Investigating the Chemical Signals of *Galerucella* spp. for Effective Biological Control of *Lythrum salicaria*

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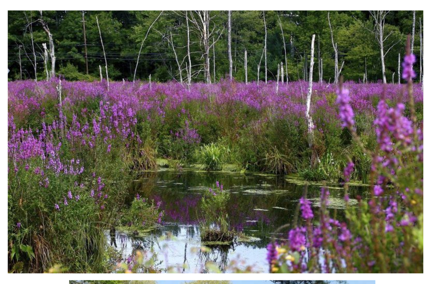
The invasive plant *Lythrum salicaria* has been a main contributor to the decline of wetland ecosystems across the North American continent. *Galerucella* spp. beetles *G. calmariensis* and *G. pusilla* are proven safe and effective biological control agents of this invasive plant; however, they are often limited to the plant colonies they are first introduced to. In order to best control the *L. salicaria* population, the mechanism which these beetles use to find new host plants needs to be better understood. · Part I of this study sought to test if *L. salicaria* emit a fragrance, presumably green leaf volatiles (GLVs), when damaged that *Galerucella* spp. beetles are attracted to in comparison to intact plant scents. · In part II, individual synthetic green leaf volatiles described in Bartelt 2008 were blended to determine if they are responsible for the behaviors observed in part I. Beetle responses to experimental conditions were observed in olfactometers, revealing an increased attraction to damaged versus intact *L. salicaria* and they were repelled by synthetic blends. This suggests the volatiles emitted from the damaged *L. salicaria* plant are independently able to assist *Galerucella* spp. beetles in host finding and demonstrates that understanding of *Galerucella* spp. beetles respond to individual plant signals is more complex than previously described. Further, mechanical damage to *L. salicaria* may enhance beetle colonization, but further development on GLVs effects is needed in order to be able to utilize synthetic blends.

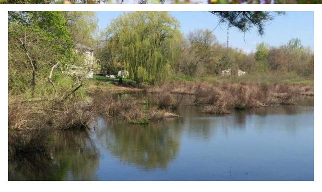
Introduction

Lythrum salicaria · Purple Loosestrife

- Flowering wetland plant invasive nonnative in North America
  - Found in all contiguous US states and Canadian Providences
  - Largest populations in New England
  - Found in all CT providences
- No native predators, hardy and prolific. Outcompetes native wetland plants like cattails
- Forms dense monotypic plant stands
- Unsuitable as cover, food, or nesting
- Overall decrease in biodiversity. Many rare/endangered species affected


Pristine indertundal wetland, Sandy Neck, Barnstable, MA © USFWS





Biological Control

- 4 species total
- 2 species: Leaf eating beetles *Galerucella calmariensis* and *pusilla*
- Extensively studied to show no further harm to native ecosystem
  - High specificity for feeding[7]
- In CT, 2 million insects released over 110 sites
- Able to skeletonize plant, slowing growth and spread[8]
- Low initial energy input for long-term effects
- Control not eradicate

Limitations:
- Host finding ineffective
- Previously believed to be largely based on sight and chance[5]
- New *L. salicaria* populations found every year

In order to Enhance the Spread of Pioneer beetles, this study sought to investigate possible Chemical Signals *L. salicaria* Expresses that *Galerucella* spp. beetles use to Identify Host Plants

Part I

Are *Galerucella* spp. beetles Attracted to Damaged *L. salicaria*?

- Evidence suggests beetle are sensitive to scents of *L. salicaria* when mechanically damaged[4], but can these scents independently attract them?
- Green Leaf Volatiles (GLVs) are expressed when a plant is damaged
  - Plant defense, Plant-Plant Communications, and Plant-Insect Interaction[6]
  - May recruit predators to protect the plant
    - Arthropod predators of *G. calmariensis* greatly reduce effectiveness of control on *L. salicaria* populations[9]

Part II

Are Previously Identified GLVs Responsible for Part I Response?

- 6 GLVs previously shown to be sensed by beetles' antennae[1]
  - No experimental studies conducted on beetle affinity
  - Preliminary data showed no attraction[1]

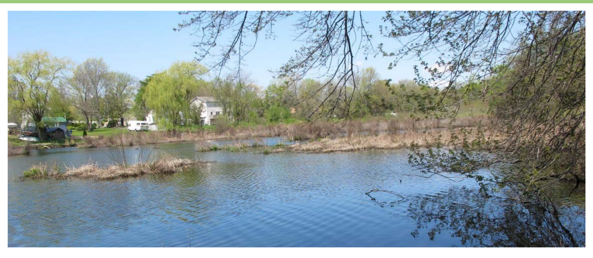
cis-3-hexenal, trans-2-Hexen-1-al, cis-3-Hexen-1-ol, trans-2-Hexen-1-ol, 1-Hexanol, and cis-3-Hexenyl acetate

- Each molecule's relative abundance and role in chemical signaling needs further investigation

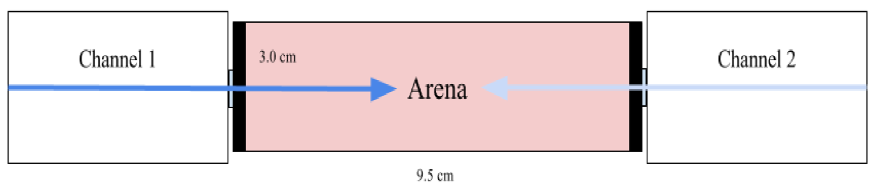
Methods and Materials

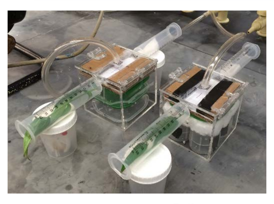
Plant and Beetle Collections

Plants grown in controlled greenhouse



Behavioral Studies





Experimental Treatments:
1. Damaged *L. salicaria* Plant Material versus 0.5 mL Mineral Oil
2. Intact *L. salicaria* Plant Material versus 0.5 mL Mineral Oil

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1. 0.5 mL Blend 1 versus 0.5 mL Mineral Oil
2. 0.5 mL Blend 2 versus 0.5 mL Mineral Oil
3. 0.5 mL Blend 2 versus Damaged *L. salicaria* Plant Material

Olfactometer:
Beetles placed in arena and exposed to two distinct scents

Blends:
1: 5x10-5 moles of chemicals, excluding trans structure chemicals, in 9.380 mL Mineral Oil
2: 5x10-5 moles of all chemicals in 9.135 mL Mineral Oil

Every minute for 30 minutes, the movements of the beetle in the olfactometer were observed and recorded

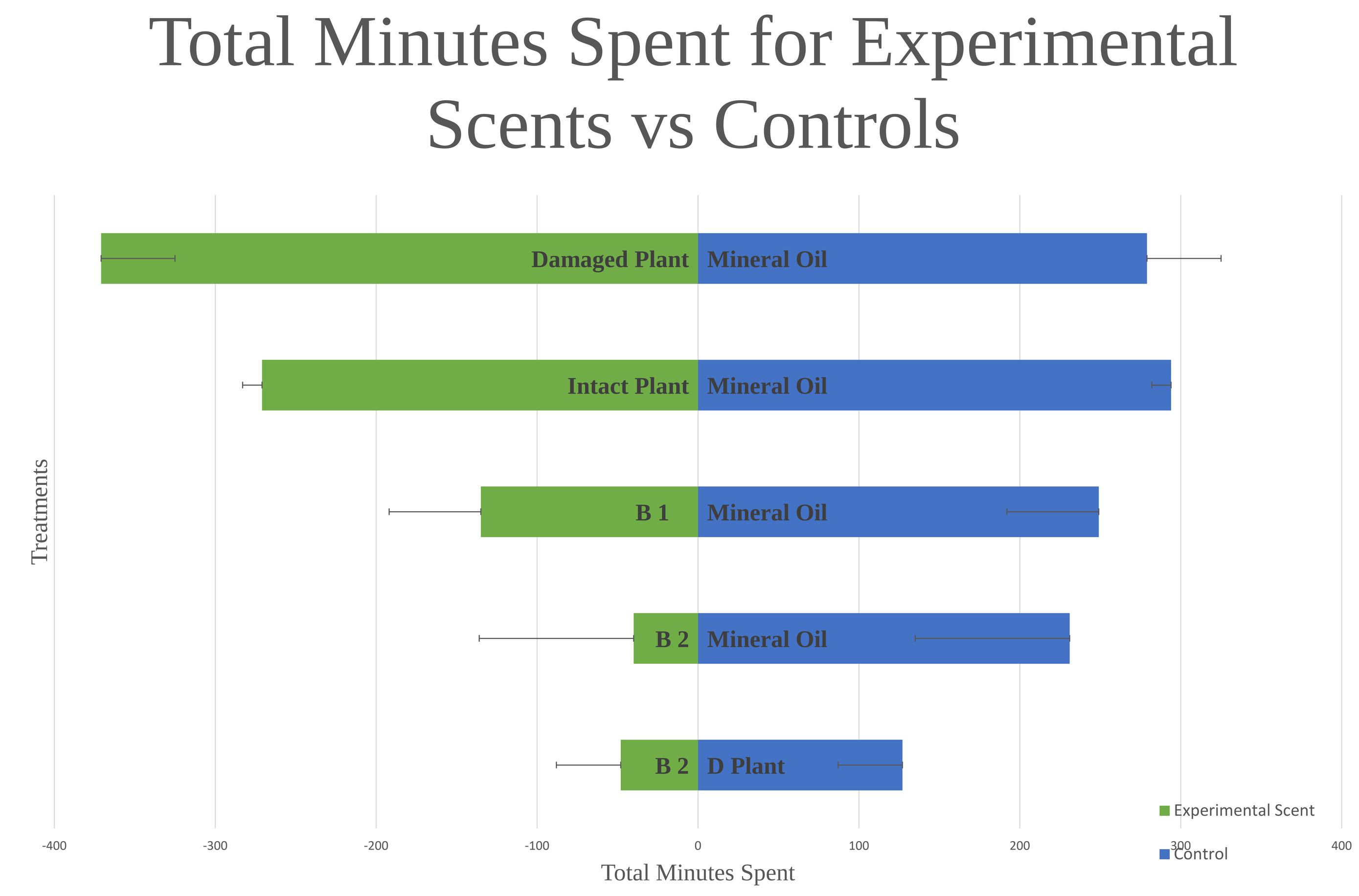
Time immediately started recording when the beetle was placed in the device
If the beetle was immobile for more than 5 minutes, they were reset to the center of the device
If they were immobile repeatedly, their data was discarded
If they were directly on the center line, whichever way their antennae were facing was recorded

Results

Part I: Beetles had Significant Attraction to Damaged Plant Scent
Part II: Beetles were Repelled by Synthetic Blends

Treatment	n	Total Minutes Observed			Percent Preference (%)	Preference Binomial Test (95% Confidence)	Chi Squared	
		Experimental Scent	Control	Total			Expected (50%)	p
1 Damaged Plant vs Mineral Oil Control	22	371	279	650	57	Significant	325	0.01
2 Intact Plant vs Mineral Oil Control	19	271	294	565	48	No Preference	283	0.5
3 Blend 1 vs Mineral Oil Control	11	135	249	384	35	Significant	192	<0.001
4 Blend 2 vs Mineral Oil Control	9	40	231	271	15	Significant	136	<0.001
5 Blend 2 vs Damaged Plant Control	6	48	127	175	27	Significant	88	<0.001

(*Figure 1*) Each beetle's movements was tracked for 30 minutes at one-minute intervals. For each treatment, the total times spent in the experimental scent and the control of all beetles tested was summed. The Preference-Binomial test determined if there was a significant preference for either scent. Chi-Squared test determined the probability that the preference was due to chance. p-value of <0.05 meant the preference was not due to chance



(Figure 2.) Error bars demonstrate the observed deviation from a 50:50 ratio. (2.1) Damaged Plant(left) vs Mineral Oil Control(right); deviation of 46 mins. (2.2) Intact Plant(left) vs Mineral Oil Control(right); deviation of 12 mins. (2.3) Blend 1(left) vs Mineral Oil Control(right); deviation of 57 mins. (2.4 Blend 2(left) vs Mineral Oil Control(right); deviation of 96 mins. (2.5) Blend 2(left) vs Damaged Plant(right); deviation of 40 mins.

Conclusions

- ***Galerucella* spp. have Adapted to Exploit Signaling Pathway of Damaged Host Plant for Detection**
  - Light mechanical damage to leaves might encourage beetle detection and retention
    - This would promote the spread of beetles to expanding *L. salicaria* populations
  - The composition of the scent and the way in which the beetles' respond to each of the various constituents must be understood
- **Each Chemical Plays a Unique Role in Signaling Their Relative Abundance may shape Beetles' Response**
  - Additionally, the repulsion to these blends suggests beetles use the signals of the damaged plant beyond locating the plant
    - Selection for less damaged plants
    - Arthropod avoidance
  - More research needed in order to utilize synthetic GLVs optimally
    - Relative abundance in natural scent
    - Individual effect on beetle
    - Arthropod response

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