sin, or collagenase, or pepsin followed by 4 M guanidinium chloride) all fail to show any selective enrichment of hyaluronic acid in the residue (F. S. Wusteman, unpublished work).

The major difference in the unextractable glycosaminoglycans which interpenetrate the network of fibrous protein in elastic cartilage lies in the relatively lower concentration of chondroitin sulphate chains (even after due allowance has been made for their lower mol. wt[8]). No way has yet been found to extract undegraded proteoglycan from these residues but their state of aggregation must be influenced by this difference in composition. Even if this were not so, the steric exclusion effect of hyaluronic acid, which is known to influence in vitro both the nucleation and fibre growth processes of collagen[8], is likely to affect the developing elastic fibre. Hyaluronic acid has been found in high concentration in another elastic cartilage, that of human epiglottis[9], and is far more abundant in elastic ligamentum nuchae than in collagenous tendon[10]. An unsulphated glycosaminoglycan has been implicated, from histochemical studies, in the formation of elastic fibres in arterial walls[11] and it may be that an involvement of hyaluronic acid in elastin fibrillogenesis has been obscured by the presence of other glycosaminoglycans with different functions in the tissue.

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Chemical and field studies on the sex pheromones of the cone and seed moths Barbara colfaxiana and Laspeyresia youngana[1,2]

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Summary. Field studies have shown that mixtures of trans-7-dodecen-1-ol and up to 10% of the cis isomer are effective attractants for male Laspeyresia youngana. Similarly male Barbara colfaxiana have been shown to be attracted to traps containing cis-9-dodecen-1-ol, and preliminary analyses indicate that the natural pheromone of this species contains a dodecen-1-ol.

The Douglas-fir cone moth Barbara colfaxiana and the spruce coneworm Laspeyresia youngana are 2 of the most serious cone ans seed pests indigenous to British Columbia. Damage is caused by larvae feeding mainly on seeds. The species overwinter as pupae (B. colfaxiana) and larvae (L. youngana) and emerge as adult moths in late spring and early summer.

Although neither species pose a serious threat to established forests, they are a problem, and the cause of economic loss in seed orchards. Since seed orchards are generally of small area (e.g. 10–20 ha), contain uniformly sized trees, usually of a single species, we believe they afford a forestry situation in which sex pehromones could be successfully used for population management. This paper presents the results of some preliminary work aimed at the isolation and identification of the sex pheromones of B. colfaxiana and L. youngana.

Table 1. Compounds screened for attractancy against B. colfaxiana and L. youngana

Alcohols	Alcohol acetates	Expoxides
trans-4-dodecen-1-ol	cis-7-dodecen-1-yl acetate	disparlure*
cis-4-dodecen-1-ol	cis-8-dodecen-1-yl acetate	
trans-5-dodecen-1-ol	trans-9-dodecen-1-yl acetate	
cis-6-dodecen-1-ol	cis-9-dodecen-1-yl acetate	
trans-7-dodecen-1-ol	trans-11-tetradecen-1-yl acetate	
cis-7-dodecen-1-ol	cis -11-tetradecen-1-yl acetate	
cis-9-dodecen-1-ol	cis-7-hexadecen-1-yl acetate*	
cis-7-tetradecen-1-ol	cis-11-hexadecen-1-yl acetate*	
trans-11-tetradecen-1-ol*		
cis-11-tetradecen-1-ol*		
cis-7-hexadecen-1-ol		
cis-9-hexadecen-1-ol*		

*Compounds not tested against B. colfaxiana

Materials and methods. The synthetic compounds field screened for attractancy (table 1) were dispensed in polyethylene vial caps (100 µg/cap) and suspended in Pherocon 2 traps. Each compound was replicated six times. For the chemical studies on B. colfaxiana pheromone, insects were reared on a 18/6 h light/dark cycle. The insects were removed from the cones at the late larval and prepupal stage, the sexes thereafter were maintained separately. After emergence the males were maintained for bioassay, while the females were sacrificed by dropping them into hexane when they were 2 days old. Bioassays were carried out using 2-day-old males in petri dishes in subdued light. The males were acclimatized in the dishes for several hours before 1 cm^2 pieces of filter paper impregnated with the test materials were introduced into the petri dishes. Intense 'buzzing' by the males was considered a positive response.

Extracts of B. colfaxiana were prepared by grinding whole, virgin female insects with hexane in a tissue homogenizer. This process was repeated twice, and the combined hexane extracts dried over magnesium sulphate prior to removal of the solvent at room temperature under reduced pressure. Gas chromatography was performed on a Perkin-Elmer 3920 gas chromatograph fitted with flame ionization detectors. All columns used were 6 × cm

1 Barbara colfaxiana (Kft.) and Laspeyresia youngana (Kft.) (Lepidoptera: Olethreutidae).
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stainless stele containing a) 10% Carbowax 20 M on Chromosorb W (80/100 mesh), b) 10% DEGS on Chromosorb W-AW (80/100 mesh) and c) 15% PDEAS on Chromosorb W-AW (80/100 mesh). Nitrogen was used as the carrier gas at a flow rate of 30 ml/min. Column chromatography of the extract was carried out, after high molecular weight material had been removed by acetone precipitation at −15 °C, on a Unisil (100/200 mesh) column (25 × 1.5 cm) eluting with hexane, hexane:ether (9:1), hexane:ether (5:1), hexane:ether (1:1) and ether.

Results and discussion. In 1975 synthetic candidate pheromones were field tested for attractancy agains B. colfaxiana and L. youngana in British Columbia. Of the 15 compounds tested against B. colfaxiana, only cis-9-dodecen-1-ol proved attractive, catching 32 males in the period 20 April to 21 May; virgin females, over the same period caught 11 male B. colfaxiana. During the period 12 June to 8 July, the 21 compounds listed in table 1 were tested against L. youngana. While virgin female L. youngana could attract only 1 male, traps baited with trans-7-dodecen-1-ol caught 69 male L. youngana. Table 2 shows that trans-7-dodecen-1-ol containing about 2% of the cis isomer is a potent attractant for male L. youngana. Increasing the amount of cis isomer decreases the attractancy; 80% of the total number of males caught were trapped with mixtures containing less than 10% cis-7-dodecen-1-ol indicating the need for, or a tolerance of small amounts of the cis isomer.

A hexane extract of 160 virgin female B. colfaxiana was split by gas chromatography; the fractions were collected and monitored by behavioural bioassay. On column a) at 170 °C the activity eluted in the 10.75–12.5 min fraction, while on column b) at 140 °C, the activity was shown to be in the 4.5–6.5 min fraction. The retention times of a monounsaturated alcohol (cis-9-dodecen-1-ol was used) under the exact same conditions were 11.45 min and 5.45 min, respectively. Treatment of crude extract with lithium aluminium hydride and hydrolysis with alcoholic potassium hydroxide did not destroy the activity of the extract, indicative of an alcohol function. Acetylation of the extract only partially reduced the activity. An extract from 1100 virgin female B. colfaxiana was subjected to column chromatography on Unisil. The active material eluted from the column with hexane:ether (1:1). Fractions exhibiting activity were combined, concentrated and subjected to gas chromatography on columns a) and c) at 180 °C. This insect derived material exhibited only one significant peak on either column, the retention times being 461 sec and 114 sec respectively. The retention times of cis-9-dodecen-1-ol on columns a) and c) under the same conditions were 460 sec and 142 sec.

Although the pheromone has not, as yet, been fully characterized, the above evidence points to a dodecen-1-ol, or a mixture of either positional or geometrical isomers of dodecen-1-ol, or both, with cis-9-dodecen-1-ol being a prime candidate. Field tests in 1976 were carried out in

Table 2. Catches of male L. youngana in traps baited with 7-dodecen-1-ol isomers[a,b]

Bait (100 µg/cap)		Catches at Bolean Lake, Falkland, B. C., 29 June–13 July
trans-7-DDOL		23
trans-7-DDOL/cis-7-DDOL	(99/1)	20
	(98/2)	37
	(97/3)	9
	(96/4)	15
	(95/5)	9
	(90/10)	23
	(80/20)	5
	(70/30)	6
	(60/40)	9
	(50/50)	6
	(40/60)	1
	(30/70)	0
	(20/80)	6
	(10/90)	0
Control (empty trap)		0
Virgin ♀ L. youngana		1

[a] Each trap was replicated 5 times except those containing the ♀♀. These traps were only replicated twice. [b] The compounds used to make up the lures were analyzed for isomer purity on a 5.18 m × 0.32 cm. Silar 10C column at 175 °C with a N_2 flow rate of 15 ml/min. The trans compound was shown to be pure while the cis compound contained 4% of the trans isomer.

Table 3. Catches of male B. colfaxiana in traps baited with 9-dodecen-1-ol (9-DDOL) isomers[a,b]

Bait (100 µg/cap)		Saanichton, 20 April–13 May	Mesachie Lake, 21 April–25 May
cis-9-DDOL		1	7
cis-9-DDOL/trans-9-DDOL	(99/1)	2	6
	(98/2)	2	6
	(97/3)	4	3
	(96/4)	2	2
	(95/5)	3	3
	(90/10)	2	5
	(80/20)	1	2
	(70/30)	0	2
	(60/40)	1	2
	(50/50)	0	0
	(40/60)	2	0
	(30/70)	0	0
	(20/80)	1	1
	(10/90)	0	0
trans-9-DDOL		0	0
Control (empty trap)		0	0
Virgin ♀ B. colfaxiana		5	8

[a] Each trap was replicated 5 times. [b] See table 1, b), cis-9-DDOL was shown to contain 0.3% of the trans isomer. No cis isomer was seen contaminating the trans-9-DDOL.

Table 4. Catches of male B. colfaxiana in traps baited with varying amounts of cis-9-dodecen-1-ol[a]

Compound	mg/cap	No. of ♂♂ trapped
cis-9-dodecen-1-ol[b]	0.1	8
cis-9-dodecen-1-ol[b]	1.0	14
cis-9-dodecen-1-ol[c]	2.25	18
cis-9-dodecen-1-ol[c]	4.5	20
Control (empty trap)	–	0
Virgin ♀ B. colfaxiana	–	13

[a] 5 replicates. [b] G. C. analysis on Silar 10C indicated a trans percentage of 0.3. [c] G. C. analysis on Silar 10C indicated a trans percentage of 17.0.

2 seed orchards where the population of B. colfaxiana was known to be low. The results of these tests are shown in table 3. Only mixtures of the cis and trans isomers of 9-dodecen-1-ol were tested since only one area of activity was obtained from both column chromatography, and from the gas chromatographic splits, and none of the other dodecen-1-ol tested in 1975 were active.

The numbers of moths trapped with the isomer mixtures are too low to be meaningful. No trap baited with synthetic chemical caught more males than those containing virgin females. From the Mesachie Lake results it can be seen that with increasing amounts of the trans isomer, the trend is decreasing attractancy. In another test the catch of male B. colfaxiana trapped with varying amounts of lure was ascertained. The results are presented in table 4 and show an increase in the number of males caught as the amount of lure is increased indicating a need to trap in a denser population.

In summary, field data from 1975 and 1976 indicate that trans-7-dodecen-1-ol is a potent attractant for male L. youngana and that the attractancy is dependent on isomer ratio. Similarly field results have shown that cis-9-dodecen-1-ol is attractive to male B. colfaxiana while the preliminary results of the chemical analyses are consistent with the natural pheromone containing a dodecen-1-ol. Further work is in progress.

The growth-retarding effect of Alden in Spirodela oligorrhiza[1]

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Summary. Alden® (10^{-5} M) retarded growth and increased chlorophyll and protein contents in Spirodela, the effects being reduced by gibberellin and benzyladenine. Alden nearly doubled activity of soluble phosphatase and increased activity of RNase.

It has been reported that 1-allyl-1-(3,7-dimethyloctyl) piperidinium bromide (Alden®, piproctanylium) effectively retarded growth of higher plants and induced some physiological responses that could be ascribed to the action of ethylene[2].

Spirodela oligorrhiza (Kurz.) Hegelm. was grown on mineral medium containing 4 mM $(NH_4)_2SO_4$ as sole N source and 1% glucose, under permanent illumination (1.1 klx) at 25 °C[3,4]. 20 ml aliquots of the medium were inoculated with 10 fronds; calcium phosphate was used as buffering agent[5]. Alden was filter sterilized or autoclaved; gibberellic acid GA_3 and benzyladenine BA were sterilized by autoclaving. Growth was assessed by counting the number of fronds or by weight[3]. Chlorophyll and protein were determined as described[4]. Samples of 100 mg fr. wt were extracted with 4 ml of 0.05 M Tris-HCl buffer, pH 7.5, and cleared by centrifugation at 3500 g at 0 °C. Phosphatase activity in the supernatant was determined at pH 6.0, and RNase activity was determined at pH 6.0 in 0.033 M citrate[5,6]. Unit of phosphatase is defined as that amount of enzyme that released 1 µmole of p-nitrophenol per min at 30 °C. Standard unit of RNase is defined as that amount of enzyme which released 1 A_{260} unit (A_{260} of 1.0/ml) of soluble nucleotide from highly polymerized yeast RNA per min at 30 °C[7].

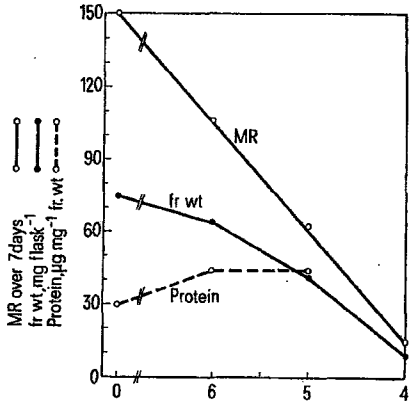

Fig. 1. Effect of different concentrations of Alden on growth and protein content in Spirodela as measured after 7 days of cultivation. Inoculum: 10 fronds per flask. Multiplication rate,

$$MR = \frac{1000\,(\log_{10}Fd - \log_{10}Fo)}{d}$$

where Fo, original number of fronds; Fd, No. of fronds on day d; d, number of days.

The multiplication rate MR of Spirodela was inversely related to the logarithm of the molar concentration of Alden (figure 1). Fronds became smaller, thicker and darker green than in the control; roots were shortened. All progeny plantlets remained attached to the mothers, forming large clusters of tightly packed overlapping fronds. Similar clusters were produced by other growth retardants[4]. Alden, in contrast to CCC and DMMC[4], did not evoke symptoms of inhibition of chlorophyll synthesis, even at concentrations completely blocking growth (5×10^{-4} M).

Growth of Spirodela was slightly stimulated by GA_3. Under the influence of BA, the MR was not changed, but fronds became larger and heavier than in the control[4]. The growth-retarding effect of Alden at the concentration of 10^{-5} M was markedly reduced by GA_3 and BA (table,

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