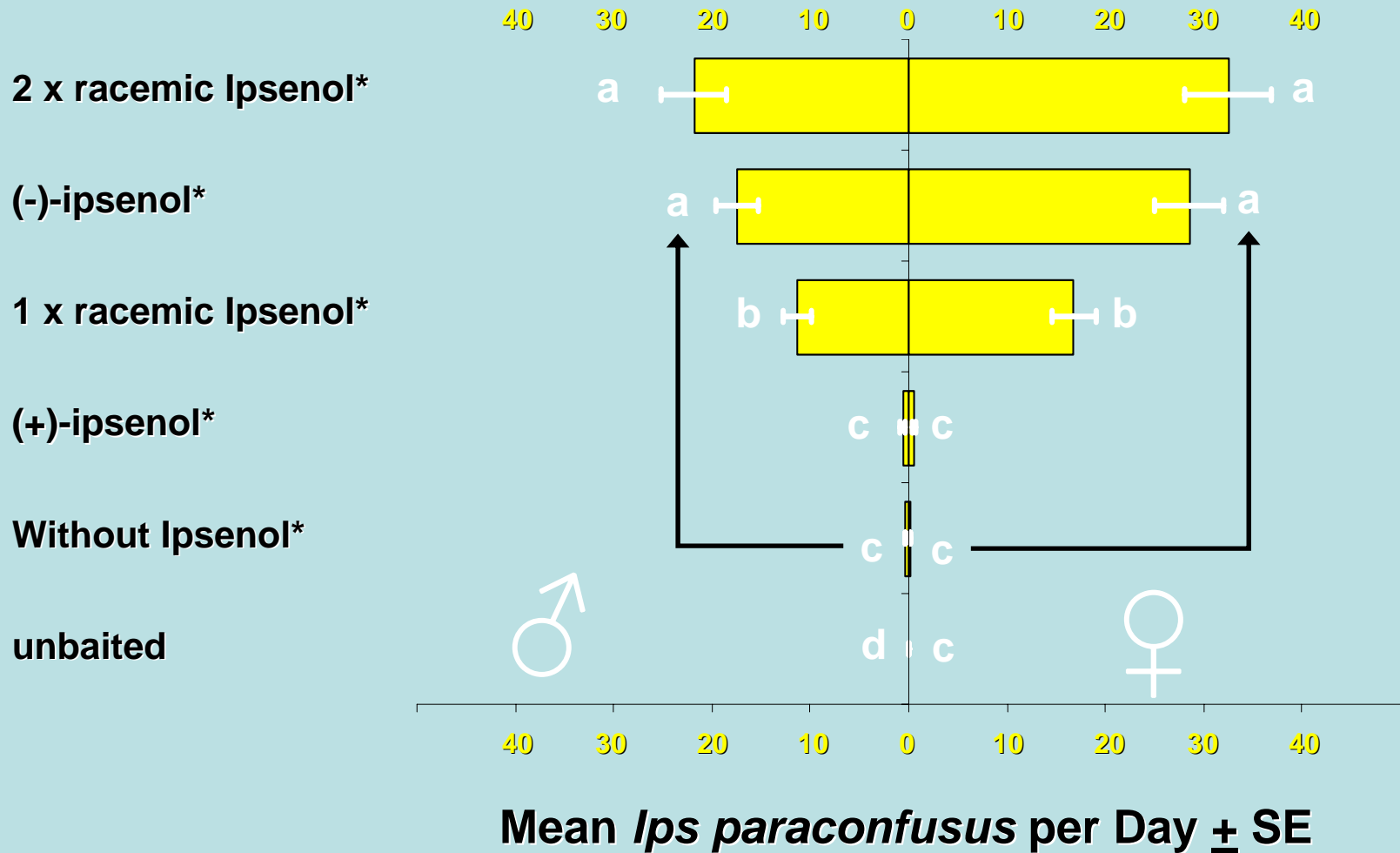


Treatments

1. **Unbaited**
2. **83% (-)-*cis*-verbenol and (+)-ipsdienol** (without ipsenol)
3. **(-)-Ipsenol, 83% (-)-*cis*-verbenol and (+)-ipsdienol**
4. **(+)-Ipsenol, 83% (-)-*cis*-verbenol and (+)-ipsdienol**
5. **Racemic ipsenol, 83% (-)-*cis*-verbenol and (+)-ipsdienol**
6. **2x racemic ipsenol, 83% (-)-*cis*-verbenol and (+)-ipsdienol**

Study 3 – Collected 13 times from August 12, 2005 to September 19, 2005

Study 3



Males: Tx 0.0001, Blk 0.0001, Time 0.0001, Tx*time 0.0001
Females: Tx 0.0001, Blk 0.0001, Time 0.0001, Tx*time 0.0001

Study 3 Summary



- *Ips paraconfusus* is attracted to the blend with (-)-ipsenol
- They are not interrupted or attracted to (+)-ipsenol in any of the blends



Conclusions

| | | | |
|------------------------|----------------------|-----------------------------------|------------------------|
| Males produce | (+)-ipsdienol | (-)-<i>cis</i>-verbenol | (-)-ipsenol |
| Commercial bait | (+)-ipsdienol | 83%(-)-<i>cis</i>-verbenol | Racemic ipsenol |
| Field | Study 1 | Study 2 | Study 3 |
| Attracted to | (+)-ipsdienol | (-)-<i>cis</i>-verbenol | (-)-ipsenol |

Most attracted to compounds that males produce



Racemic blends are more economical, does the presence of the opposite enantiomer matter?

| Field | Study 1 | Study 2 | Study 3 |
|----------------------------|------------------------------------|---|--------------------------------|
| Attracted to | (+)-ipsdienol | (-)-<i>cis</i>- verbenol | (-)-ipsenol |
| Opposite enantiomer | (-)-ipsdienol inhibits | (+)-<i>cis</i>- verbenol neutral | (+)-ipsenol neutral |
| Conclusions | Must use (+)- ipsidenol | 83% (-)-<i>cis</i>- verbenol OK | Racemic ipsenol OK |
| \$ Pricing | | \$3 vs \$20 | \$3 vs \$40 |



Changes to commercial bait?

| | | | |
|--|---------------------------|--|---|
| Commercial bait | (+)- ipsdienol | 83%(-)-<i>cis</i>- verbenol | Racemic ipsenol (1X) |
| Conclusions | (+)- ipsdienol | 83%(-)-<i>cis</i>- verbenol | 2X rac. ipsenol caught twice as much as 1X |
| Modification to commer- cial bait | | | Add another rac. Ipsenol |
| \$ Pricing | \$46 | \$3 | \$3 + \$3 |

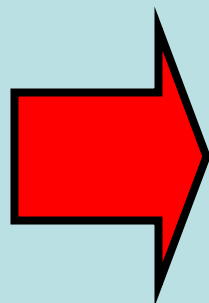


Changes to commercial bait?

(+) Ipsdienol

83% (-)-*cis*-Verbenol

Racemic ipsenol



(+) Ipsdienol

83% (-)-*cis*-Verbenol

2x Racemic ipsenol

Comparison of naturally occurring and commercial pheromone blends

Natural

| (+)-ipsdienol | (-)- <i>cis</i> -verbenol | (-)-ipsenol |
|---------------|---------------------------|-------------|
| 10 | 1 | 100 |

Commercial (Phero Tech)

| (+)-ipsdienol | 83%(-), 17%(+)- <i>cis</i> -verbenol | Racemic ipsenol 50%(-), 50% (+) |
|---------------|--------------------------------------|------------------------------------|
| 1 | 3 | 2 →4 |

Future Experiments

1. Optimize the ratios of the three components of the *Ips paraconfusus* aggregation pheromone
2. Repeat study 2, but with a racemic blend of *cis*-verbenol and all materials released at the same rate

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