ATTRACTANTS FOR FEMALE ORIENTAL FRUIT MOTH FROM SHOOT AND FRUIT ODORS

PROJECT LEADERS: Jocelyn Millar
COOPERATORS: Walt Bentley, Kent Daane, Alex Il'ichev

OBJECTIVES

1. To identify attractants for female OFM from peach shoots and ripening peach fruits.
2. To identify attractants for female OFM from their preferred host, quince.
3. To test ethylene as a possible attractant for OFM females.

PROGRESS TO THE END OF DECEMBER, 2004

1. Insects

A colony of OFM was started at UC Riverside and is now under stable continuous rearing. Female moths from this colony are used:

a. as a source of live antennae for conducting coupled gas chromatography-electroantennogram experiments to determine the compounds in peach, quince, and peach shoot extracts to which the females' antennae are most sensitive (see below);
b. as a source of test animals for conducting bioassays.

2. Collection, analysis, and identification of peach and quince odors

Methods were worked out for collection of peach shoot and fruit volatiles in the laboratory, and in situ from peach shoots on living trees in orchards, which should provide the best possible mimic of the suite of shoot odors that ovipositing female moths would encounter under natural conditions in orchards. Briefly, in the field, the growing peach shoot is gently enclosed in a glass tube, and the ends are loosely plugged with cotton batting to allow air and moisture exchange. A solid phase microextraction (SPME) device is inserted inside the tube, within a couple of millimeters of the shoot tip, to collect volatiles as they are released from the shoot tip. Similar methods have been adapted for working with cut shoots in the laboratory; the shoot is placed in a flask of water, with the top enclosed in a glass tube as described above, and an SPME device is used to collect the volatiles. For collection of volatiles from green peach and quince fruits, the green fruits were placed in a clean glass chamber and the SPME device was inserted through a port at the top of the chamber. The loaded SPME devices were thermally desorbed directly into the injection port of a gas chromatograph.

To date, the following replicated sets of analyses have been carried out, analyzing extracts by both gas chromatography-electroantennogram detection (GC-EAD) and GC-mass spectrometry. The first of these techniques produces a direct "map" showing the compounds in the extracts to which the antennae are most sensitive, i.e., the compounds that are most likely to be involved in host finding. Having located the peaks in the extracts that elicit the largest responses from the antennae, GC-MS was then used to identify most of those compounds.

1. Peach shoots at bloom. Peach shoots were cut from an experimental block maintained at Kearney Ag. Center on March 13, transported back to UC Riverside, and used for collections the next day. Extracts were analyzed only by GC-MS because we did not yet have the OFM colony in place to provide antennae for GC-EAD.
2. Peach shoot volatiles were collected directly from undamaged growing shoots on an experimental block of peach trees maintained by Jim Adaskaveg on the UCR campus. Collections were continued for a number of days in May, looking at production of volatiles during day and night independently.
3. Unripe peach fruit volatiles were sampled in July, using green peaches collected from the UCR experimental block.
4. Quince volatiles were sampled in July, using quince fruit sent to UCR by Walt Bentley.
5. Ripening peach volatiles were sampled from fruit collected from the UCR experimental block during the first week of August.
6. Peach shoot volatiles were sampled directly from trees in the UCR experimental block as described in 2 above, during the 2nd and 3rd week of September, after the fruit had been picked.

A typical GC-EAD analysis is shown in Figure 1. These analyses have provided us with a series of "snapshots" of the odors of peach shoot volatiles as they change through the season, and a comparison of the odors of green and ripening fruit. We found substantial differences between the major components of the shoot extracts throughout the season, and between green fruit and ripe fruit. Representative results from these analyses are listed in Tables 1 and 2.

The shoot odors from the March shoots were dominated by E-ß-farnesene, with much smaller amounts of a number of other sesquiterpenes, and C16-C18 hydrocarbons. In contrast, the volatiles collected from shoots later in the season were dominated by E,E-α-farnesene, with E-ß-farnesene now only present as a minor component.

It was not possible to analyze the March shoot samples by GC-EAD because the colony of OFM had not yet been established. However, GC-EAD analyses of the May and later samples showed that female OFM antennae responded to a number of components in the extracts. Furthermore, antennal responses were clearly not correlated with the quantities of compounds present: the antennae responded strongly to some trace components, whereas weak or no responses were obtained to some of the most abundant components of the extracts (e.g., Figure 1, Table 1). For example, female antennae responded strongly to methyl salicylate, cis-jasmone, and two as yet unidentified trace components in the volatiles collected from shoots in May.

The volatiles collected from unripe green peaches were dominated by a series of straight-chain hydrocarbons (Table 2). In contrast, the volatiles from ripening yellow peaches exhibited most

of the same hydrocarbons, but in addition, contained a number of typical odor compounds associated with peaches, including esters, lactones and aldehydes (Table 2). Most of these typical peach odor compounds elicited responses from moth antennae, as did a number of the hydrocarbons.

The collection and analyses of volatiles from fruits and shoots was halted in the fall when the growing season ended, and we began assembling a library of the identified compounds from commercial sources and from synthesis, and we are now ready to begin lab bioassays. Because the overall number of compounds is quite large, we plan to focus on 4 samples: shoot volatiles from March and May, and volatiles from green and ripening fruit. We have built a prototype bioassay apparatus, based on the recently reported results of Natale et a1. (2004). In particular, these authors tested a wind tunnel, a Y-tube olfactometer, and a two-choice chamber bioassay, and found that the latter was most effective. We have built a similar two-choice bioassay, consisting of a ten liter polycarbonate carboy with two holes cut in the bottom to accommodate treatment and control tubes, each fitted with an inward-pointing screen funnel at the attachment point to the carboy, and with the other end covered with screen. After the treatment and control stimuli are placed in their respective vials, 12 gravid female moths are placed in the body of the chamber, and air is pulled through the whole apparatus with a vacuum pump at 5 liters/min. The bioassay is run overnight (the moths oviposit during the dark period), and the numbers of moths in the treatment and control chambers are counted the next morning. In the first two trials with this prototype apparatus, female moths showed a preference for a reconstructed blend of the volatiles from March peach shoots.

Thus, our plan for the coming months is to build several more bioassay chambers, so that several replicates can be run simultaneously. This apparatus will then be used to test the activity of blends mimicking the odors of March and May shoots, and green and ripening peach fruits, in the laboratory. Any blends that appear to be significantly attractive to gravid female OFM will then be sent to collaborator Bentley for field trials, as soon as ovipositing OFM become active in the spring.

Table 1. Major components identified from shoot volatiles, and electroantennogram responses. Compounds are quantified as a percentage of the most abundant compound. In addition, several trace compounds (< 1% of major components) gave significant EAG responses.

Compound	**% in March Shoot volatiles**	**% in May shoot volatiles**	**EAD Active?***
Unknown	-	7.31	
α-copaene	1.49	-	
sesquiterpene	~2	-	
ß-caryophyllene	~2	-	
geranylacetone	-	2.6	
z-ß-farnesene?	3.11	-	
E-ß-farnesene	**100**	5.96	
A-caryophyllene	1.23	-	
sesquiterpene	1.12	-	
sesquiterpene	1.21	-	
Z,E-α-farnesene	-	-	
C15	11.28	-	
E,E-α-farnesene	4.11	**100**	Yes
bisabolene	1.68	-	
nerolidol		6.6	
trimethyltridecatetraene		4.52	Yes
hexadecene	2.27	-	
C16	1.49	-	
heptadecene	0.8	-	
C17	1.58	-	
unknown		4.8	
Benzyl benzoate		3.34	
C18	1.54	-	
6,10,14-triME pentadecanone	1.67	8.06	
C23		11.44	
C25		15.34	

* EAG data were not obtained for the March peach shoot volatiles data.

Table 2. Major components identified from shoot volatiles, and some electroantennogram responses. Compounds are quantified as a percentage of the most abundant compound. In addition, several trace compounds (< 1% of major components) gave significant EAG responses.

Compound	*RT GC-MS*	*% in green peach volatiles*	*% in yellow peach volatiles*	*EAD Active?*
Ethyl octanoate	10.28	-	8.4	yes
Z4-ethyl decenoate	13.01		15.5	yes
Bergamotene (?)	13.86	4.87		
5-hexyldihydrofuranone	14.33		14.6	yes
C15	14.66	88.77	43.3	
Delta-decalactone	14.70		3.8	yes
E,E-α-farnesene	14.77	0.78		yes
Octanoate esters	15.65		19.0	yes
Octanoate ester	15.72		8.3	yes
C16	15.87	4.55	2.1	
Unknown	15.94	99.3		
heptadecadiene	16.76	2.85	3.9	yes
heptadecene	16.84	6.46	3.5	
Gamma-dodecalactone	16.97		5.8	yes
C17	17.08	85.2	**100**	yes
Ethyl tetradecanoate	18.17		1	yes
C18	18.23		1.1	yes
Hexadecanal	18.46		7.0	yes
6,10,14-triME pentadecanone	18.77	7.44		
C19	19.32	28.37	63.3	yes
octadecanal	20.63		16.1	yes
C21	21.36	38.6	17.6	yes
Eicosanal	22.61		4.6	yes
C23	23.36	**100**	5.5	yes

Figure 1. Coupled gas chromatogram-electroantennogram (GC-EAD) of a peach shoot tip extract. The top trace is the GC trace showing all the compounds in the extract, separated on the basis of their size and chemical characteristics. The bottom trace is the matching trace of the responses of a live antenna of a female moth to each of those compounds as it comes off the GC.