FINAL REPORT
WTFRC PROJECT # AE-03-333A

TITLE:	Assessing the Chemical Release Behavior of Mating Disruption Products in Orchard Air
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COLLABORATORS:	Vincent P. Jones, Associate Entomologist, Jay F. Brunner, Entomologist, Mike Doeer, Research Scientist WSU-TFREC

Mating disruption has become an important integrated pest management tool for controlling codling moth injury in apple and pear orchards in the Pacific Northwest. The mating can be interrupted, or at least delayed, when there is a sufficient amount of pheromone present in the orchard air interfering with the male's capability to locate females. Unfortunately, in certain areas suppression in mating through chemical disruption appears to have diminished and may be attributable to the declining seasonal use of organophosphorus insecticides that complement mating disruption (Jones, 2002). Additionally, commercially available hand-held dispensers and sprayable formulations may not provide for efficacious pheromone release (i.e., either too much pheromone released too soon or too little release over extended time intervals) leading to insufficient season-long suppression of moth mating (Brunner, 2002a). Although the number of sprayable applications and/or number of hand-applied dispensers per acre for season-long control is being studied (Brunner, 2002b), the chemical concentration in orchard air that results in mating disruption has not been thoroughly investigated. Indirect techniques such as 1) gravimetric measurement 2) volatile trapping of vapors under laboratory chamber conditions, or 3) residual analysis of field-aged dispensers provide some notion of pheromone release but can not be interpreted as useful information on air concentration. Direct measurements of ambient pheromone concentrations in orchard air will be critically important for comparing different formulations and systems (sprayable or hand-applied) of pheromone application. The ability to measure pheromone concentration in the ambient air can provide a mechanism to more directly relate pheromone delivery system release to pest activity and crop injury.

2003 PROPOSAL OBJECTIVES:

1. Develop sensitive analytical approaches for directly measuring pheromone release from commercially available codling moth mating disruption systems
2. Measure codlemone [(*trans,trans*)8,10 Dodecadien-1-ol] concentrations in orchard air at Isomate C+ dispenser application rates of 100, 200, 400, and 800 dispensers/acre
3. Provide air concentration data that can be used in combination with dispenser release data for assessing the performance and efficacy of mating disruption products.

SIGNIFICANT FINDINGS:

- Capability to detect codlemone to the mid-picogram (trillionth of a gram) per cubic meter of air after high volume air sampling with gas chromatographic (GC) mass spectroscopy
- Codlemone found to oxidize readily under atmospheric conditions but was stable when trapped on the air-sampling medium
- Demonstrated in the orchard environment that airborne codlemone concentrations could be measured at application rates as low as 100 dispensers/Acre
- We observed sequentially higher codlemone concentrations in the orchard air environment after 100, 200, and 400 dispenser/A applications

- The highest rate of application (800 dispenser/A) did not result in increased codlemone concentrations in the orchard air
- High air temperatures in late July 2003 appeared to have a significant influence on chemical release leading to higher ambient orchard concentrations, more so than increasing application rates
- Chemical breakthrough lowered PUF trapping efficiency especially on hot days; therefore the generated air data should be viewed as lower bound estimates.

MATERIALS AND METHODS

Objective 1:

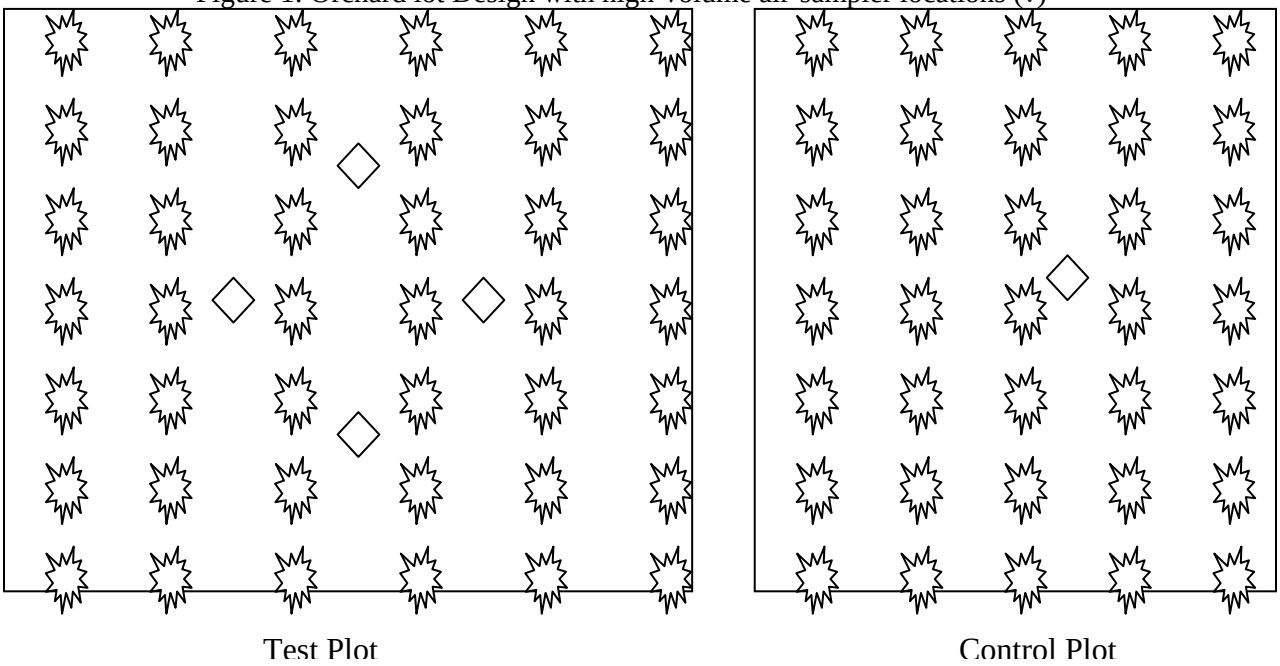
Instrument sensitivity: To quantify codlemone residues in air, original air sampling, extraction and instrumentational methods had to be developed for detecting this pheromone to the mid-to-high pigogram per cubic meter range. We first evaluated the capability of our recently acquired GC/MS in detecting and quantifying codlemone at very low concentrations. This instrument was purchased with specialized accessories that could increase the sensitivity for substances like codlemone.
Trapping efficiency: A series of experiments first were then performed to identify an efficient trapping adsorbent that could remove codlemone from air at very high (> 150 L/min) air-sampling rates. We tested two adsorbents XAD (a polystyrene resin) and PUF (a polyurethane foam).
Codlemone reactivity: Before attempting orchard evaluations, we conducted a series of specialized laboratory experiments at the University of Nevada to measure the rate of loss of codlemone under sunlight conditions and in air. These experiments were essential for understanding the atmospheric environmental fate of this highly reactive substance.
Analytical Method: The following analytical method was developed and validated before orchard air sampling:

1. Place the exposed PUF sample into a hot solvent extractor for a period of 3-hours using hexane as the extraction solvent
2. Reduce the volume of the solvent to near dryness then bring up to final volume in hexane for GC/MS determination.
3. Add the internal standard (50 µg/ml myristic acid methyl ester solution in hexane) to the final volume of the sample.
4. Analyze the extract by GC/MS using SIM (single ion monitoring mode) tuned to ions m/z 68, 81, 182 for codlemone and ions m/z 87, 143, 242 for myristic acid methyl ester.
5. For each set of samples ran on the GC/MS, at least one spiked sample was prepared by injecting a known volume of codlemone solution into the PUF. The spiked samples were extracted and analyzed in the same manner as the unknowns and control samples. Reagent blank, PUF blank samples were also routinely analyzed during the course of the study.

Objective 2: The following orchard air-sampling procedures were performed at the WSU TFREC orchards to measure airborne codlemone from Isomate C+ dispensers at various application rates:

Test Plot Design: A treatment (Block 18) and control orchard plot were established at the WSU TFREC. Enough physical distance separated the treatment from control plot to minimize possible cross-boundary source contamination (Figure 1). Four high volume air samplers were situated within close proximity to each other (ca. 50-75 feet) near the center of the treatment plot. One air sampler was placed near the center of the control plot.

Figure 1: Orchard lot Design with high-volume air-sampler locations (◊)



Chemical Treatment Program: Four application rates were sequentially performed at ca. 1-week intervals. The first 100 dispensers per acre treatment were applied to Block 18 on 7/11/03. On 7/18/03, the second series of 100 dispensers were applied (total 200 dispensers per acre). On 7/25/03, an additional 200 dispensers were applied (400 dispensers per acre) and finally on 8/1/03 an additional 400 dispensers were added to Block 18 (total 800 dispensers per acre). All dispensers used in this orchard program were aged outdoors for 1-week prior to application to minimize the pulse release effect that is usually observed for Isomate dispensers.

Air Sampling: High volume Anderson air samplers were employed to extract codlemone from orchard air. Four replicate samplers were positioned at ground level in the orchard test plot and 1 sampler in the control orchard plot (Figure 1). After each weekly dispenser application, air samples were collected three times, usually at daily intervals, before the next dispenser application was performed.

For each air-sampling event, a polyurethane foam (PUF) cartridge was inserted into the air sampler and calibrated by TFREC personnel according to standard methods. The high-volume air samplers ran for a period of ca. 5-6 hours at an average air extraction rate of ca. 140-L min^{-1} (i.e., average air mass sampled ≈ ca. 46 m^3). The exposed PUF cartridges will be immediately placed in cold storage and subsequently delivered to WSU's Food and Environmental Quality Laboratory for codlemone determination.

Field Data Book: A field data book was constructed that provides the information needed to document and construct all phases of field sampling, cold storage, shipment, and chain of custody.

Meteorological Information: Meteorological data was collected over the experimental time frame at the WSU, TFREC weather station.

Chemical Analyses: All analyses were performed according to the methods listed in Objective 1.

RESULTS AND DISCUSSION:

Objective 1:

Instrument sensitivity: Our preliminary GC/MS findings were highly encouraging. We could reliably quantify pigrogram (part per trillion) range concentrations of codlemone. In other words, the instrument could be set up in a sensitive and selective mode to pull the needle out of a haystack.

Trapping efficiency: The next question to resolve was; could a trapping medium effectively pull codlemone out of the air when using our air-sampling equipment? In our evaluations, the XAD polystyrene adsorbent provided lower recoveries (ca 65%) than PUF (ca 95%). As a result, we then chose to evaluate the breakthrough capability of PUF to retain codlemone in the adsorbent over a 3-hour period at a high volume air-sampling rate of over 200-L/min. The recovery of codlemone spiked onto the PUF was quantitative (ca 105%) over this air-sampling interval thus demonstrating the capability for this adsorbent to both efficiently remove codlemone from the air and retain this substance on the trap over extended air-sampling intervals.

Codlemone reactivity: How fast does codlemone photoreact or oxidize in air after it is released from the dispenser? We found in our investigations at the University of Nevada that airborne codlemone was surprisingly stable under atmospheric sunlight conditions but oxidized rapidly (half-life ≈ 0.5 hours) in purified filtered air. From a chemical ecology perspective, fast reactivity is highly desirable, but the fast air oxidation triggered the need to examine its behavior when trapped on PUF. Experiments were then conducted at the FEQL to compare recoveries of codlemone when using a non-oxidizing inert gas (nitrogen) and when using air. This series of experiments showed that there was no appreciable difference in recovery. In other words, codlemone should not further oxidize when trapped on the PUF over the orchard air-sampling time frame.

Analytical Method: Preliminary field air-sampling studies conducted at the FEQL demonstrated that detectable residues could be observed at concentrations equivalent to ca 200 picograms per cubic meter of air. Conservatively, we set our limit for reliably quantitating codlemone in air at 1 nanogram (1 part per billion mass) per cubic meter or air. With this level of sensitivity, we anticipated that we should be able to detect/quantify airborne release of codlemone from hand-applied dispensers in the orchard environment at rates as low as 100 dispensers per acre.

Objective 2: Table 1 and Figure 2 presents the results from the Block 18 dispenser evaluations.

Orchard Data Summary:

100 dispenser codlemone air data: Results from the averaged three air sampling dates taken 3, 5, and 7 days following the first 100-dispenser/A application showed fairly consistent codlemone air concentration behavior ranging from 1 to 1.5 nanograms per cubic meter of air. Of the 12 sampling observations, there was one replicate sample failure. The averaged mid-day air temperature for these three sampling events was 29.4 $\pm$ 2 C.

200 dispenser codlemone air data: Results from the averaged three air sampling dates taken 4, 5, and 6 days following the second 100-dispenser/A application showed less consistent codlemone air concentration behavior that ranged from 1.1 to 2.3 nanograms per cubic meter of air. Of the 12 sampling observations, there was also one replicate sample failure. The averaged mid-day air temperature for these three sampling events was 36.1 $\pm$ 2 C.

400 dispenser codlemone air data: Results from the averaged three air sampling dates taken 3, 4, and 5 days following the third application by inserting 200 dispensers/A showed reasonably consistent codlemone air concentration behavior ranging from 6.7 to 8.7 nanograms per cubic meter of air. Of the 12 sampling observations, there no replicate sample failures. The averaged mid-day air temperature for these three sampling events was 38.3 $\pm$ 0.6 C.

800 dispenser codlemone air data: Results from the averaged three air sampling dates taken 3, 4, and 11 days following the third application by inserting an additional 400 dispensers/A showed somewhat variable codlemone air concentration behavior ranging from 3.8 to 4.9 nanograms per cubic meter of air. Of the 12 sampling observations, there no replicate sample failures. The averaged mid-day air

temperature for these three sampling events was 29.6 ± 1.4 C, much cooler than the 400 dispenser air sampling period.

There was not a direct linear relationship between increased numbers of dispensers and measured airborne codlemone, although sequentially higher air concentrations in the orchard air environment were observed after 100, 200, and 400 dispenser/A applications. The highest rate of application (800 dispenser/A) did not result in increased codlemone concentrations in the orchard air. This was not a surprising observation since the very high late July air temperatures likely had a significant influence on chemical release on the 400 dispenser/acre application rate leading to very high ambient orchard concentrations of codlemone. Although the analytical method proved sensitive and reliable (laboratory spikes average recoveries 91 ± 8% ; n = 12), we did experience chemical breakthrough in the air samples, especially on the hotter days in July. Field spike recoveries ran near the end of the field study were ca. 40% of expected. As a result, the collected air data cannot be entirely viewed as absolutely quantitative due to less than desirable trapping efficiency on hot days. The generated air codlemone data, however, should be viewed as a lower bound residue estimate of actual orchard air concentrations at the various application rates.

REFERENCES:

- Brunner, J. and M. Doerr. 2002a. 2001 Pheromone dispenser analysis. WSU Tree Fruit Research and Extension Center, Wenatchee, WA. Unpublished report.
- Brunner, J. and P. Landolt, 2002b. Evaluation of sprayable pheromone and attract & kill for codling moth and leafroller control. WSU Project # 4093. *2002 Apple Entomology Review*. Washington Tree Fruit Research Commission.
- Jones, W. 2002. *Pest Management Practices Survey 2000 Report*. WSU Tree Fruit Research and Extension Center. Wenatchee, WA. http://opus.tfrec.wsu.edu/~wjones/Survey2000/.

BUDGET:

Project title: **Chemical Release Behavior of Mating Disruption Products**
PI: **Vince Hebert**
Project duration: **2003**

Salaries		
Ag Res Tech I		
(This will be a new half time position)		
April – September 2003		**$6,795**
Wages		
Time slip @ $10/hour, 800 hours		**$8,000**
Benefits		
Ag Res Tech I @ 57%	3873	
Time Slip @ 16%	1,280	
	========	
		$5,153
Goods & Services		
High volume air samplers, 3 @ $2300		**$14,900**
Solvents, gases, glassware, reagents		
Travel		
12 trips to Wenatchee @ 240 miles R/T		**$994**
TOTAL		**$35,842**

Table 1: Codlemone measured concentrations in orchard air

Air Sample ID	Date of Sampling	Averaged Codlemone Concentration (ng/m^3 ± SD)*	Mid-day air temperature (C)****
100 dispensers/acre			
07-14-03 T1 to T4	07-14-03	1.1 ± 0.20	28
07-16-03 T1 to T4**	07-16-03	1.5 ± 0.20	29
07-17-03 T1 to T4	07-17-03	1.1 ± 0.09	31
200 dispensers/acre			
07-22-03 T2 to T4	07-22-03	2.3 ± 0.75	38
07-23-03 T2 to T4	07-23-03	2.0 ± 0.45	36
07-24-03 T1 to T4***	07-24-03	1.1 ± 0.22	34
400 dispensers/acre			
07-28-03 T1 to T4	07-28-03	7.7 ± 2.0	38
07-29-03 T1 to T4	07-29-03	8.8 ± 1.4	38
07-30-03 T1 to T4	07-30-03	6.8 ± 2.6	39
800 dispensers/acre			
08-04-03 T1 to T4	08-04-03	3.8 ± 2.0	29
08-05-03 T1 to T4	08-05-03	4.9 ± 1.4	31
08-12-03 T1 to T4	08-12-03	4.7 ± 0.6	28

* The average concentration and standard deviation taken from 4 replicate air samples on that date

** Sampler 07-16-03 T1 failed and was not included in the averaged air concentration

*** Sampler 07-24-03-T3 failed and was not included in the averaged air concentration

**** Temperature reading at 2 pm on each of the air sampling dates

Figure 2: Codlemone measured air concentrations