Forest Genetics Council Seed Pest Management Program

Project Report, to April 1, 2006:

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Project Title:

Identification of an effective sex pheromone lure for the fir coneworm, *Dioryctria abietivorella,* and demonstration of its efficacy in seed orchards

Approved project funding: $20,555 US

Forest Genetics Council Seed Pest Management Project Report

Objectives:

1. To develop short and efficient syntheses of the components of the fir coneworm pheromone.
2. With project collaborators, to conduct all field trials required to optimise the attractiveness of the pheromone, including blend ratio and dose trials.
3. To test and develop release devices and formulations to provide lures with effective field lifetimes of a minimum of one month.
4. To transfer the technology, including optimised protocols for use of pheromone traps, and methods of synthesis of the pheromone, to end users and pheromone supply companies respectively.
5. To supply several hundred nonoptimised lures for use by British Columbia foresters in spring-summer of 2005.

Results:

By the end of the 2004 field season, we had determined that there were at least two key components to the fir coneworm pheromone, Z9,E11-14:Ac and 3Z,6Z,9Z,12Z,15Z-pentacosapentaene (henceforth pentaene). Thus, our goals for the 2005 field season were to work out all the details required for use of pheromone traps under typical field conditions, in preparation for transfer of the technology to pheromone companies so that traps and lures can become standard commercial products available to forest managers as needed.

Objective 1. To develop short and efficient syntheses of the components of the fir coneworm pheromone.

The first component of the pheromone, Z9,E11-14:Ac, was in theory available from Bedoukian Research, Danbury CT. However, a sample purchased from Bedoukian turned out to be a mixture of isomers. Consequently, we developed our own highly stereoselective synthesis of this compound (manusc. In prep) for use in all our field trials. Similarly, we have developed a synthesis of the pentaene component from commercially available 3Z,6Z,9Z,12Z,15Z-icosapentaenoic acid methyl ester, and this synthesis is amenable to scaling up to produce hundred-gram or larger quantities by pheromone companies (see below).

Objective 2. With project collaborators, to conduct all field trials required to optimise the attractiveness of the pheromone, including blend ratio and dose trials.
Objective 3. To test and develop release devices and formulations to provide lures with effective field lifetimes of a minimum of one month.

Field trials were conducted by project collaborators Ward Strong (B.C. Ministry of Forests) near Vernon, B.C., and secondarily by Carline Rudolph (US Forest Service) near Chico, California. In late summer, sets of lures were also sent to collaborator Richard Trudel in Quebec, for testing against eastern North American populations of the moth. Finally, lures were sent to Dan Miller (US Forest Service) for testing against the congeneric species Dioryctria ebelli in Florida, to assess whether the pentaene component in particular might form part of the pheromone blend of other pest Dioryctria species. The following sets of field trials were carried out, with the results shown:

Trial #	Object of trial	Results of trial
Pretrial 1	Detection of first moth flight	Lures containing a blend of Z9,E11-14:Ac and pentaene (1:5) in the best ratio from 2004 were deployed to detect biofix, so that formal experiments could be started
Trial 1	Test ratios of Z9,E11-14:Ac and pentaene from 1:0 to 1: 100	Ratios of Z9,E11-14:Ac and pentaene of 1:10 to 1:33 were most attractive. Z9,E11-14:Ac as a single component was not attractive
Trial 2	Hold amount of pentaene fixed at 2 mg, vary amount of Z9,E11-14:Ac from 0 to 1 mg	Ratios of 0.1: 2 mg and 0.33: 2 mg Z9,E11-14:Ac and pentaene were most attractive. Attraction dropped with 1:2 mg ratio.
Trial #	**Object of trial**	**Results of trial**

Trial 3	Test plastic capsules as release devices, using ratios of Z9,E11-14:Ac and pentaene from 10:2000 to 1000:2000 micrograms	Plastic capsules worked quite well as release devices, attracting moths for ~ 2 months. The 100:2000 microgram ratio attracted the most moths.
Trial 4	Test base blend of Z9,E11-14:Ac and pentaene (200:2000) with various possible minor components	None of possible minor components tested increased attraction in comparison to the base blend.
Trial 5	Test base blend with variable amounts of Z9-14:Ac added (0.33 to 10% of Z9,E11-14:Ac amount)	Z9-14:Ac at rates tested did not increase attraction in comparison to base blend
Trial 6	Test base blend with variable amounts of Z9-14:Ac added (0 to 0.33% of Z9,E11-14:Ac amount)	Z9-14:Ac at rates tested did not increase attraction in comparison to base blend
Trial 7	Test effects of UV stabilizers and antioxidants in the lure f	There were no clear effects of a UV stablizer and/or an antioxidant on trap catches.

Further experiments were done by project collaborators Ward Strong and Gary Grant to test different trap types. The results of those experiments are described in their reports. Furthermore, experiments parallel to those described above were carried out by project collaborator Carline Rudolph, in seed orchards near Chico, California, with very similar results. Thus, Rudolph's results served to both corroborate the findings from B.C., and strongly suggested that the Dioryctria populations in both B.C. and California are essentially the same, at least in terms of their responses to pheromone formulations.

Overall, several conclusions can be drawn from these experiments.

1. The optimum ratio of Z9,E11-14:Ac and pentaene appears to be about 100:2000 µg, released from grey rubber septa or plastic capsules. Slightly higher trap catches may be obtained with higher doses, but the increased expenditure of the rather expensive pheromone components probably is not justified by the relatively small increase in trap catches with higher doses.
2. We were not able to demonstrate any consistent effect from addition of small proportions of possible minor components of the pheromone to the base blend of Z9,E11-14:Ac and pentaene. In particular, Z9-14:Ac, which does show up in extracts of the insect's pheromone gland, had no consistent effect in ratios from 0.01 to 10% of the amount of Z9,E11-14:Ac.
3. Plastic capsules appear to hold promise as release devices, attracting moths for up to 2 months in the field.
4. During the project, experiments were often deployed for periods of several weeks, and continued to catch moths. Although not a formal test of lure longevity, this does suggest that the grey rubber septum formulations of the lures last for at least several weeks under field conditions. Ward Strong will have further information on lure longevity from the results of the survey traps that he and collaborators deployed in different areas of the province.
5. Very surprisingly, the presence or absence of a UV stabilizer and an antioxidant in the lure formulation had no major effect on trap catches. However, given the sensitivity of the pheromone structures to light and air, particularly the pentaene, we strongly recommend including antioxidizers and UV stabilizers in any lure formulations: they

certainly can do no harm, and they may do some good, and they are cheap and easy to add to formulations.

Objective 4. To transfer the technology, including optimised protocols for use of pheromone traps, and methods of synthesis of the pheromone, to end users and pheromone supply companies respectively.

There are a number of published syntheses of the first component of the pheromone, Z9,E11-14:Ac, and it is available from two companies, Bedoukian Research and Advanced Synthesis Technologies. However, based on our experience with commercial material, it will be essential to check the chemical and isomeric purity of the material supplied before using it, because we found a commercial sample of synthetic material to consist of a mixture of isomers. Furthermore, any purchase contracts for this material should contain explicit language stating minimal acceptable chemical and isomeric purities, and these should be independently checked before payment is made.

When we discovered it, the second component of the pheromone, the pentaene, was new to science. We have now found it in two lepidopteran species, and we have developed a short synthesis of the pentaene from a commercial precursor. This synthesis has now been scaled up to syntheses of several hundred grams by a contract chemical synthesis chemist (Professor Michael Chong, Waterloo University).

Since 2005, we have also been assisting a pheromone products company (Advanced Pheromone Technologies, Marylhurst, Oregon) in their development of commercial lures, including supplying them with pheromone samples for comparison with their own lures.

Objective 5. To supply several hundred nonoptimised lures for use by British Columbia foresters in spring-summer of 2005.

To assist in ongoing efforts to monitor flights of Dioryctria abietivorella throughout the province, several hundred lures containing the best blend from the 2004 trials were shipped to project collaborator Ward Strong in March, 2005. A further 500 lures will be sent to collaborator Strong by midApril of this year 2006.

Output and Deliverables:

We have identified, synthesized, and fully tested the critical components of the pheromone blend of the fir coneworm. Furthermore, we have worked out the optimised doses and blend ratio for field use, and obtained estimates of field longevity of lures. In parallel studies, Ward Strong has determined the best type of traps to use. The technology is being transferred to a pheromone products company, and lures for this species should soon be readily available for use by forestry personnel.

The first publication on this pheromone has been published as a rapid communication (Millar, J.G., G.G. Grant, J.S. McElfresh, W. Strong, C. Rudolph, J.D. Stein, and J.A. Moreira. 2005. (3Z,6Z,9Z,12Z,15Z)-Pentacosaene, a Key Pheromone Component of the Fir Coneworm Moth, *Dioryctria abietivorella*. J. Chem. Ecology, rapid communication published online May9/05; print version vol. 31:1229-1234), and a full publication, with full data on field trials, is in preparation .

Financial:

No changes to budget or expenditures.

Employment: Project required part time services of one senior staff research associate and one postdoctoral chemist.

Signature Block:

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

Signature:_____

2005-6 Financial

	Totals, 2005-6
a) Salaries and benefits	21,599
b) Equipment	
c) Travel	
d) Materials & supplies	1,452
e) Other expenses (publication costs)	605
f) Administrative Costs	1,183
TOTALS	**$24,839 CAN = $20,555 US**