



Forest Genetics Council Seed Pest Management Program

Project Report: Reports are due no later than March 31, 2002.

Name of Project Leader Jocelyn G. Millar	For Official Use Only
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Name of Organization:
Dept of Entomology, University of California

SPM Project #

Mailing Address: *(Please fill the following section where there have been changes.)*
Dept of Entomology, University of California, Riverside CA 92521, USA

Telephone:
1 909 787 5821

Fax:
1 909 787 3086

E-Mail Address:
Jocelyn.millar@ucr.edu

Financial Officer: Margaret Fehn

Project Title: *Copy from application*
Identification of an effective sex pheromone lure for the fir coneworm, *Dioryctria abietivorella*, and demonstration of its efficacy in seed orchards.

Approved Project Funding: \$27,000 CAN

Forest Genetics Council Seed Pest Management Project Report

Objectives:

1. To reexamine the pheromone blend of this species using coupled gas chromatography-electroantennogram detection to analyze extracts and locate possible missing components.
2. To develop methods of obtaining pheromone components of very high chemical and stereoisomeric purity.
3. To formulate and load possible pheromone blends for field testing by project collaborators in Canada and northern California.

Results:

Briefly (1 or 2 pages maximum) describe the results of the project and how they have met the needs and objectives. Where the procedure employed or results are different explain why they differ and how they impact on the objectives of the project, include significant changes in projected dates for completing specific activities, and team members.

1. GC-electroantennogram detection studies on pheromone extracts.

Live moths were reared out of shipments of infested cones from northern California. Virgin female moths were used for preparation of crude pheromone extracts, whereas antennae from male moths were used as living detectors in electroantennogram studies. Multiply

replicated pheromone extracts were prepared by a) dissection and solvent extraction of pheromone glands from calling female moths, b) collection of pheromone onto an adsorbent as the pheromone was released by calling females, and c) by wiping the extruded pheromone glands of both calling and non-calling females with a solid phase microextraction fiber (SPME). All these extracts were analyzed by coupled gas chromatography-electroantennogram detection, with candidate pheromone compounds identified by comparisons of retention times on polar (DB-WAX) and nonpolar (DB-5) GC columns with those of standards. The electroantennogram detector, which was several orders of magnitude more sensitive than the flame ionization detector of the GC, consistently detected only two compounds in the extracts: Z9,E11-14:Ac, and trace amounts of either Z9- or E9-14:Ac. The GC usually was able to detect the Z9,E11-14:Ac in extracts, but the second component was always below the limits of detection for the FID detector. Although the GC detector did detect a number of other compounds in the various extracts, none of them elicited antennal responses. Thus, at first glance, the evidence from GC-EAD studies indicates that the pheromone should only consist of one or possibly two components.

However, it is still possible that there may be other components to the pheromone, for the following reasons. First, the EAD peaks are relatively broad, and it is difficult to determine whether a response is due to a single compound, or to two or more components, such as isomers, that have very similar GC retention times. If there was a trace of a second component that eluted close to the major component, it would be difficult to detect it, because the amount would be too small to register on the GC. Nevertheless, the possibilities for a trace component are very limited, because very few compounds would have structures so similar to that of the major component that they could not be well separated on one or both of the GC columns used for conducting the GC-EAD analyses.

Second, there are cases known whereby some lepidopteran pheromone components give weak EAD responses because the insect's antenna contains only a relatively small number of receptors tuned to those components. Thus, there could be another trace component that our equipment has not yet picked up. As soon as more moths are available, we will continue these studies, using concentrated extracts from a number of female moths, in the hopes of detecting a minor component that has up till now escaped detection

2. Purification of pheromone components.

One possible reason that the reconstructed pheromone blend has worked poorly may lie with the purity of the synthetic pheromone components that we and others have used to prepare trial blends. Specifically, it is well known that the attraction of male moths to pheromone lures can be strongly antagonized by the presence of small amounts of inhibitors, to the extent that trap captures drop essentially to zero. These inhibitors are frequently isomers or analogs of the pheromone components, and are used in nature to minimize cross-attraction between species that have one or more pheromone components in common. Thus, the chemical and isomeric purity of synthetic pheromones can be of the utmost importance, but it can also be remarkably difficult to achieve.

We have pursued two main routes to attack this problem, specifically focusing on the purification of the major component of the pheromone, Z9,E11-14:Ac. First, we have purified the technical grade pheromone on custom-made medium- and high pressure liquid chromatography columns packed with silica gel impregnated with silver ions, which form transitory complexes of different strengths with cis and trans double bonds. With both columns,

we were able to produce Z9,E11-14:Ac and Z9-14:Ac of >99% chemical and isomeric purity. These materials were used to formulate lures tested in the 2003 field season.

However, these chromatographic methods are very expensive and tedious to use, because it is only possible to separate a few milligrams of purified pheromone per hour, and they use considerable volumes of solvent. Consequently, we have also invested a major effort into trying to develop a method for purification of Z9,E11-14Ac and similar compounds, based on low-temperature recrystallization of derivatives. This method is widely used in industry, and has numerous advantages over chromatographic separations. In particular, this method can be used to purify compounds in quantities ranging from a fraction of a gram to many tons, in one batch. Thus, we have experimented with formation of a wide variety of derivatives, in the hopes of forming one which was suitably crystalline. These attempts have included:

1. Low temperature crystallization of Z9,E11-14:Ac, or the corresponding alcohol or acid. These compounds did not crystallize from either polar or nonpolar solvents at temperatures that were readily accessible (-20°C or higher).
2. Formation of naphthyl, biphenyl, 3,5-dinitrobenzyl, and other esters of Z9,E11-14:OH. None of these derivatives crystallized from either polar or nonpolar solvents.
3. Formation of the bisulphite adduct of Z9,E11-14:Ald, followed by attempted recrystallization from water-alcohol mixtures. Whereas the adduct did precipitate out as an amorphous powder, the powder proved to be almost as impure as the starting material.
4. Formation of amine salts of the Z9,E11-14:COOH. The resulting amine salts precipitated out of solution as oils rather than forming crystalline complexes.

Despite the discouraging results to date, we are persisting with these efforts, because if successful, it will provide a method of obtaining pheromone of very high purity in multigram quantities, in one simple batch process. Furthermore, if a method can be developed, it should be broadly applicable to a wide variety of lepidopteran pheromones, thus eliminating a substantial technical and cost bottleneck that hinders the continued development of a number of pheromones.

3. Formulation and loading of lures.

Several sets of lures were loaded with test pheromone blends prepared from both purified and nonpurified synthetic pheromone components. These lures were tested by project collaborators at field sites in northern California, British Columbia, and Quebec. The results of field trials are discussed in the report of co-PI Gary Grant. From the low overall trap catches, it is clear that the reconstructed pheromone blends are not yet correct.



Output and Deliverables:

List the specific products produced and where available (i.e. reports and information). If they differ from the proposed outputs or deliverables indicate how and why. Where reports are part of the product please include a copy.

Financial:

Please indicate if there are differences between the actual and the approved expenditures and why. Indicate the impact if any on the project. Financial reporting is done through the Government General Ledger. However it is the responsibility of the project leaders to ensure that the General Ledger and the project expenditures coincide.

Employment:

Signature Block:

Name (Project Leader): **Jocelyn G. Millar**

Signature: _____



Forest Genetics Council Seed Pest Management Program

2001/2002 Financial (maximum one page)

Note: Information in this section will be routinely and publicly released

	Quarter				Total Year 1
	1 st	2 nd	3 rd	4 th	
a) Salaries and benefits					
b) Equipment					
c) Travel					
d) Materials & supplies					
e) Other expenses (attach list)					
f) Administrative Costs					
TOTALS					