

**PHEROMONE-BASED TRAPPING AND LIFE HISTORY OF THE FIR
CONEWORM, *Dioryctria abietivorella* (LEPIDOPTERA:PYRALIDAE)
IN BRITISH COLUMBIA**

by

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B.Sc. (Biochemistry), University of Victoria, 1981

**THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF PEST MANAGEMENT
in the Department
of
Biological Sciences**

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Degree: **Master of Pest Management**

Title of Thesis:

**PHEROMONE-BASED TRAPPING AND LIFE HISTORY OF THE FIR
CONEWORM, *Dioryctria abietivorella* (LEPIDOPTERA:PYRALIDAE),
IN BRITISH COLUMBIA**

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Title of Thesis/Project/Extended Essay

Pheromone based trapping and Life History
~~of the~~ of the Fir Cone worm, *Dioxyctria abietivorella* ♀
(Lepidoptera: Pyralidae) in British Columbia

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ABSTRACT

The Douglas-fir coneworm (DFCW), *Dioryctria abietivorella* (Groté) is a major cone and seed pest of conifers in North America. Research objectives were to expand current life history knowledge, determine the major sex pheromone component and establish the seasonal flight pattern in coastal and interior British Columbia (BC). Coupled gas chromatographic-electroantennographic detection (GC-EAD) analyses of female DFCW pheromone gland extract revealed *Z9,E11*-tetradecadienyl acetate (*Z9,E11*-14:OAc) as the major candidate pheromone component. Traps baited with *Z9,E11*-14:OAc captured DFCW males and genitalia dissection confirmed their identity. Seasonal flight in interior BC commenced in the last week of May, peaked in June and July, and terminated mid August. In coastal BC, flight commenced one month later and terminated in the third week of September. Based on cone sampling and subsequent rearing, DFCW may overwinter as an egg, larva or pupa. Further research is needed to determine the potential for pheromone-based monitoring or control of the DFCW in Douglas-fir seed orchards.

DEDICATION

To my family for enduring my absences and the joyful welcomes on my return.

QUOTATION

*Research is Meaningless-Unless you act upon it. ... G. Geall (Acumen May/June 1995
Inside Guide Magazine Ltd., Toronto, Ontario)*

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1.0 INTRODUCTION

1.1 Seed Production in British Columbia

Seed production is the first step in growing seedlings for reforestation. Currently in British Columbia (BC) about 225,000,000 seedlings are planted annually. To produce the required seedlings, seed is collected from natural stands or from trees in seed orchards. Conifer seed orchards contain grafted, genetically selected even aged trees of a single species grown in evenly spaced rows with a grass cover similar to commercial tree fruits (Turgeon *et al.* 1994, Grant 1994). Orchard trees are induced chemically and/or culturally to produce an annual crop. In 1994, 16% of the seedlings grown in BC for reforestation were from seed orchard seed. By the year 2000, half of all planted seedlings will be grown from orchard seed (Barber 1993). Seven percent of these will be Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco. Currently, there are more than 80 managed orchards growing 12 conifer species (Bennett 1994) in the BC seed orchard program (Table 1).

There are 22 major and numerous minor insect and disease species which attack these 12 conifer species, seven to eight species can be pests on each conifer host (Hedlin 1974, Furniss and Carolin 1977, Miller and Ruth 1988, Bennett 1993). The Douglas-fir coneworm (DFCW), *Dioryctria abietivorella* (Groté) (Lepidoptera:Pyralidae) is a major pest of Douglas-fir cones, and can destroy 25-100% of natural stand seed in a single year (Kulhavy *et al.* 1976, Ruth *et al.* 1982, Mossler *et al.* 1992, Turgeon and de Groot 1992).

Table 1. 1993-(Interior BC) and 1994-(Coastal BC) production and approximated value of seed from BC seed orchards.

Location Common name <i>Species</i>	Number of Orchards	Annual Seed Production (Kg)	\$/Kg	Total Annual Value (\$ Thousands)
Coastal BC				
Coastal Douglas-fir <i>Pseudotsuga menziesii</i> <i>var. menziesii</i>	14	450.4	930	418
Western Hemlock <i>Tsuga heterophylla</i>	11	56.9	1725	98
Western Redcedar <i>Thuja plicata</i>	6	46.6	1680	78
Yellow-Cedar <i>Chamaecyparis</i> <i>nootkatensis</i>	3	4.3	3727	16
Sitka spruce <i>Picea sitchensis</i>	4	27.2	1005	27
Engelmann spruce <i>P. engelmannii</i>	1	5.1	900 ¹	4.5
White spruce <i>P. glauca</i>	2	0.4	828	0.3
Western white pine <i>Pinus monticola</i>	3	0	1125	0
Pacific silver fir <i>Abies amabilis</i>	2	0	300	0
Interior BC				
Interior Douglas-fir <i>Pseudotsuga menziesii</i> <i>var. glauca</i>	4	0	800 ¹	0
Interior spruce <i>P. glauca x engelmannii</i>	21	868.6	828	719
Lodgepole pine <i>P. contorta</i>	15	17.0	1838	31
Western larch <i>Larix occidentalis</i>	2	0	1890	0
Western white pine <i>P. monticola</i>	2	26.9	1125	30

Source: BC Ministry of Forests: Coastal Seed Orchards.

¹ Costs estimated using figures from a similar species.

Seed orchard crop management practices, particularly the unnatural induction of annual crops, can allow build-up of pest populations which utilize cones and seeds as a food source (Turgeon *et al.* 1994). In turn, seed orchards characteristically require pest management systems to maximize seed production (Turgeon and de Groot 1992, Turgeon *et al.* 1994).

1.2 Pest Management in Seed Production

Investigations of cone and seed insect biology and damage to conifer cones used for reforestation was begun in 1912 by the United States Department of Agriculture (USDA). This work concentrated on describing western American cone and seed insects and their parasites but was discontinued after five years (Keen 1958). Interest in the natural history of cone and seed insects resumed in the 1950's in the United States when DDT provided a cheap option for insect control (Keen 1958). Preliminary biological data collected earlier in the century provided the basis for damage assessment (Buffam 1965, Coulson and Franklin 1970a, 1970b, Sartor and Neel 1971, Dale and Schenk 1978), monitoring (Hedlin 1964, Dewey 1972, Schenk *et al.* 1972, Yates 1973a,b, Yates and Ebel 1975, DeBarr *et al.* 1975 Dale and Schenk 1978) and control work (Koerber *et al.* 1975, Miller 1979). Concurrently, French (Roques 1986), Scandinavian (Annala 1982) and Pakistani (Ghani and Cheema 1973) researchers also began documenting cone and seed insect damage. Publications began to appear on damage appraisal, sampling (DeMars 1975, Kozak 1964, Miller 1986, Krober *et al.* 1960) and chemical control (Haverty *et al.* 1986, DeBarr and Berisford 1983, Cameron and DeBarr 1989). Information on the "recognition, biology and

importance” of seed destroying insects in Canada, Mexico and USA was compiled into a single volume by Hedlin *et al.* (1980).

In 1982, an international Cone and Seed Working Party was established as part of the International Union of Forestry Research Organizations (IUFRO). Integrated control in seed orchards is documented in its proceedings. Turgeon and de Groot (1992), Turgeon *et al.* (1994) and de Groot *et al.* (1994) recently summarized the seed orchard pests and the status of pest management in Canadian seed orchards. In the last decade, an ecological approach to orchard pest management evolved with the investigation of host-insect interactions and attractive semiochemicals (Turgeon *et al.* 1994).

Seed orchards in BC have benefited from the development of integrated pest management programs since 1980 (Miller 1979, 1985, Bennett 1993, Turgeon and de Groot 1994). Sanitation, fertilization and water management comprise the cultural controls for minimizing pest infestations. Pre-harvest monitoring of cone and seed insects includes conelet sampling in western redcedar, interior spruce, and Douglas-fir for dipteran and lepidopteran eggs during the spring pollination period. Sampling is destructive, time consuming and does not detect summer feeding pests such as coneworms, *Dioryctria* spp. and seed wasps, *Megastigmus* spp. (Hedlin 1974, Turgeon *et al.* 1994). After spring sampling, cones are not sampled again for pest damage until after harvest, in part due to staff reductions in summer and lack of sampling methodology. Therefore summer infestations are detected after damage has occurred.

1.3 *Dioryctria abietivorella* as a Seed Production Pest

The DFCW is a pest of maturing Douglas-fir cones from June to September (Hedlin 1974, Ruth 1980, Turgeon and de Groot 1994). It is one of six major cone and seed insect pests of Douglas-fir (Hedlin 1974, Ruth 1980, Bennett 1993) and often occurs in association with the Douglas-fir cone moth, *Barbara colfaxiana* (Kearfott) (Keen 1958, Hedlin 1974, Ruth 1980, Miller and Ruth 1988, Smith 1992). Damage is "sporadic but intense" (Johnson and Hedlin 1967). Larval feeding on cones begins during pollination, peaks when cones are half grown and continues until cones are fully mature. Coarse frass without resin and some webbing on the outside of cones indicate infestations by fir coneworms (Keen 1958, Hedlin 1974, Hedlin *et al.* 1980, Ruth 1980). They can destroy up to 100% of the seeds in a cone (Keen 1958, Hedlin 1974, Ruth 1980, Miller 1985).

DFCW larvae feed on at least 20 species of conifers in six genera (Table 2) (Keen 1958, Furniss and Carolin 1977, Hedlin 1980, Miller and Ruth 1988, Turgeon and de Groot 1992, 1994). These include most of the commercially important species in BC. *Dioryctria* spp. are attracted to diseased or damaged host tissue (stem and branch wounds) on spruce and pine, and have been found on all parts of these hosts except roots.

No control or monitoring programs for *Dioryctria* spp. are currently in place in BC seed orchards because: 1) the life cycles are not well documented, making effective control strategies difficult to devise, 2) damage may be sporadic from year to year, and 3) most damage occurs in late summer when crops are maturing and orchard seed pests are not monitored because of low staffing levels and lack of sampling methodology.

A pheromone-based monitoring program could provide an effective means of monitoring for the DFCW (Figure 1). Numbers of captured DFCW males early in the season may indicate whether control measures are justified, and late in the season if there will be abundant developmental stages to overwinter and cause damage the subsequent year.

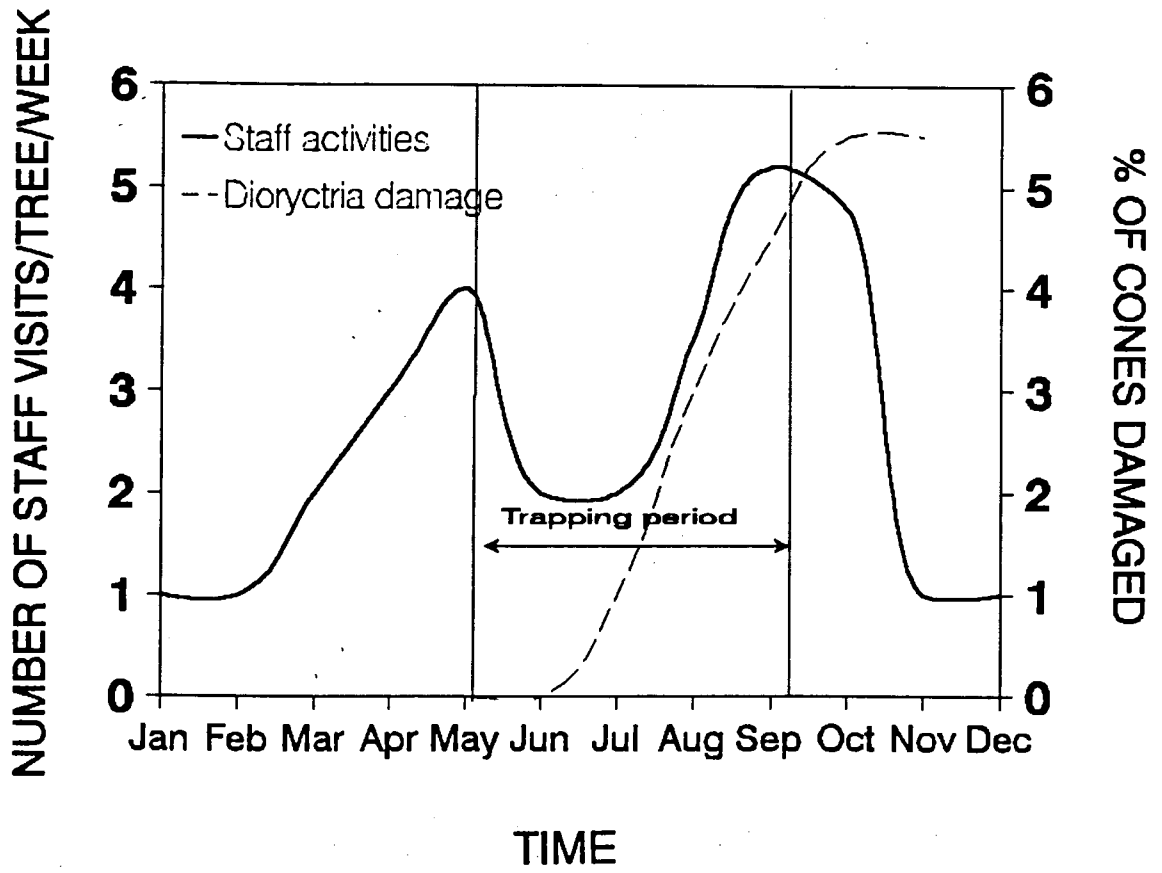
1.4 Biology of *D. abietivorella*

Although DFCW has been successfully reared in the laboratory (Ebel 1959, Fatzinger 1973, Trudel *et al.* 1995; G. G. Grant, Great Lakes Forestry Centre, Canadian Forest service, Sault Ste. Marie, Ontario, pers. comm.), its behaviour and life history are not well known (Figure 2) (Keen 1952, Hedlin 1974, Ruth 1980, Miller 1985, Miller and Ruth 1988, Mosseler *et al.* 1992, Turgeon and de Groot 1992, 1993). Larvae of all sizes are easily sampled and are often observed in the summer (Lyons 1957, D. R. Ruth, Pacific Forestry Centre, Canadian Forest Service, Victoria, BC, unpublished field notes 1960-1980). They are thought to leave the cones in the fall and pupate in the soil (Keen 1958, Hedlin 1974) or to overwinter as last instar larvae in cocoons on the ground (Keen 1958, Hedlin *et al.* 1980). Adults eclose in early summer (Keen 1958, Hedlin 1974). Oviposition and adult flight have not been observed in wild populations. Eggs laid during late summer may hatch the following spring (Keen 1958; Hedlin 1974). In the laboratory at 27°C eggs hatch in about 2-3 days, larvae pass through 5 or 6 instars at 25°C over 23 days and the pupal stage lasts 10-14 days (Trudel *et al.* 1995). DFCW adults are 20-30 mm long with grey-brown zigzag-lined forewings and dusty white unmarked hindwings (Keen 1958; Hedlin 1974, Hedlin *et al.* 1980, Ruth 1980).

Table 2. Hosts of *Dioryctria abietivorella*

Host Genus and Species	Structures Attacked	References
<i>Pinus</i>		
<i>contorta</i>	young pine trees,	Hedlin <i>et al.</i> 1980
<i>monticola</i>	cambium, rust galls,	Hedlin 1974
<i>flexilis</i>	graft unions, bole	Heinrich 1956
<i>banksiana</i>	injuries, twigs, cones	Turgeon & de Groot 1992
<i>resinosa</i>		Furniss & Carolin 1977
<i>ponderosa</i>		Evans 1982
<i>sylvestris</i>		Keen 1958
<i>Picea</i>		
<i>glauca x engelmannii</i>	cones, rusty cones	Furniss & Carolin 1977
<i>sitchensis</i>	cones previously attacked by other insects	Fidgen & Sweeney 1995
<i>engelmannii</i>		Turgeon & de Groot 1992, 1994
<i>rubens</i>		
<i>marina</i>		Ruth <i>et al.</i> 1982
<i>glauca</i>		Hedlin 1974
<i>Abies</i>		
<i>amabilis</i>	cones	Ross & Evans 1957
<i>grandis</i>		Hedlin 1974
<i>concolor</i>		Keen 1958
<i>lasiocarpa</i>		Furniss & Carolin 1977
<i>Pseudotsuga</i>		
<i>menziesii</i>	cones, twigs, buds, grafts	Ruth 1980, Hedlin 1974
<i>Tsuga</i>		
<i>heterophylla</i>	cones	Personal observation
<i>Larix</i>		
<i>occidentalis</i>	cones	Hedlin <i>et al.</i> 1980

Figure 1. Seasonal activities of BC seed orchard staff, incidence of *D. abietivorella* damage, and time period for potential pheromone-based monitoring of *D. abietivorella* adults.



Other *Dioryctria* spp. in BC are difficult to separate from DFCW based only on wing morphology. Species in the grey-brown colour group (Table 3) are reliably distinguished only by their genitalia. The nearctic and transcontinental DFCW (Hedlin *et al.* 1980) was distinguished on the basis of male genitalia from palearctic *D. abietella* by Monroe in 1959 (Fidgen and Sweeney 1995).

1.5 Pheromone-based Monitoring of *Dioryctria* spp.

Interest in pheromones of cone feeding *Dioryctria* began when Fatzinger (1971) and Asher (1970) reported pheromone-based mating behaviour in *D. abietella* (Denis & Schiffermüller). Fatzinger (1972) located the pheromone gland on female abdominal segments eight and nine. Males respond to pheromone gland extracts, but require other stimuli such as visual or auditory cues before mating occurs (Fatzinger and Asher 1971).

Verified or candidate pheromone components are now known for eight *Dioryctria* spp. (Table 4), and have been successfully field tested as monitoring tools for *D. amatella* (Hulst), *D. disclusa* Heinrich, *D. clarioralis* (Walker), *D. auranticella* (Groté), *D. reniculelloides* Mutuura and Munroe and *D. merkei* Mutuura and Munroe in pine orchards of the southeastern United States (Hanula *et al.* 1981, 1984a, 1984b, Cameron and Debarr 1989, Debarr and Berisford 1981, Mayer *et al.* 1982, 1984, 1986, Debarr *et al.* 1982, Pasek and Dix 1989, Grant *et al.* 1987, 1993) and for *D. resinosella* in red pine orchards in Wisconsin (Grant *et al.* 1993) (Table 4).

Figure 2. Current knowledge of *Dioryctria abietivorella* life history.

Month	J	F	M	A	M	J	J	A	S	O	N	D
Egg					?	?						
Larva	████████████████					?	██					
Pupa				?	?	?						
Adult						?	?	?				

Compiled from: Turgeon & de Groot (1994)

Table 3. *Dioryctria* spp. found in British Columbia.

Colour Group	Species	Common name, if any
grey-brown		
	<i>D. abietivorella</i> (Groté)	fir coneworm
	<i>D. cambiicola</i> (Dyar)	
	<i>D. contortella</i> Mutuura, Munroe & Ross	
	<i>D. monticolella</i> Mutuura, Munroe & Ross	
	<i>D. okanaganella</i> Mutuura, Munroe & Ross	
	<i>D. pentictonella</i> Mutuura, Munroe & Ross	-
	<i>D. pentictonella vancouverella</i> Mutuura, Munroe & Ross	
	<i>D. pseudotsugella</i> Munroe	
	<i>D. reniculelloides</i> Mutuura & Munroe	spruce coneworm
	<i>D. tumicolella</i> Mutuura, Munroe & Ross	
rusty-red		
	<i>D. auranticella</i> (Groté)	ponderosa pine coneworm
	<i>D. rossi</i> Munroe	-

Source: Reference Collection, Pacific Forestry Centre, Canadian Forest Service, Victoria, BC. (pers. observations)

Table 4. Field tested sex pheromone components or attractants of *Dioryctria* spp.

Species	Pheromone Component/Attractant	Reference
<i>D. abietella</i>	Z9,E11-14:OAc ¹	Löfstedt <i>et al.</i> 1983,1986
<i>D. amatella</i>	Z11-16:OAc ²	Meyer <i>et al.</i> 1986
<i>D. auranticella</i>	Z9-14:OAc ³	Pasek & Dix 1989
<i>D. clarioralis</i>	Z9-14:OAc; E9-14:OAc ⁴ ; Z11-16:OAc	Hanula <i>et al.</i> 1984 Meyer <i>et al.</i> 1984
<i>D. disclusa</i>	Z9-14:OAc	DeBarr <i>et al.</i> 1982 Hanula <i>et al.</i> 1984 Meyer <i>et al.</i> 1982
<i>D. merkei</i>	Z9-14:OAc; E9-14:OAc	Hanula <i>et al.</i> 1984 Meyer <i>et al.</i> 1982
<i>D. reniculelloides</i>	Z9-14:OAc; Z7-12:OAc ⁵	Grant <i>et al.</i> 1987
<i>D. resinosella</i>	Z9-14:OAc; Z9-14:OH, ⁶ E9-14:OAc; Z9-12:OAc ⁷	Grant <i>et al.</i> 1993

¹ Z9,E11-tetradecadienyl acetate; ² Z11-hexadecenyl acetate; ³ Z9-tetradecenyl acetate; ⁴ E9-tetradecenyl acetate; ⁵ Z7-dodecenyl acetate; ⁶ Z9-tetradecenol; ⁷ Z9-dodecenyl acetate

In BC pheromone-based monitoring of *Dioryctria* spp. would integrate well with current seed orchard practices. Pheromone baited traps provide simple, inexpensive, selective and user-friendly monitoring tools (Cameron 1981, Grant 1990, Debarr and Berisford 1981, Turgeon *et al.* 1994). If correlations could be established between DFCW trap catches and cone damage, pheromone-based monitoring would provide time to prepare and implement control measures. Traps would be monitored when seed orchard staff are limited and when cone sampling is impractical. Pheromone-based monitoring would be a much better tactic than light trapping which has been used to determine seasonal flight of *Dioryctria* spp. (Powers 1969, Yates 1973a, 1973b, Yates and Ebel 1975), but is not feasible for use in seed orchards because of the large numbers of non-target insects that are captured (Debarr and Berisford 1981).

1.6 Objectives

My objectives were to:

1. identify and field test candidate pheromone components for the DFCW,
2. determine through pheromone-based trapping the seasonal flight period of the DFCW in interior and coastal British Columbia,
3. monitor the incidence from June to October of DFCW larval instars and pupae, and
4. determine where the DFCW overwinters and at what developmental stage.

2.0 PHEROMONE IDENTIFICATION AND FIELD TESTING

2.1 Materials and Methods

In 1993, attempts to rear wild larvae collected in BC to the adult stage failed. Thus in October of 1994, laboratory-reared male (n=20) and female (n=24) DFCW pupae were obtained from Dr. G.G. Grant (Canadian Forest Service, Sault Ste. Marie, Ontario). One-half of the emergent adults were used in conducting a mating experiment and as voucher specimens for preservation. The remainder were sent to Regine Gries at Simon Fraser University for GC-EAD analysis.

Adults were aged for two days prior to pheromone analyses. Six hours into the scotophase, pheromone glands of eight calling virgin females were extracted in hexane for 5 min. Aliquots of one female equivalent of pheromone extract were subjected to coupled gas chromatographic-electroantennographic detection (GC-EAD) analyses (Arn *et al.* 1975) on three fused silica columns (30 m x 0.25 or 0.32 mm ID) coated with DB-5, DB-210 or DB-23 (J & W Scientific, Folsom, California).

E9, E11-Tetradecadienyl acetate (*E9,E11*-14:OAc) was purchased from Sigma Chemical Co. (St. Louis, MO 63178). *E9, Z11*-tetradecadienyl acetate (*E9,Z11*-14:OAc), *Z9, E11*-tetradecadienyl acetate (*Z9,E11*-14:OAc) and *Z9, Z11*-tetradecadienyl acetate (*Z9,Z11*-14:OAc) were obtained from Dr. Ezra Dunkelblum, Institute of Plant Protection, Volcani Center, Bet Dagan 50250, Israel. *E9, E12*-tetradecadienyl acetate (*E9, E12*-14:OAc), *Z9, E12*-tetradecadienyl acetate (*Z9, E12*-14:OAc), *E9, Z12*-tetradecadienyl acetate (*E9, Z12*-14:OAc), and *Z9, Z12*-tetradecadienyl acetate (*Z9, Z12*-14:OAc) were

purchased from the Research Institute for Plant Protection, Binnenhaven 17, Wageningen, the Netherlands. Z9-Tetradecenyl acetate (Z9-14:OAc) was obtained from Sigma Chemical Co. If indicated chemicals were purified by high performance liquid chromatography (HPLC) [Waters 625 LC equipped with a reverse phase Nova-Pak C₁₈ (3,9mm x 300mm column)]. Chemicals used experimentally were >97% chemically and >98% geometrically pure.

Field experiments were conducted from April 10 to October 31, 1994 at the Mt. Newton Seed Orchard (48°38'N, 123°25'W), near Victoria (coastal BC) and at the upper Ashnola River in Cathedral Provincial Park (49°13'N, 120°00'W) near Keremeos (interior BC) (Figure 3). Wing traps (Phero Tech Inc., Delta, BC) were deployed in randomized complete blocks. Treatments were randomly assigned to traps within each block. Traps were placed in a grid pattern with 50 m between blocks and 50 m between traps.

Traps were hung from Douglas-fir trees 4 m above ground (Figure 4) and baited with grey rubber septa (West Company, Phoenixville, PA) impregnated with candidate pheromone components in HPLC grade hexane. Trap tops and sticky bottom were spaced 5 cm apart (G.L.DeBarr, USDA, Forest Service, Athens, GA, pers. comm.). Traps were initially checked biweekly; following capture of the first DFCW male, traps were checked weekly for the duration of the flight period.

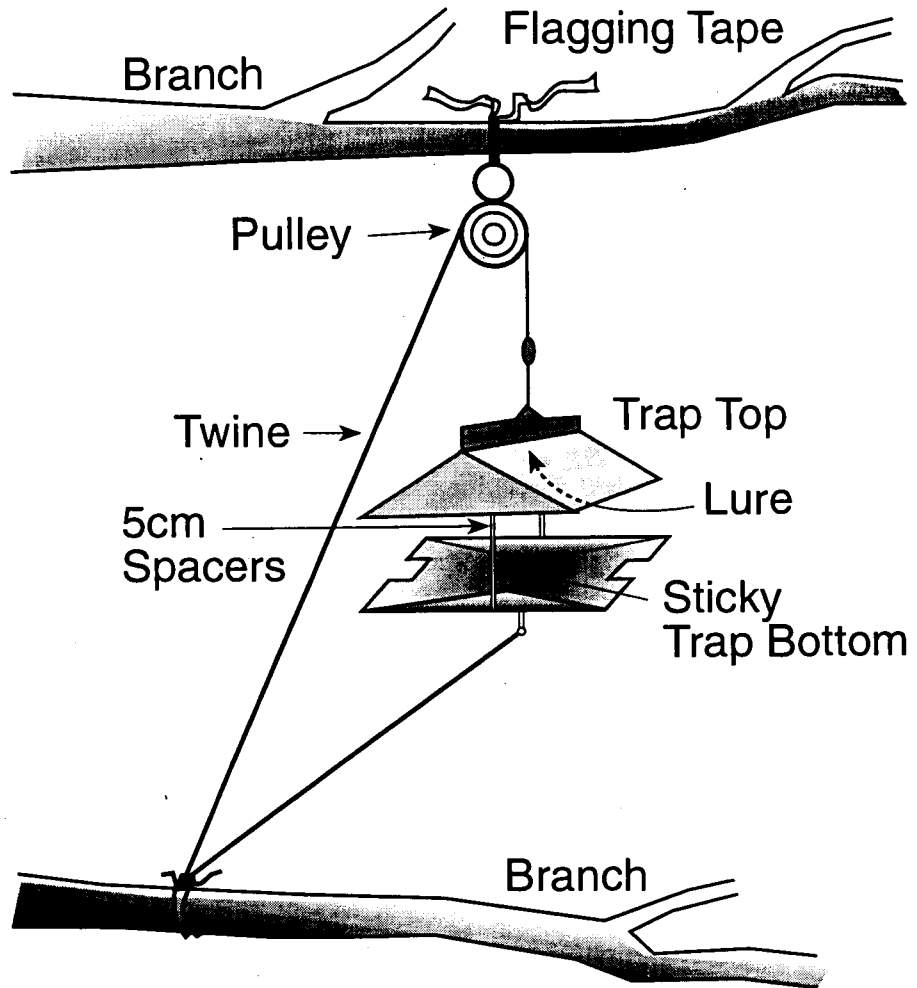
A five-treatment experiment with six replicates (=blocks) in coastal BC and ten replicates in interior BC tested attraction of DFCW males to Z9,E11-14:OAc (100 µg) alone and in binary and ternary combination with Z9-14:OAc (1 µg) and

Figure 3. 1994-Field sites for pheromone-based trapping of *Dioryctria* spp. in coastal (1) and interior (2) Douglas-fir regions.



- ① Mt. Newton Seed Orchard, Saanichton
- ② Upper Ashnola River, near Keremeos

Figure 4. Pulley system for trap placement. The trap was tied to plastic twine which was threaded through a pulley tied with flagging tape to a branch in the tree crown. A ladder is needed to place the trap, but at the end of the season the pulley can easily be removed by pulling on the twine. Pulley and spacers are modifications from DeBarr *et al.* 1982.



Z9,E12-14:OAc (1 µg). Lures were replaced at 3-week intervals. The fifth treatment was an unbaited (control) trap.

Data analyses were conducted on cumulative male moth capture by block within treatment for each site. Treatment differences were compared using the nonparametric Kruskal-Wallis test (Chi-Square approximation) (SAS 1988).

For taxonomic determinations, captured moths were removed from sticky traps with a 20-min. ethyl acetate soak (Murphy 1985). Genitalia were then prepared according to instructions by J. Troubridge (Agriculture Canada, Vancouver, BC), J. Sweeney (Canadian Forest Service, Fredericton, NB) and J.-F. Landry (Centre for Land and Biological Resources Research, Agriculture Canada, Ottawa), and were tentatively identified by S. Sopow, University of Victoria, and then sent to J.-F. Landry, for verification.

2.2 Results

2.2.1 Pheromone Analysis

GC-EAD analyses of female DFCW pheromone gland extract revealed 5 consistently EAD-active components (Figure 5). Based on their retention indices on several GC columns with different retention characteristics, compounds 1, 2 and 4 were hypothesized to be Z9-14:OAc, Z9,E12-14:OAc and Z9,E11-14:OAc, respectively. When gas chromatographed under the same conditions as female DFCW pheromone gland extract, these three synthetic compounds coincided with EAD-active compounds 1, 2, and 4. Employing a slower temperature program, all 4 co-injected geometrical isomers of

Figure 5. Representative recording (N=3) of flame ionization detector (FID) and electroantennographic detector (EAD: male *D. abietivorella* antenna) responses to one female equivalent of pheromone gland extract. Chromatography: DB-23 column; temperature program: 100°C (1 min.) 10°C/min. to 200°C.

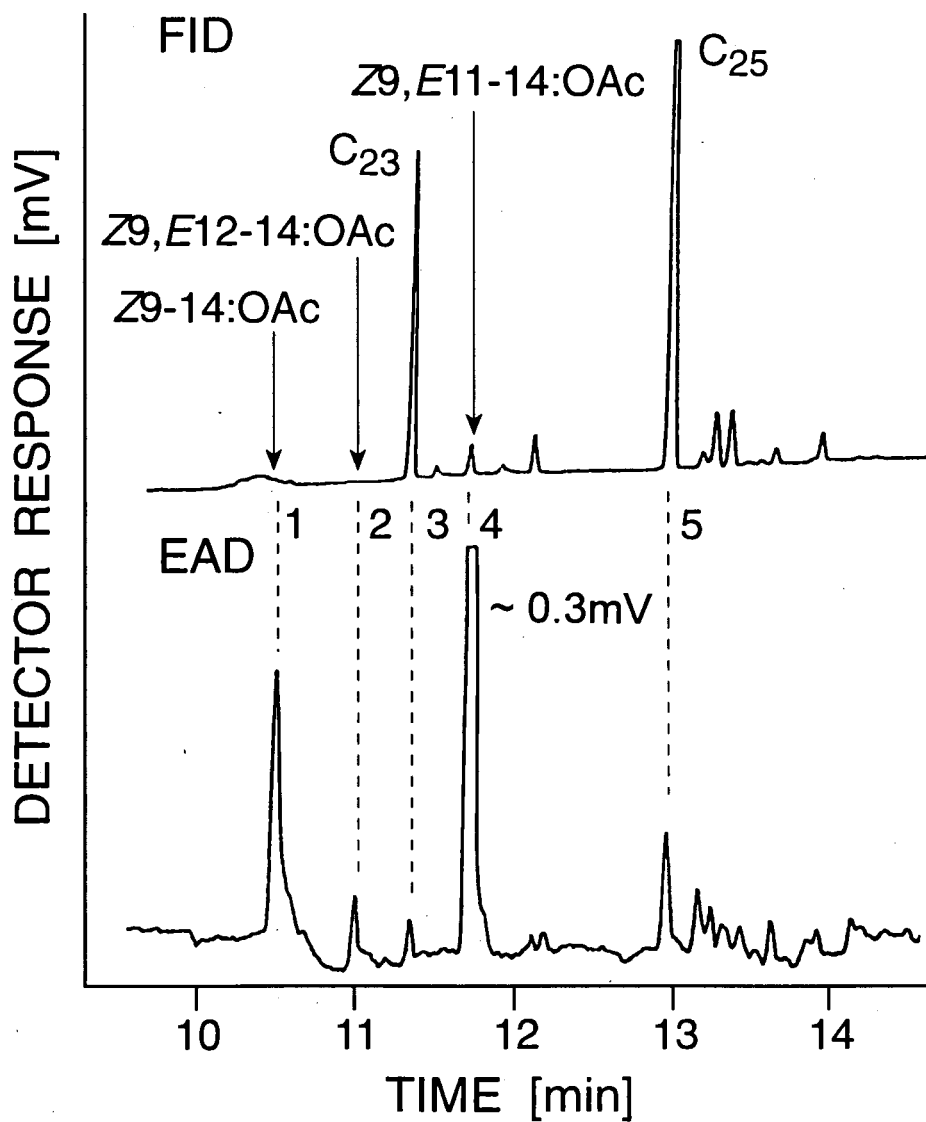


Figure 6. Electroantennographic detector (EAD: male *D. abietivorella* antenna) responses to one female equivalent of female *D. abietivorella* pheromone gland extract (top) and to 50 *pg* standards of co-injected 9,12-14:OAc geometrical isomers (middle and bottom). Chromatography: DB-23 column; temperature program: 50°C (1 min.) 20°C/min. to 100°C, then 5°C/min. to 200°C. Corresponding FID traces are omitted. Each recording was conducted with a different male *D. abietivorella* antenna.

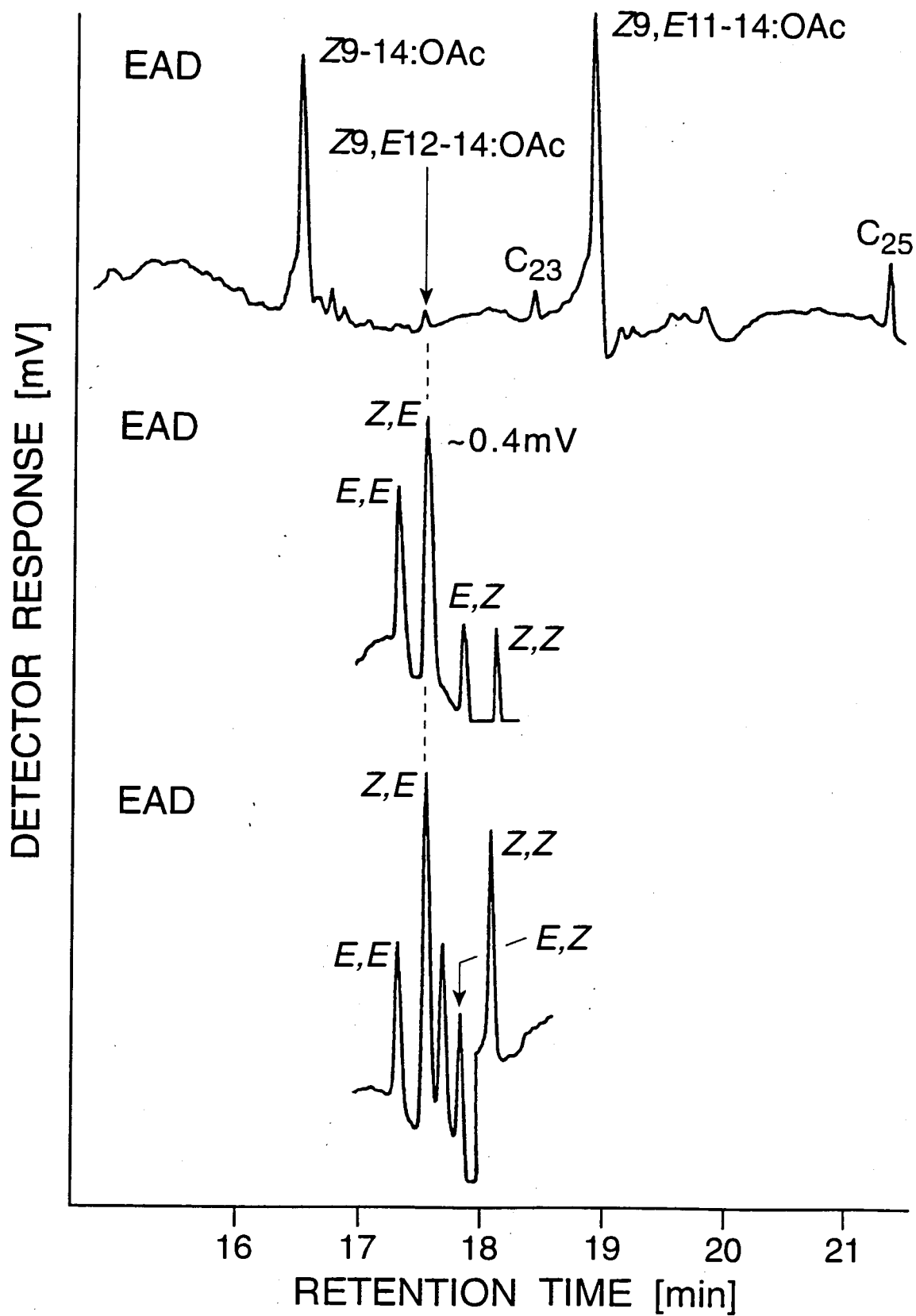
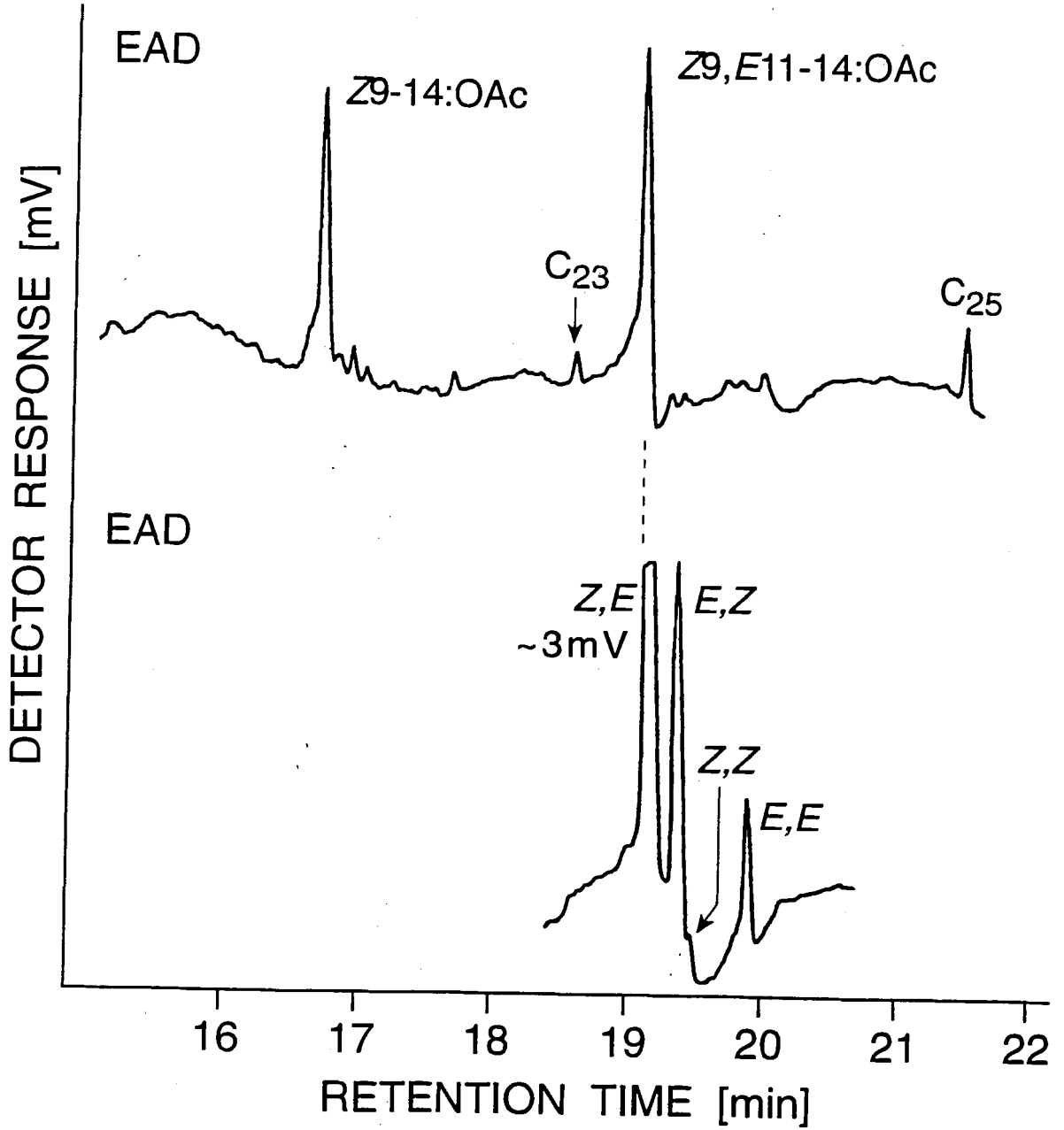


Figure 7. Recording (N=2) of electroantennographic detector (EAD: male *D. abietivorella* antenna) responses to one female equivalent of female *D. abietivorella* pheromone gland extract (top) and to 50 *pg* standards of co-injected 9,11-14:OAc geometrical isomers (bottom). Chromatography: DB-23 column; temperature program: 50°C (1 min.) 20°C/min. to 100°C, then 5°C/min. to 200°C. Corresponding FID traces are omitted. For each recording, a different male *D. abietivorella* antenna was used.



9,12-14:OAc were separated. In this analysis, Z9,E12-14:OAc coincided with female-produced component 2 and elicited the strongest antennal response (Figure 6).

Similarly when all four isomers of 9,11-14:OAc were separated, Z9,E11-14:OAc coincided with female-produced 4 and was consistently most EAD-active (Figure 7). It remains unknown whether antennal responses 3 and 5 (Figure 5) were indeed to tricosane (C₂₃) and to pentacosane (C₂₅) or to compounds of different functionality, co-chromatographing with C₂₃ and C₂₅.

Occurrence of Z9,E11-14:OAc at approximately 50 pg per female equivalent of gland extract (Figure 5) and FID-undetectability of Z9-14:OAc and Z9,E12-14:OAc justified field testing at the 100:1:1 ratio.

2.2.2 Field Experiments

Sixty pyralid moths were captured at both trapping sites, five of which were identified as *Myelopsis alatella* (Hulst). The fifty-five DFCW (Figure 8) captured included fifty-two males and three females (females were always alone in the traps). All treatments were similarly attractive, except for unbaited control traps which did not attract a single pyralid (Table 5). Because mean trap catches were low over the trapping period, and not normally distributed non parametric data analyses were used.

For the interior site, comparison of the four pheromone treatments (excluding unbaited controls) revealed no significant differences between treatments. Thus the single component lure and the components blends were shown not to be different from one another (Kruskal-Wallis test (Chi-Square Approximation); n=10 df=4, p=0.19).

Comparison of all treatments including controls revealed significant differences between

Figure 8. *Dioryctria abietivorella* male captured in a sticky trap at the upper Ashnola River on July 22, 1994. Note the characteristic zigzag-lined forewing and the dusty white unmarked hindwing. The lure comprised Z9,E11-14:OAc (100 µg) plus Z9,E12-14:OAc (1 µg).

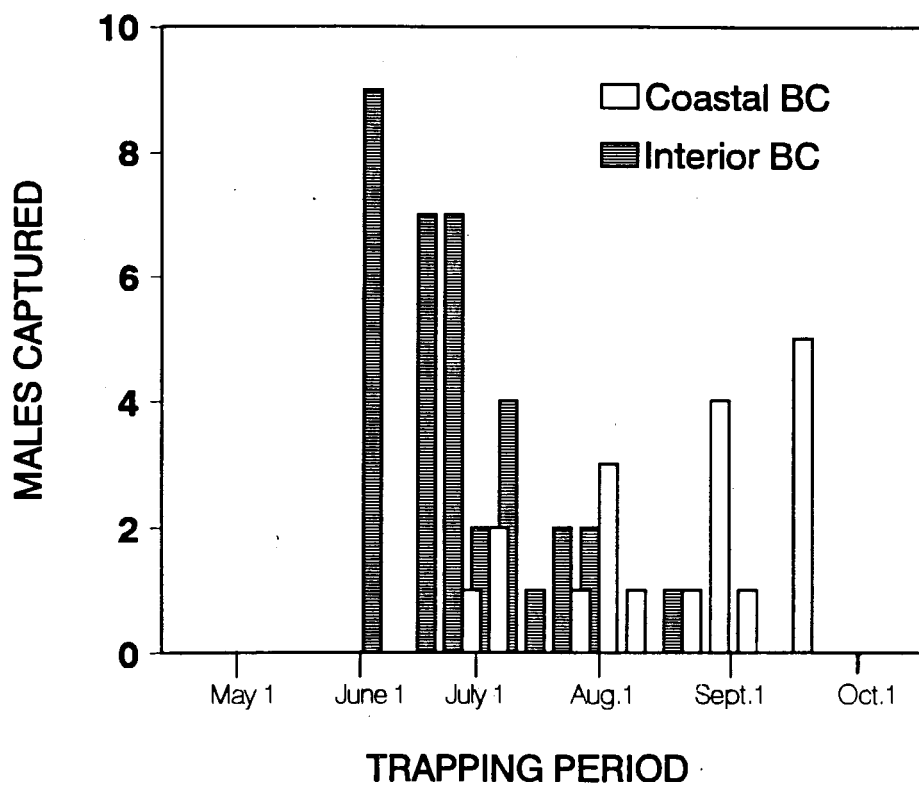


Table 5. Trap catches (all replicates summed) of male *D. abietivorella* at coastal (Mt. Newton) and interior (Upper Ashnola River) locations in British Columbia, 1994.

Treatment	Number of male <i>D. abietivorella</i> captured ^a		
	Ashnola n=10	Mt. Newton n=6	Ashnola plus Mt. Newton
1) Z9,E11-14:OAc (100 µg)	12	6	18
2) Z9,E11-14:OAc (100 µg) Z9-14:OAc (1 µg)	5	4	9
3) Z9,E11-14:OAc (100 µg) Z9,E12-14:OAc (1 µg)	6	3	9
4) Z9,E11-14:OAc (100 µg) Z9-14:OAc (1 µg) Z9,E12-14:OAc (1 µg)	13	3	16
5) unbaited (control)	0	0	0
Totals	36	16	52

^a Traps deployed April 19-October 19, 1994 and April 10-October 31, 1994, at Ashnola River and Mt. Newton, respectively.

Figure 9. Seasonal distribution of trap captures of *D. abietivorella* males at the Ashnola River (interior BC) and Mt. Newton Seed Orchard (coastal BC). Three *D. abietivorella* females were captured at the coastal site between July 1 and August 15. Five *Myelopsis alatella* males were captured at the Ashnola River between May 21 and June 15, 1994.



treatments, suggesting trap catches in pheromone baited and unbaited traps significantly differed (Kruskal-Wallis test (Chi-Square Approximation); $n=10$ $df=4$, $p=0.0001$).

At the coastal site, baited and unbaited traps could not be shown to be significantly different from one another based on block totals within treatment (Kruskal-Wallis test (Chi-Square Approximation); $n=6$, $df=4$, $p=0.31$). But when all baited traps were pooled then there is some indication that baited traps caught significantly more moths than the unbaited controls (Kruskal-Wallis test (Chi-Square Approximation); $n=6$, $df=2$, $p=0.0021$).

At the upper Ashnola River site (interior BC), captures of DFCW males commenced in the last week of May, peaked in June and July and terminated in mid-August, encompassing a trapping (flight) period of 12 weeks (Figure 9). At Mt. Newton (coastal BC), captures (flight) began the first week of July and terminated in the third week of September for a total of 14 weeks. Thus, the interior DFCW flight began and ended one month earlier than the coastal flight in 1994.

3.0 SEASONAL INCIDENCE OF *D. ABIETIVORELLA*

3.1 Materials and Methods

Cone sampling was conducted in seven coastal and three interior sites in BC known to have DFCW infestations. Coastal sites were managed seed orchards within the Coastal Douglas-fir and Coastal Western Hemlock biogeoclimatic zones (Biogeoclimatic Zones of BC 1992 Base Map, Ministry of Forests, Victoria, BC). They included Cowichan Lake Research Station (on Lake Cowichan), Bowser Seed Orchard (Bowser), Sechelt Seed

Orchard (near Sechelt), Yellow Point Seed Orchard (near Nanaimo) and Saanich, Nootka and Mt. Newton Seed Orchards (near Victoria). Interior sites were natural, mature stands within the Interior Douglas-fir zone and included D'Algaards Farm, and lower and upper Ashnola River (all near Keremeos).

Because objectives of this study were to determine the life history of the DFCW and obtain specimens for GC-EAD analysis (rather than to assess abundance of cone damage) only Douglas-fir cones with visible frass (July-October) were collected. Cones were collected monthly from June to September at all sites in 1993. In 1994, interior sites were excluded because there were no cones. *Dioryctria* spp. larvae and pupae were removed from the cones, counted and put into rearing (Appendix I). Parasites and predators were identified to family.

To search for overwintering DFCW, litter and duff was collected in November, 1992 from around western white pine, *Pinus monticola* Dougl. ex D. Don in Lamb., saplings at the Cowichan Lake Research Station. The saplings were infected with white pine blister rust, *Cronartium ribicola* J.C. Fisch. ex Rab., and secondarily by DFCW. This litter and duff was placed in plastic trays and overwintered at 20°C. A small amount of this litter and duff was screened in March of 1994 to search for DFCW cocoons.

In November 1993, litter and duff was collected in the upper Ashnola River from around mature Douglas-fir trees heavily infested (40% of cones damaged) with coneworms that year. This material was covered with plastic and kept overwinter in an unheated greenhouse at Saanich Seed Orchard near Victoria, BC.

3.2 Results

3.2.1 Cone Sampling

In 1993 (Table 6), of 743 damaged cones collected, 154 (21%) were infested with *Dioryctria* spp., 11% of these with >1 larvae. The remaining 79% had either no larvae or larvae of the Douglas-fir cone moth. *Dioryctria* spp. larvae were first found on July 9 and mid-August in the lower and upper Ashnola River sites, respectively and were detected until October. Of the few *Dioryctria* spp. pupae recorded most were present in August and September cone samples (Table 6). Parasitoids emerged from 47% of the larvae collected after mid September at the Ashnola River (Appendix I). A large number of small larvae collected at the coastal sites in September 1993 were later determined (after rearing) to be late instar *Holcocera* spp. instead of early instar DFCW. Only seven DFCW adults could be reared.

Of 677 cones with visible frass collected in 1994 (Table 7), 313 (46%) were infested with specimens of *Dioryctria* spp.; 17% of these had early instars, 40% had late instars, 43% had pupae, and 15% of late instars put into rearing were parasitized (Appendix I). Thirty-nine percent of infested cones had >1 larvae. In both years at all collection sites, several larval instars plus pupae were found in cones sampled in late July, August and September. Several *Dioryctria* spp. larvae were found on conelets in April.

Ichneumonid endoparasitoids and tachnid ectoparasitoids, apparently one species of each, were found associated with DFCW-damaged cones. Similar parasites were found by Keen (1958). Parasitism ranged from 4-55% at the various collection sites. A possibly

predatory beetle larva was occasionally encountered in cone samples from interior BC.

Cones with *Dioryctria* damage often contained only an earwig, *Forficula* sp.

(Dermaptera: Forficulidae), a type of insect known to be predatory on lepidopteran larvae (Radford 1992).

3.2.2 Soil Sampling

In February 1993, four DFCW adults (confirmed by examination of genitalia) emerged from litter and duff collected from Cowichan Lake in the fall of 1992. No cocoons were recovered from the screened material examined from the same site. No moths emerged from 1000 liters of litter and duff taken at the upper Ashnola River site in late November 1993.

Table 6. Incidence of *Dioryctria* spp. in cones collected in 1993.

Location	Site	Date	no. cones examined	no. cones infested	no. <i>Dioryctria</i> spp. larvae/pupae	no. <i>Dioryctria</i> spp. reared ¹	no. <i>Dioryctria abietivorella</i> emerged	no. parasitoids
Interior								
	D'Algaards Farm	June 10	41	0	0	0	0	0
	D'Algaards Farm	July 9	51	3	3/0	3	0	0
	D'Algaards Farm	Aug. 25	101	12	14/4	8	1	0
	Lower Ashnola	July 9	25	4	4/0	3	0	8
	Lower Ashnola	Aug. 26	123	27	41/3	19	3	1
	Upper Ashnola	July 11	12	0	0	0	0	0
	Upper Ashnola	Aug. 26	126	50	68/0	55	1	1
	Upper Ashnola	Sept. 18	126	20	7/12	19	1	9
Coast								
	Yellow Point	July 27	32	10	9/1	10	1	1
	Yellow Point	Sept. 8	26	4	4/0	1	0	0
	Yellow Point	Oct. 3	5	1	1/0	1	0	0
	Cowichan Lake	Aug. 5	5	3	3/1	4	0	1
	Cowichan Lake	Aug. 30	2	0	0	0	0	0
	Cowichan Lake	Sept. 8	45	11	10/1	11	0	0
	Sechelt	Aug. 15	40	7	5/0	5	0	0
	Campbell River	Sept. 9	9	2	2/0	1	0	0
Totals			743	154	171/22	140	7	21

¹ For rearing methods, see Appendix I.

Table 7. Incidence of *Dioryctria* spp. in cones collected in 1994.

Location	Site	Date	no. cones examined	no. cones infested	<i>Dioryctria</i> spp. larvae early/late instars	<i>Dioryctria</i> spp. pupae	<i>Dioryctria</i> spp. reared ¹	<i>Dioryctria abietivorella</i> emerged	no. parasitoids
Interior	Keremeos (all sites)	June 15	No cone crop in 1994	-	-	-	-	-	-
Coast	Cowichan lk.	Aug. 10	5 (pine graphs)	0	0/5	-	-	-	-
		Aug. 15	160	96	1/64	11	73	17	1
	Bowser	Aug. 18	100	33	0	0	0	-	-
		Aug. 29	100	25 ²	2/2	0	4	0	0
	Sechelt	Aug. 30	130	58	0/46	11	46	8	1
		Sept. 8	60	25 ²	0/3	0	3	0	1
	Saanich	Oct. 12	127	76 ²	0	0	0	0	0
		Totals	677	313	3/120	30	126	25	3

¹For rearing methods, see Appendix I.

²Most specimens collected from these sites appeared to be early instar *Dioryctria* spp., but in subsequent rearing were determined to be *Holcocera* spp.

4.0 DISCUSSION

4.1 Sex Pheromone Components of *D. abietivorella* and Potential Applications

The three acetates, Z9-14:OAc, Z9,E11-14:OAc and Z9,E12-14:OAc to which male DFCW antennae responded in GC-EAD analyses (Figure 5) are reported as sex pheromone components in other *Dioryctria* spp. (Löfstedt *et al.* 1983, 1986, Pasek and Dix 1989, Debarr *et al.* 1982, Grant *et al.* 1987,1993). The strong antennal response elicited by Z9,E11-14:OAc, its attractiveness to males in the field (Table 5, Figure 5), and its role as a pheromone component for the palearctic species, *D. abietella* (Löfstedt *et al.* 1986) all support the conclusion that it is the major sex pheromone component for the DFCW.

In southern loblolly pine seed orchards (Table 4), the sympatric species *D. disclusa*, *D. merkei*, *D. amatella* and *D. clarioralis* are all cross-attracted to Z9-14:OAc, whereas Z9-14:OAc inhibits the response of *D. amatella* to its principal pheromone component Z11-16:OAc (Hanula *et al.* 1984). Similarly, *D. reniculelloides* and *D. auranticella* are sympatric with *D. abietivorella* and respond to Z9-14:OAc (Grant *et al.* 1987, Pasek and Dix 1989) but were not captured in this study in traps baited with Z9,E11-14:OAc and Z9-14:OAc. This could be attributed to inhibition by Z9,E11-14:OAc but could also be caused by the low dose of Z9-14:OAc, or by low population levels.

At a 1 µg dose, Z9-14:OAc and Z9,E12-14:OAc did not significantly enhance trap catches (Table 5), but a 3-30 µg dose may be required for optimal attraction of *Dioryctria*

spp. (Grant *et al.* 1993). Similarly, potential synergistic behavioural activity of geometrical isomers of all three acetates needs to be investigated. For example, addition of 12% of *E9-14:OAc* to *Z9-14:OAc* enhanced attraction of male *D. clarioralis* over that of the *Z*-isomer alone (Meyer *et al.* 1984). However, the consistent response by DFCW males to all lures containing *Z9,E11-14:OAc* indicates that it could be developed as a single-component lure for DFCW monitoring. Because examination of genitalia confirmed conspecificity of males captured in coastal and interior BC and males obtained from Eastern Canada, *Z9,E11-14:OAc* might also be used for monitoring DFCW in Eastern Canada.

Because captures of male *Dioryctria* spp. increase with trap height (Grant *et al.* 1993, Debarr *et al.* 1992) effective pheromone-based monitoring of DFCW requires standardization of trap placement, as well as dose. As for other insects, trap catches should then be related to subsequent larval populations and ultimately to percentage of damaged cones per tree and/or seed per cone (Sweeney 1994, Sweeney *et al.* 1990). Our traps were at the top of the trees in the coastal seed orchards but were not in the crowns in the mature stands in the interior.

Pheromones may further be considered for mass trapping or mating disruption of the DFCW (Carde and Minks 1995). The small size (<2 ha) of seed orchards and high value of their seed crops would justify operational costs. At a density of 8 traps/0.1 ha, traps competed with each other and mean captures of *D. merkei* declined (Hanula *et al.* 1984)

suggesting partial disruption of orientation. However, in an attempt to disrupt mating of *Dioryctria* spp. trap catches of males declined but not damage (Turgeon *et al.* 1994).

4.2 Biology of *D. abietivorella*

The DFCW has been reported to be univoltine (Hedlin *et al.* 1980), bivoltine (Fogel 1979), “apparently variable” (Hedlin 1974) and univoltine with a partial second generation (Keen 1958). According to catches in pheromone-baited traps (Figure 9) and sampling of developmental stages (Tables 6,7), the DFCW is most likely univoltine with an extended flight period from June to September (Figure 10).

A bimodal flight period has been reported for *D. ebeli* (reported as *D. abietella*) in Florida (Merkel and Fatzinger 1971), peaking in late May until July and late August to late October. Based on laboratory observations (Trudel *et al.* 1995) and black light field trapping (Merkel and Fatzinger 1971), male and female *Dioryctria* spp. emerge concurrently. Assuming a 1:1 sex ratio throughout the flight season, as reported for *D. ebeli* (reported as *D. abietella*) in Florida (Merkel and Fatzinger 1971), and assuming that females oviposit within 2-5 days post-emergence, eggs (although not yet found in nature) must be present between June and September. Consistent with this hypothesis, all larval instars were present in cones collected in August (Table 7). Larvae collected in early summer matured directly to adults, whereas most of those collected in late summer overwintered as late instar larvae in a cocoon. Other overwintering stages could include eggs laid in late summer, or like *D. auranticella*, first instar larvae in hibernacula

Figure 10. Current knowledge of the life cycle of *D. abietivorella* in BC. Solid bars indicate previously reported data (Turgeon and de Groot 1992), open bars indicate information obtained from this study and the gray bar indicates the hypothesized period for the presence of the egg stage.

Month	J	F	M	A	M	J	J	A	S	O	N	D
Egg						▨						
Larva	■			▬			■					
Pupa				?	?	?	▬					
Adult						▬						

(Pasek and Dix 1989). Pupae found in August and September are unlikely to overwinter because adults emerged soon after collection, and male DFCW are still captured in pheromone-baited traps in September.

Recovering of DFCW adults from litter and duff around rust-infested western white pine at Cowichan Lake supports evidence that late instar larvae leave feeding sites to overwinter in the ground. If such larvae can have an extended diapause like *B. colfaxiana* (Hedlin *et al.* 1982) as an adaptation to the periodicity of natural cone crops, this may, in part, explain the sporadic occurrence of DFCW infestations in seed orchards.

The extended activity period and offset between coastal and interior DFCW flights (Figure 9) probably lie behind reports of multiple generations and bimodal yearly flight periods (Keen 1958, Hedlin 1974, Ruth 1982, Hedlin *et al.* 1980). The results of season-long trapping (Figure 9) suggest that the DFCW is univoltine with an extended emergence and flight period, and indicates that the activity periods of the geographically separated coastal and interior populations are offset by one month. Similar lack of synchrony occurs in geographically separate populations of *Dioryctria* spp. in the southern USA (Hanula *et al.* 1985).

5.0 RECOMMENDATIONS

1. Because DFCW pheromone identification was conducted with specimens from Sault Ste. Marie, Ontario, DFCW from coastal and interior BC should be collected, reared and the pheromone blend analyzed by GC-EAD. In subsequent field experiments all antennally active components and geometrical isomers need to be tested singly, in

- different ratios, and in all possible combinations. Attractiveness of the optimal lure needs to be compared to that of virgin DFCW females.
2. Because DFCW oviposition sites are still unknown, an intense DFCW infestation should be located and cones, branches and bark crevices searched for eggs. Alternatively, potted Douglas-fir should be placed in large cages, and female DFCW oviposition sites and behaviour video-taped.
 3. In DFCW infestation sites, light traps should also be used to determine the ratio of captured males and females throughout the flight season.
 4. Captures of DFCW males in pheromone-baited traps should be correlated with populations of immature DFCW, e.g. eggs and larvae, as well as with cone damage which begins to occur three weeks after the beginning of the flight.
 5. Using DFCW trap catches and cone damage data from various locations, a model should be developed to predict DFCW damage. Pheromone-based DFCW monitoring could then be integrated into BC seed orchard pest management programs.
 6. As there are currently no operational pheromone-based monitoring programs for cone and seed insects in BC, seed orchard managers have no experience in using this technique. Training regarding trap placement and moth identifications could be provided at workshops during annual seed orchard staff meetings.
 7. Taxonomic work on *Dioryctria abietivorella* and *Dioryctria* spp. must be continued.

APPENDIX I

Rearing method 1993

Larvae and pupae were removed from cones and placed individually in plastic snap top pill bottles (10 ml). Larvae were supplied weekly with spruce budworm diet obtained from Herb Grey of the Pacific Forestry Centre, Forestry Canada, Victoria BC. Specimens were kept at 20⁰C until Nov. 30 1993. Subsequently, vials containing specimens were buried in soil and placed outside at Saanich Seed Orchard to overwinter. When they were retrieved and brought to the lab on February 1, 1994, most of the specimens were either dead or still in the larval stage.

Rearing method 1994

Specimens were treated the same as in 1993, until Nov. 4, 1994 when they were transferred to petri dishes containing a 2 % agar medium (Davis 1983). Fifty percent of the specimens were overwintered in an unheated greenhouse and 50% were maintained at 10⁰C, in a photoperiod of 12 hrs. light:12 hrs. dark and 60-70% relative humidity; after Dec. 16, 1994 the temperature was lowered to 4⁰C; after Feb. 24, 1995 all specimens were slowly warmed to 20⁰C.

Parasitoids

After overwintering, parasitoids were collected from rewarmed storage containers both in 1993 and 1994.

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