AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: Analyzing Semiochemical Properties of a Noxious Weed and a Specialist Herbivore to Enhance Weed Control in Perennial Cropping Systems

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SUMMARY:
Field bindweed (*Convolvulus arvensis* L., FIG. 1) is difficult to manage in both dryland and irrigated systems. It causes dramatic yield loss, particularly in perennial crops ranging from caneberries to mint. Due to the regenerative capacity of field bindweed, chemical control is only marginally effective and successful management of this weed requires an integrated approach.


Figure 1 – Field bindweed can greatly reduce crop vigor and yields of blueberries, mint, and other prized perennial crops in Oregon.

Pheromone techniques have been used widely to monitor pest species, but there are less than a dozen published reports of pheromone monitoring for biological weed control agents. Over the past few years, our team has been field-testing the female-produced sex pheromone of *T. luctuosa* (bindweed moth), one of the two approved biocontrol agents for field bindweed. We have determined that the compound can be applied to synthetic lures and used to detect activity patterns of male bindweed moths from an established population. This method also has utility to provide estimates about the current range and dispersal capacity of the bindweed moth. However, in order to improve the efficiency of field bindweed biocontrol in agricultural settings, we need to be able to attract female moths.

Plant-insect research projects present a unique opportunity for collaboration across disciplines. That is, analytical chemists have the knowledge base and tools available to detect compounds, and biologists can propose studies that investigate some of these biologically-important compounds in terms of how they affect ecological processes. We know from the literature that plants produce ‘odors’ that are detected by pest insects, and that feeding by pests can initiate the plant to produce specific biochemicals called herbivore-induced plant volatiles (HIPVs).

These can in turn attract parasitoids and predators, thus effectively protecting the plant against further damage. Some insects use the host plant signals as cues for oviposition (egg-laying). In our case, we were interested in quantifying what, if any, volatile organic compounds (VOCs) are produced by field bindweed in order to better understand the defenses used by the weed and the trophic levels that affect biocontrol success.

OBJECTIVES:

1. Evaluate emission of plant volatiles produced by field bindweed that may be influential as host recognition cues for *T. luctuosa* moths.
2. Determine if male *T. luctuosa* moths produce pheromones to attract female moths, and if so, identify compounds that could be reproduced on synthetic lures.

PROCEDURES:

Patches of field bindweed (*Convolvulus arvensis*, COAR) were identified and 'treated' by exposing the weed to currently approved biological control agents. *Tyta luctuosa* (TYLU) is a small owlet moth, the larvae of which defoliate bindweed shoots. *Aceria malherbae* (ACMA) is a gall-forming mite that cannot be seen with the naked eye; the damage to leaf tissue is a consequence of the mite inhabiting the plant, and leads to reduced plant vigor over time. Uninfested plants with and without simulated mechanical damage were used as controls.

<u>OBJ. 1 METHOD A</u> - Direct headspace analysis of *in situ* field bindweed was conducted by placing actively growing plants into a glass collection chamber fitted to a negative pressure pump (FIG. 2). Strands of COAR with open blooms were tested separately from those without. Solid Phase Micro-extraction (SPME) fibers were exposed to each sample, allowing any compounds present to adhere to the fiber material, which was then desorbed by a Hewlett Packard 5973 gas chromatograph interfaced to an Agilent GC mass selective detector. Coupled gas-chromatography/mass-spectrometry (**GC-MS**) analysis was performed and peaks of interest were compared to a computerized library of constituent chemicals matching either the retention time, mass spectra, or both.

Figure 2 – Experimental setup to collect volatile compounds from bindweed. Insects use plant 'odors' as behavioral cues.

<u>OBJ. 1 METHOD B</u> – In year two, we were able collaborate with the Halsey Lab (OSU Microbiology Dept.) and explore another, more detailed form of analysis that provides on-line monitoring of volatile compounds by means of Proton-Transfer Reaction Mass Spectrometry (**PTR-TOF-MS**). This method is considered novel because it provides a way to visualize VOCs in real time and can yield better detection of low-molecular weight compounds.

OBJ. 2 - On three separate occasions, we received or collected *T. luctuosa* moths at various life stages, in an effort to rear useable moths for experimental lab trials. Unfortunately, none of the attempts were fruitful. Rearing of this insect has been a perpetual challenge, and therefore we have shifted our focus to testing wild populations in the field.

SIGNIFICANT ACCOMPLISHMENTS:

With OBJ. 1, Method A, we successfully sampled field bindweed as it was actively growing in a field. This is important because VOC profiles can change when plant tissues are damaged, so it was imperative to reliably collect field samples. The specific collection method we employed is common in the environmental chemistry and hazmat sector, but to our knowledge, has never been used to analyze an invasive weed. We were able to improve on the originally proposed methods, and reduce contact and transferring of samples, thereby reducing risks of contamination or loss of compounds.

The new collaboration we established with the Halsey lab (OBJ. 1, Method B) will be very beneficial if we move forward with this project. By using PTR-MS, it is possible to visually observe processes as they happen, thus reducing error in sample collection and data analysis. The analytical equipment is highly sensitive, and in fact, the specific model we worked on is one of only 7 machines worldwide. The Halsey lab research technician was excellent to work with, and was equally excited about cross-disciplinary collaboration.

Although we were unable to produce male moths for OBJ. 2., it should be noted that a member of our research team has recently identified male-produced compounds from another species of moth (Choi et al., 2016 J. Chem. Eco. v.42), and his findings will contribute to our future investigations. Volatiles produced by male Lepidoptera are often related to both the biology of the host plant, and the female sex pheromone. Dr. Choi's discovery marks the first time a noctuid moth female sex pheromone has been isolated from legs of conspecific males.

Both analytical methods were successful, and each has different strengths (see above). We hope to be able to continue this project, to better discern the effects of mechanical damage, herbivory, prior exposure to biocontrol agents, etc. The preliminary findings are encouraging, and suggest that the tested methodologies are suitable for qualitatively identifying VOC production of plant-insect systems.

Ecologically-significant findings of specific semiochemical compounds are listed in Table 1 and the text below. At this phase, we are not specifically identifying the compounds, in order to preserve the integrity of the intellectual property.

> Terpenes – TRP1 represents the monoterpenes, which can only be distinguished as a group. They were present when bindweed had been fed on by *T. luctuosa* caterpillars (FIG. 3). Monoterpenes are known to elicit antennal responses from many other moth species, and are sometimes included in pheromone blends to make sampling more effective.

SLX1 and 2 – The siloxane signatures are likely contaminates of the sampling process, because the SPME fibers that were used are coated with a type of siloxane, although heptasiloxane, a related structure, is a confirmed phytochemical. Future efforts with different SPME fibers could further delineate this finding.

OH1 - This compound was present at a low level in both shoot and root tissue of the plant. It is also a known component of the female bindweed moth's pheromone (TYLU). Other species of moths use plant compounds as part of their pheromone signature, but this relationship has never before been proven for TYLU.

OH5 – is known to be a wound-response signal in plants. It was detected only via PTR-MS, and increased in magnitude when plant tissue was exposed to herbivory by either agent or mechanical damage. The fact that is was detected in unexposed samples (Table 1, COAR), is because stem tissue was clipped at soil level prior to analysis. That is, it was 'wounded', but not by herbivores.

TRP2 and OH2 – are other wound-response products and exhibited the same pattern as OH5. TRP2 is a precursor to compounds that are thought to provide protection against high temperatures and other stressors. Not all plant species produce it, and at this time, we have found only one other documented case of Convolvulaceae production.

Aromatics (ARO1-3) – This related group of compounds were identified in both types of sampling. They are known as kairomones (signals that are detected by a different species, and usually harm the producer). In this case, the emission of ARO2 in response to feeding by TYLU caterpillars (FIG. 3) could be considered an infochemical, either for TYLU moths or its predators.

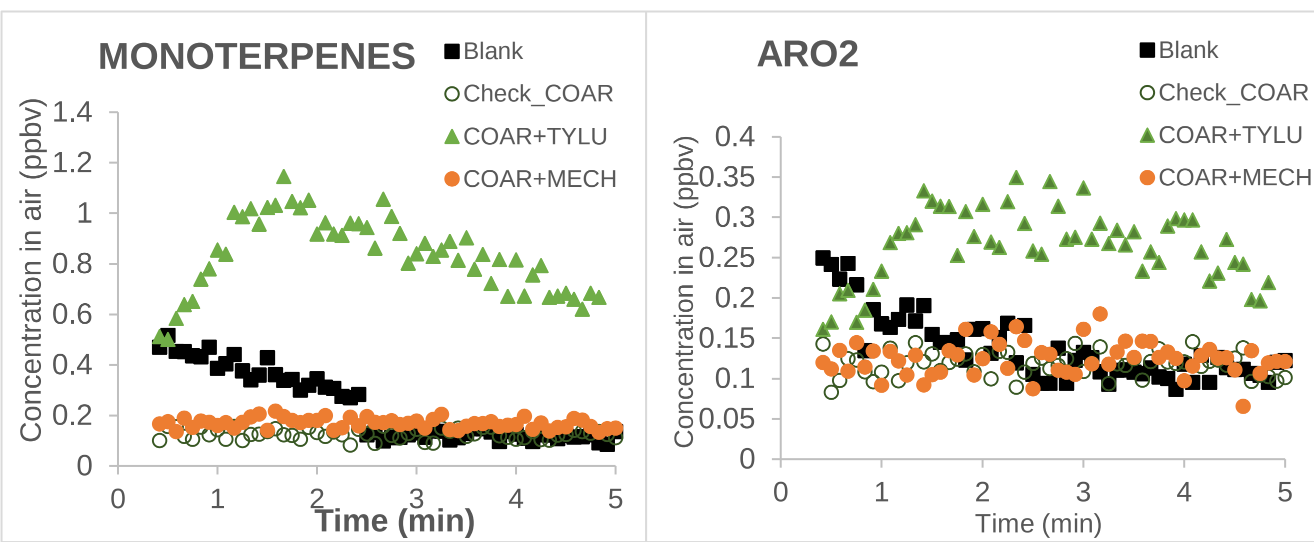

Figure 3 – Monoterpenes and one specific aromatic compound were detected from field bindweed plant tissue (COAR) that had been damaged by T. luctuosa caterpillars (TYLU), but not mechanical damage (MECH) nor mites.

Table *1* - Compounds of interest as identified by SPME/GC-MS (◊) or PTOF-MS (→ = present; ↑ = increased response). COAR=*C. arvensis*, TYLU=*T. luctuosa*, ACMA=*A. malherbae* , mech = mechanical damage (non-insect).

Group	Identifier	COAR				TYLU		ACMA	
		bloom	no bloom	root	mech.	lure	on COAR	on COAR	COAR + ACMA year prior
Terpenes	TRP1						↑		
	TRP2				↑		↑	↑	↑
Sulfides	SLF1		→		→		→	↑	↑
Siloxanes	SLX1	◊	◊						
	SLX2	◊	◊			◊	◊		
other Aliphatic	OH1			→					→
(ALD, OH, esters)	OH2		→		↑		↑	↑	↑
	OH3								→
	OH4	◊	◊						
	OH5		→		↑		↑	↑	↑
Aromatic	ARO1	◊				◊			
	ARO2	◊					◊, ↑		
	ARO3	◊	◊			◊	◊		

BENEFITS & IMPACT:

The data gleaned from this project have provided proof of concept that field bindweed does indeed have unique compounds that are detectable in the headspace of the plant. Of these, at least two can be detected by insects, and could lead to behavioral changes such as increased oviposition. We feel that the discoveries made from this project will have direct and applied use for field bindweed management. If, for instance, we can replicate host-plant chemicals, we may be able to focus herbivory of the biocontrol agents, which would improve their efficacy from a management standpoint.

Overall, there is a renewed interest in biocontrol of bindweed because of our work. We have worked with stakeholders of various crops (mint, blueberries, raspberries, vegetables), all of whom are interested in incorporating biocontrol, if we could make it compatible with their current weed management plan. We continue to be contacted by county agencies from across Oregon, small farmers, and homeowners who would like more information on this system.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: None

FUTURE FUNDING POSSIBILITIES: Our research team will be applying for an OREI/Organic Transition grant (USDA-NIFA), FY2017, application in progress. Farms transitioning to organic production methods are plagued by field bindweed, and would likely benefit from an integrated weed management tactic such as biocontrol.