

# Application of SPME to Insect Semiochemicals

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The SPME technique has become an extremely useful tool in our laboratory for the detection of biologically active materials from a variety of sources. Coupling SPME with the gas chromatography with electroantennogram detection (GC-EAD) provides a powerful method for detection of compounds that could function as semiochemicals. Several projects in which the SPME technique was used for the analysis of a liquid or headspace for biologically active components will be described briefly below.

## I. Volatiles from Fruit

Survival and successful reproduction in phytophagous insects depends on locating an appropriate host plant. One sensory cue that has been exploited for this purpose is host odors, and one group in which these odors have been implicated in maintaining host fidelity, as well as shifts to new hosts via sympatric speciation, are the fruit flies belonging to the *Rhagoletis pomonella* species group. Previous studies by others on host odor discrimination in this group have been stymied because of problems in identifying the key host-volatile compounds involved. We have successfully applied two techniques that have allowed us to overcome previous problems. The SPME technique combined GC-EAD is being used for the identification of new key volatiles and for the precise analysis of antennal responses to host odors. The project focused initially on two key agricultural pests, the apple maggot, *R. pomonella*, and the blueberry maggot, *R. mendax*.

Various cultivars of apple and blueberry were analyzed using a combination of the SPME technique to sample the headspace volatiles and GC-EAD to detect the key volatiles as assessed by the fly's antennae. The key volatiles were then collected in larger quantities by airborne collection on Porapak Q, characterized, and the synthetic blends used to compare behavioral responses of the two species on colored spheres in a flight tunnel. Headspace volatiles sampled by SPME of 5 apple cultivars showed that there were 5 compounds (esters) in all cultivars that gave reproducible and high EAD responses with both apple and blueberry maggot antennae. There compounds were as follows (average ratio from all cultivars): butyl butanoate (13); propyl hexanoate (5); butyl hexanoate (50); hexyl butanoate (50); and pentyl hexanoate (6). This blend differed from the previously identified apple volatile blend, which was as follows: hexyl acetate (35); (E)-2-hexenyl-1-yl acetate (2); butyl 2-methylbutanoate (8); propyl hexanoate (12); hexyl propanoate (5); butyl hexanoate (28); and hexyl butanoate (10).

Headspace volatiles sampled by SPME of 7 blueberry cultivars showed that there were 4 compounds (all different from those found in apple) in all cultivars that gave reproducible and high EAD responses with both apple and blueberry maggot antennae. There have been identified, but still classified as unpublished data. It was interesting to find that apple maggot and blueberry maggot antennae gave similar EAD-response patterns to apple and blueberry volatiles, and to the individual synthetic compounds identified from both fruit. EAD responses were generally higher to the apple volatiles with antennae from both species.

It was interesting to find that EAD analysis of higher concentrations of volatile components obtained by the usual Porapak collection technique by previous investigators did not reveal the few active components from the host volatiles as exhibited with the SPME sampling technique. Antennal responses are probably more discriminating among volatile components when they are presented at low concentrations obtained by SPME - similar to what the fly might be exposed in the field.

## II. Apple Leaf Midge

We were contacted by Jeremy Heath (Nova Scotia) to characterize the sex pheromone of the apple leaf midge. Females were placed in a small glass vial and the SPME technique used to sample the headspace. The combination of SPME and GC-EAD revealed a very active component eluting from the GC capillary column. The technique quickly revealed that the females were releasing a sex pheromone and retention times on polar and non-polar GC columns were obtained for this material. The small amount of material obtained by the SPME did not allow for the characterization of the material, but it provided the basis for further research on this species.

## III. Striped Cucumber Beetle

Dr. Michael Hoffmann (Cornell University) contacted us to assess the possibility of finding aggregation attractants for the striped cucumber beetle. Apparently, they aggregate in large numbers in the field during their Spring flight. Utilizing the SPME technique combined with GC-EAD we analyzed headspace from plants alone, beetles alone, and beetles feeding on plants (squash) for compounds that elicited EAD activity. A few small peaks of EAD activity were found with the plants alone and the beetles alone, but several new peaks of activity were found reproducibly with beetles feeding on plants. It was interesting to find that compounds in the commercial lure (TIC) for striped cucumber beetle (2,4,6-trimethoxybenzene, indole, and trans-cinnamaldehyde) were not as active with the EAD technique as compounds from the beetles feeding on plants, and only indole was detected in the plant volatiles. Characterization of some of the compounds obtained by the SPME collection method could lead to new aggregation compounds for this pest species.

#### **IV. Elephant Urine**

Another interesting application of the SPME was found in a cooperative project with Bets Rasmussen (Oregon Graduate Institute). Female Asian elephants use a pheromone to signal to males their readiness to mate. This pheromone is released in their urine during oestrus and before ovulation. The urine elicits a high frequency of flehmen responses (a distinct trunk movement that facilitates chemosensation via the vomeronasal organ) from Asian bull elephants. Urine was collected from female Asian elephants and the active material isolated through a series of purification steps. It was finally identified as (Z)-7-dodecenyl acetate, which is used by my moth species as a pheromone component.

Quantitative analysis of Z7-12:OAc in urine of cycling female elephants was performed with the SPME technique. Five milliliters of urine obtained from different stages were each placed in 7-ml glass vials. Standard heptadecane in hexane (5  $\mu$ g/5  $\mu$ l) was then added in each urine sample along with a micromagnetic stir bar. The solution was stirred at room temperature for 2 min with a fused silica fiber (100  $\mu$ m non-bonded polydimethylsiloxane phase fiber installed in the SPME manual fiber holder) immersed in the solution. The SPME syringe then was injected into the GC injection port and the plunger pushed down to desorb the fiber for 5 min while running the GC program. An analysis of the detector response to hexadecane and Z7-12:OAc standards (1 ng/1  $\mu$ l) showed that the response areas were close to 1. The urinary concentration of Z7-12:OAc was measured at 4 hormonally distinctive periods during the female estrous cycle: luteal, early follicular, mid-follicular, and immediately prior to ovulation. Male response to female urine was quantitatively distinct during these periods, and they are characterized by decreasing amounts of serum progesterone levels. SPME analysis of whole urine in the different periods gave the following results for the titer of Z7-12:OAc (Rasmussen et al., 1997): Luteal = not detectable;

Early Follicular = 0.48 mg/ml; Mid-Follicular = 13 mg/ml; and Pre-ovulatory = 33 mg/ml. It was also interesting to find that analysis of the whole urine without any purification steps provided a much higher quantity of Z7-12:OAc than was found in solvent extracts of the urine.

#### **V. Grape Thrips**

Another interesting example of using the SPME on a liquid resulted from a request by Todd Ugine in Prof. Greg English-Loeb's research group on grapes to check out a liquid droplet found at the abdominal tip of a thrips when it was touched with a forceps. It was difficult to get a clean extract from a single thrips, and so the SPME noodle was touched directly to the droplet and analyzed by GC-EAD. An antennal response was found for one of the few compounds exhibited on the GC tracing. Repeating the SPME technique and injecting on the GC/MS revealed that the compound eliciting activity was juglone.

#### **VI. Predaceous Mite**

Prof. Jan Nyrop approached us recently with the challenge of looking for a possible kairomone used by predaceous mites to locate their prey. We put some predaceous mites (*Amblyseius fallacis*) in a small glass vial with the prey *Tetranychus urticae* (two spotted spider mite) feeding on bean leaves. The headspace was sampled by SPME for 0.5 hr and then analyzed by GC-ELG. In this case it is ELG because it was an electro-leg-ogram in which a front leg of the mite was connected between the electrodes for detection of active materials eluting from the GC. This method allowed us to detect a compound from the headspace volatiles that reproducibly elicited a response from the leg.

### References Cited

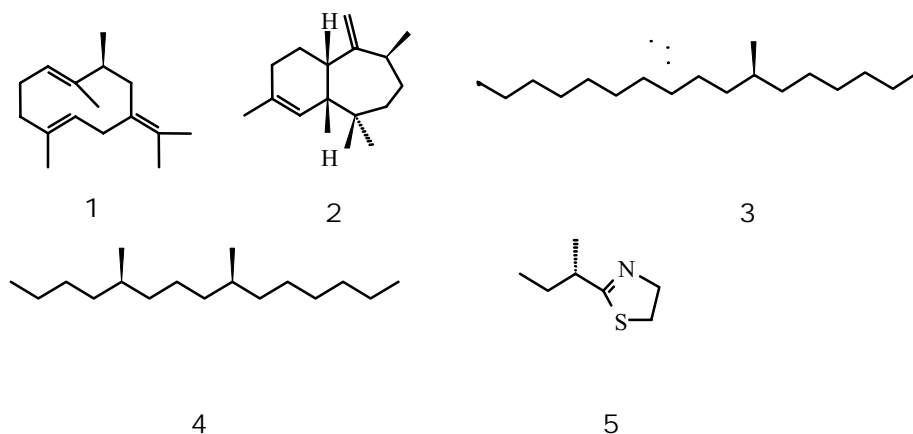
- Rasmussen, L. E. L., Lee, T. D., Zhang, A., Roelofs, W. L., and Daves, Jr., G. D. 1997. Purification, identification, concentration and bioactivity of (Z)-7-dodecen-1-yl acetate: Sex pheromone of the female Asian elephant, *Elephas maximus*. Chem. Senses 22: 417-437.

## Recent Results in Pheromone Synthesis

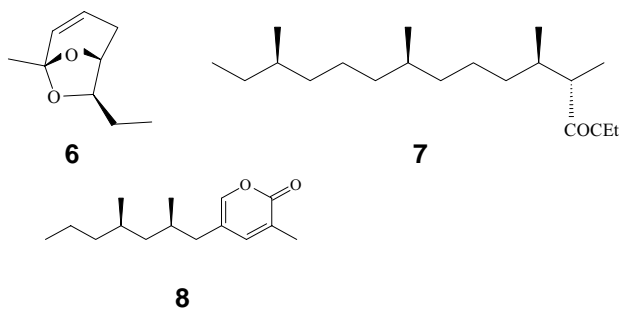
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Enantioselective synthesis of chiral pheromones is the essential part of pheromone chemistry to determine their absolute configuration. Our recent results on this subject will be illustrated by the following examples: **1** (9-methylgermacrene-B) and **2** (3-methyl- $\alpha$ -himachalene), the male sex pheromone of the sandfly *Lutzomyia longipalpis*; **3** and **4**, the female sex pheromone of the spring hemlock looper *Lambdina athasaria*; and **5**, the pheromone component of the male mouse *Mus musculus*.



New enantioselective syntheses of the following pheromones will also be discussed; **6**, the pheromone component of the male *Mus musculus*; **7**, the pheromone of the pine sawfly *Microdiprion pallipes*; and **8**, the female sex pheromone of the brownbanded cockroach *Supilla longipalpa*.



## Exploratory Researches on New Control Techniques of Insect Pheromones

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Significant advances have been made to improve pest management efforts while reducing chemical pesticide use in 20<sup>th</sup> century. Particularly semiochemicals can be used to lure pest insects to traps and sample populations. This monitoring information can then be combined with other observations to improve control programs through better targeting and timing.

The semiochemicals in addition to their use in traps for detection, monitoring and survey successfully, also can be used in several other ways for control or suppression of insect population: (a) Mass trapping. Actually mass trapping is useful in a very limited number of specialized cases. One successful case in China is that control of an important forest pest, poplar clearwing moth *Paranthrene tabaniformis* in Northeast China by using mass trapping (Du et al., 1984,1985). The successful reasons for this case is firstly male moth of these pest only mates with female once. Second, population is low and to be suitable for mass trapping. Other successful cases are boll weevil (Ridgway et al., 1990), Oriental fruit fly (Stainer et al., 1965) and so on. (b) Mating disruption. Mating disruption has been successful in controlling a number of insect pests, for example Pink bollworm, *Pectinophora gossypiella* (Li et al., 1982; Kinya Ogawa, 1996); oriental fruit moth *Grapholita molesta* (Kirsch et al, 1993); Codling moth, *Cydia pomonella* (Charmillot, 1990; Thomson, 1996); European grape moth *Eupoecilia ambiguella*, Grapevine moth *Lobesia botrana* and so on. More than 20% of the grape growers in Germany and Switzerland employ this technique to control the important insect pests injured the grape and produce their wine without using insecticides. (c) Combination of baits with toxicants, pathogens, or chemosterilants (Plimmer et al., 1982;). An example of the combination of toxicant with a bait is the use of such a preparation in the screwworm control program (Coppedge et al., 1978,1980). Control of codling moth, *Cydia pomonella* L by an attractive and kill formulation (Charmillot et al., 1996) and a new technique of pheromone/virus combination (Du et al.,1996; Jackson,D.M.et al, 1992).

The use of sex pheromones for monitoring, mass trapping and mating disruption to control several agricultural and forest insect pests in China has over three decade. A recurring problem with using pheromone for control of insect pests is the cost of the product to the end-user. At present, the Research Institute supports the most of pheromone products in China, not by Companies. Most of Chinese fine-chemical industries are not interested in pheromone production from a commercial viewpoint. Secondly, scientists in China are devoid of putting their efforts to technology transfer when the applied aspects grow more satisfying. These problems will be solve by Yunnan Xinlian Eco-Scientific & Technological Industry Co. LTD. which organized and invested by Yunnan Xianlian Chemical Industries, Shanghai Institute of Entomology, Chinese Academy of Sciences, and Department of Chemistry, Yunnan University and found in this year. There are great deals of pheromone technologies they have to learn.

Using pheromone for control of insect pests in China, firstly the farmers appear to be unhappy if the technique of using pheromone is so expensive with little practical benefit, particularly for crops with low economical value. A lot of farmers in China prefer to use the mass trapping for control of insect pests, the reasons for this are (1) it is easy to use; (2) they can see a large number of insect pests in traps and (3) not so expensive. But, most cases of mass trapping tests in China have been very discouraging in past years. Actually mass trapping is often deemed to be uneconomic or at least noncompetitive with other means of control.

Therefore, more time and efforts has been spent on studies of using pheromone trap as a mean of producing a highly effective and cheaper trapping system for spreading virus (Du,et al.1966) or the sterile males, and to explore male orientation inhibitor and female calling behavior interrupter in order to replace with expensive sex pheromone. The discussion will center on our efforts to set up laboratory and large field-cage tests for feasibility of this control techniques prior to their use in larger field trials.

### Male orientation Inhibitor

It has been suspected for a long time that the brush-like organs found in males of many Lepidopterous species may store and disseminate a pheromone during courtship (Aplin and Birch,1968 ). Table (1) shown some male pheromones found in Noctuidae. We identified 10 components from extracts of *H.armigera* male hairpecil (See Table 1). The data of bioassay in the wind tunnel shown that male moths of cotton bollworm produced the highest response to the binary components of Z-9-16:Ald and Z-11-16:Ald in a ratio of 5:95 at the dosage of 0.4  $\mu$  g. When Z-11-16:

OH was added to the binary blend, 5% of the alcohol completely inhibited male orientation behavior. The results of inhibitory tests on field large-cage by spraying or coating the inhibitor on plastic tubes indicated that egg hatch rate was reduced about 60-80 % in compared with control (See Table.2). Further researches will conduct to screen a simple and effective compound.

**Table 1. Some Male pheromones found in Noctuidae**

Insect Pests	Male Pheromone	Reference
<i>Pseudaletia unipuncta</i>	2-Phenyl ethanol	Birch et al. 1976
<i>Heliothis virescens</i>	16:Ac, 16:OH, Z-11-16:Ac, 18:COOH 18:OH, 14:COOH,, 16:COOH, 18:COOH	Teal et al. 1989
<i>Helicoverpa armigera</i> .	14:OH, 16:OH, 18:OH, Z-11-16:OH, 14:Ac, 16:Ac, 18:Ac, 14:COOH,16:COOH; 18:COOH	Huang et al. 1992,1996
<i>Helicoverpa.subflexa</i>	14:OH, 16:OH, 18:OH, 14:COOH,18:Ac 16:COOH, 18:COOH, 16:Ac, Z-9-14:Ac	Huang et al.1992
<i>Mythimna separata</i>	Benzaldehyde	Clearwater . 1972
<i>Peridroma saucia</i>	Benzaldahyde,Benzyl alcohol , Benzoic acid	Grant et al. 1972

#### Female calling behavior interrupter

We know that Z-11-16: OH in *H.armigera* male hairpencil plays a role of inhibitory on male orientation behavior. However, We don't know what is biological function of RCOOH in male hairpencil on female calling behavior. Actually, odors of some RCOOH and its analogues would interrupt female calling behavior. Based on bioassay, the percentage of female calling behavior in scotophase by treatment of RCOOH odors was reduced by 50-70% compared with the control. Therefore, we assumed that one of the biological functions of special fatty acids in *H.armigera* male hairpencil would be to terminate female calling behavior effectively.

Simulated filed-cage tests also shown that some unsaturated RCOOH and its analogues could be to interrupt female calling behavior effectively. Large field-cage test by spraying of Z-9-18: COOH solution shown egg hatch rate was decreased by about 70% in compared with control. (See Table.2). It is clear that unsaturated RCOON could be a chemical signal to exert significant effects on female calling behavior. From the above tentative results, the female calling behavior interrupter would be a valuable and promising behavior mediator for practical use.

#### Pheromone trap combined with virus

Webb(6) and Knipling (44) emphasize those systems employing sex attractants in attraction-annihilation strategies to be most useful against low-level populations. Thus, use of attraction-annihilation might be feasible against pests, such as the peach tree borer, *Synanthedon exitiosa* (say), that cause economic damage at low-population densities, or against eruptive outbreak pests, such as the gypsy moth, either before the population has reached damaging levels or after the population has been reduced by insecticide or other means.

The mechanism of this technique is that the male attracted by the sex pheromone are acted upon by an special affector, which could be a container or trap with a surface treated with virus formulation. Then the male returns to the field and mates with the native females, spreading the virus through the mating. Theoretically, this technique can cause an epidemic effect on the population (Kennedy et al.,1977). For example, the population of *Trogoderma glabrum* (Herbst) was successfully suppressed by using the pheromone to lure this dermestid to a protozoan pathogen. The pathogen was then disseminated effectively by the beetles as they moved into the storage area.

**Table. 2 Field-Cage Test of *H.armigera* Male Orientation Inhibitor and Female Calling behavior Interrupter**

Field-cage test	Inhibitor or Interrupter	Hatching Rate of Egg %
A	Z-11-16:OH	24.6
	Z-9-18:COOH	35.5
	Control	100.0
B	Z-11-16:OH	15.7
	Z-9-18:COOH	5.2
	Control	100.0
C	Z-11-16:OH	19.0
	Z-9-18:COOH	15.8
	Control	100.0

Three basic studies have been conducted to investigate the potential of using a virus-dispersal technique for controlling cotton bollworm, *H.armigera*. One study centered on the transmission of the virus; (a) to males attracted to virus-contaminated pheromone lures, particularly in relation to the males showing random movements and hairpencil displays on the lure, and (b) to the eggs laid by females mated with virus-infected males. The second study followed the transmission of virus to offspring of *H.armigera* parents that contained a sublethal infection of the virus. The third study involved the collection of data on the epidemic spread of viurs in a population with a sublethal virus infection and its large field-cages tests. Table (3) summarized some simulated tests on pheromone/virus control technique.

Our results in this study provide new information regarding the technique of pheromone/virus combination and clearly show that (1) males entered in trap contacted with virus formulation by movements and hairpencil displays around the lure. Over 80% of the males entered in the trap are contaminated; (2) the female mated with the infected male laid eggs covered with virus. About 78-88% of hatching larvae was died by virosis, and only 12-24% of hatching larvae was survived. (3) The simulated test in the field cage, the mortality of hatching larvae caused by virosis has attained 21-33%. These experimental data are still theoretical because we have not actually tested in the field. The problem now facing us is how we can used this technique most effectively. In order to achieve a good efficacy by using pheromone/virus technique, the special affector should be developing to simple device with a good virus formulation and special lure which can attract males and female both. Further investigations are needed to develop special lure, which would be a valuable for this technique. For example, Chinese farmers often use poplar tree leaves to catch *H.armigera* female as monitoring or controlling tool over 30 years. The results of GC-MS and GC shown known 20 compounds from steam-distillate of poplar tree leaves. The results of bioassay on the wind tunnel indicated that *H.armigera* female will respond to special combined blend of some compounds strongly (Du and Gregg ,et al. 1999. Unpublishing data). It will make this technique effectively.

**Table 3. Simulated tests on pheromone/virus control techniques**

Insect Pests	Virus	Trap	Virus Formulation	%Virus Induced Larvae Mortality	Ref
<i>H.virescens</i>	AcNPV	Contamination station	Powder	0.7-11.9	Jackson et al.1992
<i>P.interpunctella</i>	IMMGV	Pyrex cake pan	Freeze-dried powder	16.0	Patrick et al.1993
<i>H.armigera</i>	AcNPV	Two-pans trap	Micro-Particle	21.0-33.0	Du et al.1996

Autographa californica nuclear polyhedrosis virus--AcNPV

A homologous granulosis virus—IMMGV

#### **Pheromone traps combined with chemo-sterilant**

As suggested by Kennedy et al (1981), traps could also be designed to spread the sterile males within an insect population by capturing, contacting with chemosterilant, and then returning into the field. Theoretically, this suggestion seems reasonable. In this study we wanted to evaluate the feasibility of the pheromone/chemosterilant combination technique as a control tool. The research has followed the necessary sequence of :1 ) effects of the chemo-sterile males on female reproductive capacity; and 2) the simulated test of the pheromone/chemosterile combination technique in a wand tunnel.

The results shown that the Asian corn borer males treated with hair oil formulation have higher percentage of

the sterilization in dosage of 10 g/m<sup>2</sup> and contact time of <10 sec. The results indicated that a sterile male would make 6 females to lose reproductive capacity through multiple mating. The simulated test in wind tunnel proved that the average sterility of the eggs was 56.9% in the test group vs. 25.7% in the check group. The average sterility of the eggs in the test group was increased by 45.2% when compared with the check group.

According to our preliminary study, the pheromone/chemosterilant combination technique is many superiors to the mass trapping because a sterile male can make 1-6 female to lose reproductive capacity through mating. For the chemosterilant used in this combination technique, one of the most important conditions is safety. This means that the chemosterilant used in the pheromone trap should be harmless to nontarget organisms and not pollute or damage the environment. Meantime, the chemosterilant used in this way may operate harmoniously with other biological control agents. From a scientific viewpoint, the pheromone/chemosterilant combination technique represents an ideal form of insect pest control to be used in agricultural IPM system. Meantime the technique could also combined virus with chemosterilant both in affector in order to enhance its potency.

## LITERATURE CITED

- Aplin,R.T. and Birch,M.C. 1968. Pheromones from the abdominal brushes of male noctuid Lepidoptera. *Nature* 217:1167-1168.
- Birch,M.C.,Grant,G.G., Brady,U.E.1976. Male scent brush of *Peridroma saucia* chemistry of secretion. *Ann.Entomol.Soc.Am.* 69:491-492.
- Charmillot,P.J.1990.Mating disruption technique to control codling moth in Western Switzerland. In *Behaviour-modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants* (eds R.L.Ridgway, R.M.Silverstein and M.N.Inscoe), Marcel Decker Inc., New York, pp.65-182.
- Charmillot P.J., D.Pasquier, A.Scalco and Hofer.D. 1996.Essais de lutte contre le carpocapse *Cydia pomonella* L. Par un proc attracticide. *Mitt schweiz ent Ges* 69:431-439.
- Clearwater,J.R.1972. Chemistry and function of a pheromone produced by the male of the southern armyworm, *Pseudaletia separata*. *J.Insect.Physiol* 18:781-789.
- Coppedge,J.R.,J.L.Goodenough,A.B.Broce,F.H.Tannahill,J.W.Snow,M.M.Crystal,H.D.V.Petersen. 1978. Evaluation of the screwworm adult suppression system (SWASS) on the Island of Curacao. *J.Econ.Entomol.* 71:579-584.
- Coppedge,J.R.,H.E.Brown,J.L.Goodenough and F.C.Tannahill.1980.Field performance of a new formulation of the screwworm adult suppression system. *J.Econ.Entomol.* 73:411-414.
- Du, Jia-wei, Xian-han Tang, Shao-fu Xu and Jian-wu Mial. 1996. Developing new control techniques with insect sex pheromones. *Proc.Int.Symp.on Insect Pest Control with Pheromones*, In Korea . (ed.by K.S.Boo, K.C.Park, J.K.Jung) pp.16-28.
- Du, Jia-wei, Shao-fu Xu, Xiao-jie Dai and Zhang Xi. 1984. Field test on controlling poplar clearwith moth, *Paranthrene tabaniformis* Root by mass trapping. *Contr.Shanghai Inst.Entomol.* 4:53-58
- Du, Jia-wei, Shao-fu Xu, Xiao-jie Dai and Zhang Xi. 1985. Strategies for control poplar clearwing moth *Paranthrene tabaniformis* Root by mass trapping. *Contr.Shanghai Inst.Entomol.* 5:19-24
- Grant,G.G.,Brady,U.E.,and Brand,J.M. 1972. Male armyworm scent brush secretion:identification and electroantennogram study of major components. *Ann.Entomol.Soc.Am.* 65:1224-1227.
- Knipling,E.F. 1979. *The Basic Principles of Insect Population Suppression and Management*. Agric.Handbook 512, U.S.Dept. Agric., Washington, 659pp.
- Kirsch,P. And Lingren,B. 1993. Chmmercial advancement on pheromone related monitoring and control technology. In *insect Pheromones, Proceedings of an IOBC/WPRS Working Group Meeting, 11-14 May 1993, Chatham, UK*, Bulletin OILB/SROP,16,pp.121-127.
- Huang, Yong-ping.,Shao-fu,Xu.,and Jia-wei Du. 1992. Studies on the male hairpencil pheromone of *Helicoverpa armigera*. *Contributions from Shanghai Institute of Entomology.* 12:5-12.
- Huang,Yong-ping.,Shao-fu,Xu., Xian-han Tan.,Zhang-wu Zhao and Jia-wei Du. 1996. Male orientation inhibitor of cotton bollworm:identification of compounds produced by male hairpencil glands. *Entomologia Sinica.* 3(2)172-182.
- Li, Wen-gu,Zhi-yi Luo, J.W.Jenkins and C.C.Doane.1982.Demonstration of disrupting mating communication for control of *Pectinophora gossypiella* Saunders with Nomate PBW. *Contr.Shanghai Inst.Entomol.*3:1-8
- Jackson,D.M.,Brown,G.C.,Nordin,G.L, and D.W.Johnson. 1992. Autodissemination of a Baculovirus for management of tobacco budworms (Lepidoptera:Noctuidae) on tobacco. *J.Econ.Entomol.* 85(3):710-719.
- Ogawa K. 1996. The key to success in mating disruption. Papers presented at the 1996 IOBC Conference on *Technology Transfer in Mating Disruption* in Montpellier,France
- Patrick V.Vail., Darlene F.Hoffmann and J.Steven Tebbets. 1993. Autodissemination of *Plodia interpunctella* (Hubner) (Lepidoptera:Pyralidae) granulosis virus by healthy adults. *J.stored Prod.Res.* 29(1):71-74.
- Plimmer,J.R.,M.N.Inscoe and T.P.McGovern. 1982. Insect attractants. *Ann.Rev.Pharmacol.Toxicol.* 22:297-320.
- Ridgway,R.L., M.N.Inscoe. and Dickerson, W.A. 1990. Role of the boll weevil pheromone in pest management. In

- behaviour-Modifying Chemicals for Insect management: Applications of Pheromones and Other Attractants* (eds R.L.Ridgway,R..M. Silverstein and M.N.Inscoe), Marcel Dekker, Inc., New York, pp.437-471.
- Stainer,L.F., W.C.Mitchell, E.J.Harris, T.T.Kozama and M.S.Fujimoto. 1965. Oriental fruit fly eradication by male annihilation. *J.Econ.Entomol.* 58:961-964.
- Teal,P.E.A.,and J.H.Tumlinson. 1989.Isolation, identification, and biosynthesis of compounds produced by male hairpencil glands of *Heliothis virescens* (Lepidoptera:Noctuidae). *J.Chem.Ecol.*15(1)413-427.
- Thomson, D. 1996. Confusion amongst codling moth fellows continues: a commercial perspective on the implementation of codling moth mating disruption in North America. Paper presented at *the 1996 IOBC Conference on technology Transfer in Mating Disruption. In Montpellier* (France)
- Toth,M. 1982. Male produced pheromone of *Mamestra brassicae* (L) (Lepidoptera:Nuctuidae):Its possible role in courtship behaviour and study of other effects. *Acta Phytopathologica Academiae Scientiarum Hungaricae*, 17(1-2)123-132.
- Web,R.E. 1982.Mass trapping of the gypsy moth. In *Insect Suppression with Controlled Release Pheromone Systems*, Vol.II, (Kydonieus,A.F. and Beroza,M.,eds), CRC Press, Boca Raton, pp.27-56.

# Interacting Factors in Chemical Ecology: Case Studies on Conifer Growth Inhibition and Habitat Modification by Ericaceous Plants

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Chemical ecology of any ecosystem, natural or managed is complex involving a wide range of biochemical interactions resulting into production, transformation and accumulation of an array of chemical compounds. A multitude of factors such as parent material, climate, vegetation, disturbance regime, all play important roles in developing and functioning of an ecosystem. All ecosystems have certain buffering abilities to withstand man-made management interventions. It is argued that by human interventions complex natural ecosystems can be converted into simpler and more vulnerable ecosystems. However, the newly transformed ecosystems can be sustainable provided that the management interventions do not interfere with the basic functional characteristics of the system. The intensity of management is directly related to the buffering ability of an ecosystem. Sustainable management regimes must be devised within this buffering limit. An understanding of the basic bio-physico-chemical response of an ecosystem to management regimes is a prerequisite for its sustainable management. Knowledge of vegetation response to disturbance and their effects on soil chemistry can give prior indications of ecosystem stress. Case study of a *Kalmia*- black spruce ecosystem showed that multiple ecological processes such as competition, allelopathy, allelochemical-nutrient interactions, and conifer-mycorrhizal relationships following disturbance play important roles in regeneration of conifer forests.

## Introduction

Chemical ecology of any ecosystem, natural or managed is complex. It involves a wide range of biochemical interactions resulting into production, transformation and accumulation of an array of secondary plant and microbial metabolites in soil many of them are allelochemicals (Rice 1984; Inderjit et al. 1999). The type of compounds and their rate of production and accumulation in an ecosystem depend on a multitude of factors such as vegetation type especially the dominant plants and their age, ecosystem disturbance regime, environmental stresses and soil physical, chemical and biological characteristics (Fig. 1). The process is further complicated in managed ecosystems such as agricultural and intensively managed forestry and horticultural systems where additional synthetic chemicals are applied as fertilizers, insecticides and pesticides. The cropping pattern such as crop rotation, method and frequency of harvesting also impose significant influence on physical, chemical and biological properties of soil environment. These have long- and short-term implications for the ecological integrity of the ecosystems. Objectives of the present paper are i) to describe the interacting nature of the many physical, chemical and biological factors that affect the chemical ecology of an ecosystem, ii) to conceptualize the effects of management interventions on ecosystem function and integrity and iii) present specific case studies showing the effects of disturbance induced vegetation change and the inhibitory effects of ericaceous plants on conifer regeneration.

## Ecosystem integrity and Management interventions

Management interventions can transform complex natural ecosystems into simpler ecosystems with new sets of ecosystem properties and new dynamic equilibria (Fig. 2). The integrity and equilibria of the transformed ecosystems can be maintained by implementing carefully designed management regimes that do not interfere with the overall functioning of the system. Conversion of one ecosystem into another by human intervention is not uncommon. All agricultural and most of the pastoral and horticultural systems are developed from natural forests by human. However, the long-term sustainability of the converted ecosystem is the issue at hand. The intensity and style of management have direct impact on sustainability of the system.

An understanding of chemical ecology of an ecosystem can give a cautionary signal as to its health and integrity before the basic properties of the system change. All ecosystems have certain buffering ability to withstand some degree of natural and human induced management interventions. In order to maintain the basic integrity of the system the management interventions must not only exceed the limit of its buffering ability but must make up for the deficiencies resulting from the periodic management induced stress. High intensity management interventions involving repeated disturbance and chemical input may gradually degrade and eventually break down the ecological

integrity of the managed ecosystems (Fig. 3). Low input traditional agriculture using traditional cropping pattern, cultivation method and indigenous crops is an example of sustainable ecosystem management in the developing world that has been practiced for hundreds of years. In the developed nations mechanized agriculture with high input of fertilizers, insecticides and pesticides are used to grow genetically altered crops often with irrigation. Generally speaking it provides higher yields as long as the agriculture inputs are assured. However, the long-term sustainability of the ecosystem properties and continuous high yield under this management regime is questionable. Careless management in either system can bring about disastrous consequences, for example, salination of agricultural lands in some tropical countries from deep tube well irrigation for growing high yielding varieties of rice and wheat.

### **Ecosystem disturbance and vegetation change: Response of ericaceous plants**

Many temperate forests of Europe and North America characterized by cool and moist climate and ericaceous understory. Their humus rich forest floors have slow decomposition, high paludification and nutrient sequestration. After forest canopy removal by harvesting and fire the ericaceous plants regenerate vigorously and interfere with conifer regeneration (Fraser 1993; Fraser et al. 1993; Page 1970; Mallik 1996). In some nutrient poor sites the disturbance induced ericaceous growth can transform forests into heathlands precluding tree regeneration (Mallik 1995). These plants are quite resilient to disturbance, equipped with efficient regeneration strategies after disturbance and are adapted to nutrient poor soils (Mallik 1993). Within 6-8 years after disturbance they can spread rapidly forming almost continuous cover.

### **Response of Conifers**

Planted as well as naturally regenerating conifers exhibit growth check symptom with stunted growth and chlorotic foliage (Table 1). After a few years of normal growth in the presence of heather (*Calluna vulgaris*), Sitka spruce (*Picea sitchensis*) seedlings become yellow (chlorotic) and their growth remains stagnant (Leyton 1954; Weatherell 1953). This type of growth check was widely observed in conifers five to seven years after planting in *Calluna*-dominated heathlands in Britain (Gimingham 1972). Similar growth-check phenomenon is also observed in the presence of salal (*Gaultheria shallon*) in regenerating western hemlock (*Thuja heterophylla*), Sitka spruce and western red cedar (*Thuja plicata*) sites on old growth clearcuts in coastal British Columbia, Canada (Germain 1985; Weetman et al., 1989a,b). Amabilis fir (*Picea amabilis*) may also experience growth check in presence of *Vaccinium* spp. in certain higher elevation Hemlock-Amabilis fir clearcut sites of Vancouver Island, British Columbia (A.U.Mallik, pers. com.). Inderjit and Mallik (1996a) reported on growth-check of black spruce (*Picea mariana*) in presence of *Ledum groenlandicum*, from the Clay belt region of northern Ontario, Canada. Inadequate natural regeneration of Norway spruce (*Picea abies*) in presence of bilberry (*Vaccinium myrtillus*) has been reported by several authors from the sub-Alpine spruce forests of France (Andre et al. 1986; Pellissier 1993). Plantation failures characterized by stunted growth and eventual death of black spruce in presence of *Kalmia angustifolia* has been reported on nutritionally poor sites of central and eastern Newfoundland (Page 1970; Titus et al. 1995; Mallik 1995). Growth inhibition of jack pine (*Pinus banksiana*) in presence of *Kalmia* understory has been reported in New Brunswick, Canada by Krause (1986). A low growing member of the Empetraceae *Empetrum hermaphroditum*, was found to be responsible for germination inhibition and seedling growth of Scots pine (*Pinus sylvestris*) in northern Sweden (Zackrisson and Nilsson 1992).

### **Mechanisms of conifer growth inhibition: Competition, allelopathy and soil nutrients**

Ericaceous plants generally possess very efficient vegetative regeneration strategies. They are quite resilient to disturbances such as forest fires and logging and their recovery after disturbance is fast. *Kalmia* cover can increase from 30% at harvest to 65% within 5 years and 85% within 15 years (van Nostrand 1971). The rapid vegetative growth of these plants results in high accumulation of litter on the forest floor (Damman 1971). Litter of ericaceous plants contains a wide range of secondary metabolites, some of which are phyto- and fungi-toxic (Jalal and Read 1983a,b). The litter is characterized by high N capital but low nutrient availability. A combined effect of allelopathy, resource competition and soil nutrient imbalance created by various phenolic compounds may result conifer growth inhibition. The following mechanisms are suggested to explain the ericaceous-induced growth inhibition in conifers:

Shortly after disturbance most understory ericaceous plants grow vigorously by vegetative reproduction and can attain high ground cover. Stem base sprouting, underground rhizomatous growth and repeated branching and to a limited extent layering contribute to this rapid spread of ericaceous plants after forest canopy removal. Moreover, vegetative sprouting and seed regeneration in many ericaceous plants are stimulated by cutting and fire (Mallik and Gimingham 1985; Mallik 1991; Messier and Kimmins 1990). This aboveground growth is accompanied by the proliferation of an extensive network of roots and rhizomes and thus enabling them highly competitive in the initial phase of forest succession (Mallik 1993, 1994). It has been suggested that resource competition, (such as soil available N and light) is the

main cause for reduced growth of western hemlock, Sitka spruce and western red cedar in presence of salal (Weetman *et al.* 1990; Messier 1992). Most vigorous sprouting and rhizomatous growth of salal occur under 70% of natural light (Huffman *et al.* (1994). By having morphological and physiological plasticity in allocating above- and below-ground biomass in relation to available light some broad-leaved ericaceous plants with sun and shade leaves such as *Kalmia*, *Vaccinium* spp. and salal become better competitors (Huffman *et al.* (1994; Bunnell 1990; Smith 1991; Sabhasri 1961; Sabhasri and Ferrel 1960; Mallik 1993).

Allelochemical inhibition of germination, and primary root growth and as well as seed predation have been suggested as the main reasons for failure of natural regeneration of Norway spruce in the presence of *Vaccinium myrtillus* (Pellissier 1994), Sitka spruce in the presence of *Empetrum hermaphroditum* (Zackrisson and Nilsson 1992), black spruce, red pine and balsam fir (*Abies balsamea*) in the presence of *Kalmia* (Mallik 1987; Mallik and Roberts 1994; Thompson and Mallik 1989). Growth-check of planted seedlings of these and other tree species is also thought to be due to allelopathic interactions between the understory shrubs and their associated conifers. Several secondary plant metabolites, mainly phenolic compounds such as *o*-hydroxyphenylacetic, *p*-hydroxybenzoic, vanillic, *p*-coumaric, ferulic, syringic and *m*-coumaric acids, found in the leaves and humus of *Kalmia*, *Ledum* and salal have been implicated in growth inhibition of black spruce, Sitka spruce and western hemlock (Zhu and Mallik 1994, de Montigny and Weetman 1990; de Montigny 1992; de Montigny *et al.* 1991). Wollenweber and Kohorst (1994) have extracted epicuticular leaf flavonoides from *Kalmia* and salal and speculated that these may also have growth inhibitory effects on conifers. Germination and root growth inhibition of Norway spruce in the presence of fresh leaves and humus of *V. myrtillus* are attributed to high concentrations of caffeic acid in them (Gallet and Lebreton 1994). Seasonal variation in the concentration of several phenolic compounds and batatasin-III in *E. hermaphroditum* leaves are thought to be responsible for its allelopathic suppression of seed germination of tree species (Zackrisson and Nilsson 1992; Nilsson and Zackrisson 1992, Nilsson *et al.* 1998; Wallstedt 1998). High concentration of tannins in leaves and inflorescence of salal are thought to be inhibitory to the growth of western hemlock, western red cedar and amabilis fir (Preston *et al.* 1998).

Organic molecules releasing from living and dead tissue of ericaceous plants can induce toxicity to conifers and their mycorrhizae as well as can create nutrient imbalance in soil making it unfavorable for conifer growth (Fig. 4). Most sites dominated by ericaceous shrubs have deficiency in available N (Inderjit and Mallik 1999). Inderjit and Mallik (1996b) demonstrated that water leachates of *Kalmia* can bring about significant change in nutrient concentrations of humus and mineral soil. They found significantly higher pH, lower concentrations of total phenols and available N and higher concentrations of Fe, Zn, K, Ca, Mg and Mn in soil amended with *Kalmia* leaves compared to the unamended control soil. These results support the views of Damman (1971) and Meades (1983, 1986) that a long-term occupancy of *Kalmia* in a site may bring about permanent change in soil nutrient characteristics which is unfavorable for conifer regeneration. However, toxicity effect on conifers and soil nutrient changing ability of phenolic compounds vary depending on the type of phenolic compounds, their concentration and residency time in soil (Inderjit 1996; Inderjit *et al.* 1999). Soil micro-organisms can transform many innocuous compounds including common plant phenolics and terpenoids into toxic forms and they also can degrade toxic compounds into non-toxic forms or concentrations (Inderjit *et al.* 1999; Blum and Shafer 1988; Blum 1998). Their growth inhibitory effects on conifers depend on their bioactive concentrations in soil. Ericaceous plants produce large quantities of polyphenolic compounds (e.g. tannins, humic acids, melanins and quinines) that can bind soil organic N as calcitrant protein-phenol complexes (Bull 1970; Ladd and Butler 1975; Tackechi and Tanaka 1987; Mole and Waterman 1987; Kuiters and Dennemann 1987). Bending and Read (1996a) have shown that ericoid mycorrhizae are able to utilize protein N that is complexed with tannic acid by means of enzymatic degradation whereas ectomycorrhizal fungi associated with conifers could not obtain N from the same source. They also demonstrated that ericoid mycorrhizae have the ability to utilize tannin as a carbon source, a feature other mycorrhizae do not have. The works of Leak and Read (1989, 1991) and Bending and Read (1996b) suggest that ericoid mycorrhizal associations have resulted from the selective force of low available nitrogen environments.

The above mechanisms may help explain why and how ericaceous plants could suppress conifer regeneration. Under field conditions these mechanisms can work simultaneously and/or sequentially to induce the growth inhibitory effects (Fig. 5). The site characteristics and climatic conditions play important roles in the growth inhibition process. In case of *Kalmia*-induced regeneration failure of black spruce in Newfoundland, Mallik (1995) suggested that the extent of regeneration failure will be determined by the combined effects of climate, site type, vegetation composition, type and frequency of forest disturbance and the resiliency, regeneration strategies, competitive abilities and allelopathic properties of *Kalmia*. Similarly, Messier and Kimmins (1991) and Prescott and Weetman (1996) suggested that competition for nutrients and declining site fertility after the assart flush are the major reasons for Sitka spruce, western hemlock and western red cedar growth check in young plantations dominated by salal. Zackrisson *et al.* (1997) demonstrated a three-way interaction between conifers, ericaceous plants and feathermoss in immobilizing nutrients which may cause growth inhibition in conifers in northern Sweden. Andre (1994) suggested that high phenolic contents of a *Vaccinium*-dominated humus layer in the forest floor restricted the development of mycorrhizae of Norway spruce seedlings in sub-Alpine forests. Figure 6 summarizes the dynamics of conifer-ericaceous interactions where competition, allelopathy and nutrient sequestration all play a role in creating unfavorable conditions for conifer regeneration (Mallik 1998). The mechanism and extent of conifer growth inhibition depend on the site type and the ericaceous species involved.

## Habitat heterogeneity and forest succession

Although *Kalmia* proliferation after clear cut and fire is common in nutrient poor *Kalmia*-black spruce forests of eastern Canada resulting into retrogressive succession there is evidence that trees eventually re-establish following a period of arrested succession. (i.e retrogressive succession) as long as the sites are not repeatedly disturbed (Meades 1983; A.U. Mallik unpublished data). Preliminary field observations suggest that mineral soil exposed by fire and micro-sites that were not previously occupied by *Kalmia* may allow black spruce regeneration after disturbance. These 'safe sites' provide favorable seedbed conditions for tree seedling establishment and thus may act as nuclei for regeneration of new forests. Site characteristics, response of the particular ericaceous species to disturbance, degree of habitat heterogeneity, and time since disturbance will determine the duration of retrogressive succession and the rate of progressive succession after that. For example stagnation of black spruce growth in presence of *Ledum* lasts for about 7 years after planting in northern Ontario (Inderjit and Mallik 1996a) where as black spruce growth inhibition in presence of *Kalmia* in central Newfoundland may last for over 20 years. Forest canopy closure in some nutritionally poor sites with high *Kalmia* cover may take 50 years and indeed in some cases the forest canopy closure may never happen.

## Vegetation control, site preparation and reforestation

Success of forest regeneration in areas with high ericaceous cover is often predicated upon the control of ericaceous plants. Regeneration behavior of these plants after disturbance are such that the traditional vegetation control strategies such as commonly used herbicides, scarification, cutting and burning are of limited success (Anonymous 1990). Among the herbicides tested, Garlon and a microbially produced herbicide, Bialaphos provide some control when applied in late summer (Jobidon 1991). Mulching can control some ericaceous plants by destroying their vegetative buds but the high cost of treatment and the difficulty of operating a mulching machine in stony soil makes this option impractical (Mallik 1991). Ploughing and liming has been a standard site preparation practice in Scotland for afforestation for many decades (Gimingham 1972). However, after a few years of normal growth, planted seedlings exhibit growth-check again with symptoms of N deficiency. Scarification of *Kalmia*- and salal-dominated sites also failed to improve conifer growth-check and visible N deficiency symptoms (Kumi 1984; Weetman *et al.* 1989a,b, 1990; Prescott and Weetman 1996; Mallik 1995). Repeated application of fertilizer-N (600 - 1344 kg per ha) was found to be effective in reducing *Kalmia* and salal growth and releasing conifer growth (Prescott *et al.* 1993, 1995). High concentrations of N and P are thought to disrupt the functioning of ericoid mycorrhizae which in turn may affect the growth of the ericaceous plants (Read 1982). Prescott and Weetman (1996) demonstrated that combined treatments of i) salal removal by cutting or herbicide followed by ii) dense planting of conifers and iii) subsequent N and P fertilization can stimulate the conifer growth and eradicate salal by shedding. Smith (1991) reported that salal growth can be reduced temporarily by prescribed burning.

Black spruce seedlings pre-inoculated with mycorrhizal fungi such as *Paxillus involutus*, *Laccaria laccata* and E-strain, were able to overcome *Kalmia*-induced growth inhibition of black spruce in greenhouse experiments (Mallik *et al.* 1998). Appropriate field trials are necessary to validate the results of the controlled experiments since the inoculated fungi compete with the naturally occurring microbial populations (McAfee and Fortin 1986).

## 6. Conclusions

We can draw a few conclusions from the above discussion: 1) chemical ecology of a natural ecosystems is complex and it gets further complicated by the input of chemical fertilizers, insecticides and pesticides under managed conditions. 2) Depending on the degree and frequency of human interventions natural ecosystems can be converted into managed ecosystems with different sets of ecological characteristics. 3) To be sustainable the ecosystems must be managed in such a way that the basic ecological integrity of the system remain unchanged. 4) Knowledge of vegetation response to disturbance and their effects on soil chemistry can give prior indications of ecosystem stress. 5) An in-depth study of *Kalmia*- black spruce ecosystem showed that multiple ecological processes such as competition, allelopathy, allelopathy-nutrient interactions, and mycorrhizae all play important roles in conifer forest regeneration.

## References

- Anonymous 1990. Impact of chemical and manual treatments on salal and conifer development-Project 2.17. FERDA Research Memo, No. 158, B. C. Ministry of Forests, Canada, pp 1-3.
- Andre, J. 1994. Regeneration de la pessiere a myrtle: allelopathie, humus et mycorhizes. *Acta Botanica Gallica* 141: 551-558
- Andre, J., Gensac, P., Pellissier, F. and Trosset, L. 1986. Regeneration des peuplements d'epicea en altitude: allelopathie et mycorrhization. *Revue d'Ecologie et de Biologie du Sol* 24: 301-310.

- Bending, G.D. and Read, J.R. 1996a. Effects of soluble polyphenol tannic acid on the activities of ectomycorrhizal fungi. *Soil Biology and Biochemistry* 28: 1595-1602.
- Bending, G.D. and Read, J.R. 1996b. Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi. *Soil Biology and Biochemistry* 28: 1603-1612.
- Blum, U. 1998. Effects of microbial utilization of phenolic acids and their phenolic acid breakdown products on allelopathic interactions. *Journal of Chemical Ecology* 24: 685-708.
- Blum, U. and Shafer, S.R. 1988. Microbial populations and phenolic acids in soils. *Soil Biology and Biochemistry* 20: 793-800.
- Bull, A.T. 1970. Inhibition of polysaccharase by melanine enzyme inhibition in relation to mycolysis. *Archives of Biochemistry and Biophysics* 137: 345-356.
- Bunnell, F.L. 1990. Reproduction of salal (*Gaultheria shallon*) under forest canopy. *Canadian Journal of Forest Research*, 20:91-100.
- Damman, A.W.H. 1971. Effects of vegetation changes on the fertility of a Newfoundland forest site. *Ecological Monograph* 41: 253-270.
- de Montigny, L. 1992. An investigation into the factors contributing to the growth check of conifer regeneration on northern Vancouver Island. Ph. D. Thesis, Forest Sciences Department, University of British Columbia, Vancouver.
- de Montigny, L. and Weetman, G.F. 1990. The effects of ericaceous plants of forest productivity. In: B.D. Titus, M.B. Lavigne, P.F. Newton and W.J. Meades (eds). Forestry Canada, Information Report N-X-271, pp. 83-90.
- de Montigny, L., L. Lowe, and C. Preston. 1991. Evidence for allelopathy by salal in conifer plantations of Northern Vancouver Island. SCHIRP Research Abstracts, University of British Columbia, Vancouver, B.C.
- Fraser, L. 1993. The influence of salal on planted hemlock and cedar saplings on northern Vancouver Island. M.Sc. Thesis, Forest Sciences Department, University of British Columbia, Vancouver, B.C., 166pp.
- Fraser, L., Turkinton, R. and Chanway, C.P. 1993. The biology of Canadian weeds. 102. *Gaultheria Shallon* Pursh. *Canadian Journal of Plant Sciences* 73: 1233-1247.
- Germain, A. 1985. Fertilization of stagnant Sitka spruce plantations on northern Vancouver Island. M. Sc. Thesis, University of British Columbia, Vancouver.
- Gimingham, C.H. 1972. Ecology of heathlands. Chapman & Hall, London.
- Gallet, C., and Lebreton, P. 1994. Evolution of phenolic patterns and associated litter and humus of a mountain forest ecosystem. *Soil Biology and Biochemistry* 27: 157-165.
- Huffman, W.D., Zasada, J.C. and Tappeiner II, J.C. 1994. Growth and morphology of rhizome cuttings and seedlings of salal (*Gaultheria shallon*): effects of light intensities. *Canadian Journal of Botany* 72: 1702-1708.
- Inderjit 1996. Plant phenolics in allelopathy. *Botanical Review* 62: 186-202.
- Inderjit and Mallik, A.U. 1996a. Growth and physiological responses of black spruce (*Picea mariana*) to sites dominated by *Ledum groenlandicum*. *Journal of Chemical Ecology*, 22: 575-585.
- Inderjit and Mallik, A.U. 1996b. The nature of interference potential of *Kalmia angustifolia*. *Canadian Journal of Forestry Research*, 26: 1889-1904.
- Inderjit and Mallik, A.U. 1999. Nutrient status of black spruce (*Picea mariana*) forest soils dominated by *Kalmia angustifolia*. *Acta Oecologica* 20: 87-92.
- Inderjit, Cheng, H.H. and Nishimura, H. 1999. Plant phenolics and terpenoids: Transformation, degradation, and potential for allelopathic interactions. In Principles and practices of plant ecology, Inderjit, K.M.M. Dakshini and C.L. Foy (eds.), CSC Press, Boca Raton, FL. pp. 255-266.
- Jalal, M.A.F., and D.J. Read. 1983a. The organic acid composition of *Calluna* heathland soil with special reference to phyto- and fungitoxicity. I. Isolation and identification of organic acids. *Plant and Soil*, 70:257-272.
- Jalal, M.A.F., and D.J. Read. 1983b. The organic acid composition of *Calluna* heathland soil with special reference to phyto- and fungitoxicity. II. Monthly quantitative determination of the organic acid content of *Calluna* and spruce dominated soils. *Plant and Soil*, 70:273-286.
- Jobidon, R. 1991. Control of *Kalmia* with bialophos, a microbially produced phytotoxin. *Northern Journal of Applied Forestry* 8: 147-149.
- Krause, H.H. 1986. Ericaceous vegetation as a site factor in jack pine growth of New Brunswick plantation. Proceedings of IUFRO Workshop, 7-10 October 1985, Fredericton, N.B., Canadian Forest series Fredericton, N.B., 182pp.
- Kuiters, A.T. and Dennemann, C.A.J. 1987. Water soluble phenolic substances in soils under several conifer and deciduous tree species. *Soil Biology and Biochemistry* 19: 765-769.
- Kumi, J. 1984. Effect of repeated fertilization and straw application to the organic layers under jack pine and seedling response. M.Sc. Thesis. Faculty of Forestry, University of British Columbia, Vancouver, 111pp.
- Ladd, J.N. and Butler, J.H.A. 1975. Humus-enzyme systems and synthetic organic polymer-enzyme analogs. In *Soil Biology* Vol. 4. (E. A. Paul and A.D. Mc.Larne, Eds.). Pp. 143-193. Marcel Dekker. New York.
- Leak, J.R. and Read, D.J. 1991. Experiments with ericoid mycorrhiza. In *Techniques for the Study of Mycorrhiza. Methods in Microbiology*. Vol. 23. J.R. Norris, D. J. Read and A.K. Varma, eds. pp 435-459. Academic Press. London.

- Leak, J.R. and Read, D.J. 1989. The effects of phenolic compounds on nitrogen mobilization by ericoid mycorrhizal system. *Agriculture, Ecosystems and environment* 29: 225-236.
- Leyton, L. 1954. The growth and mineral nutrition of Spruce and Pine in heathland plantations. Commonwealth Forestry Institute, Oxford, Institutional Paper, 31: 1-109.
- Mallik, A.U. 1987. Allelopathic potential of *Kalmia angustifolia* to black spruce. *Forest Ecology and Management*, 20:43-51.
- Mallik, A.U. 1991. Cutting, burning and mulching to control *Kalmia*: results of a greenhouse experiment. *Canadian Journal of Forest Research* 67(5):1309-1316.
- Mallik, A.U. 1993. Ecology of a forest weed of Newfoundland: Vegetative regeneration strategies of *Kalmia angustifolia*. *Canadian Journal of Botany*, 71:161-166.
- Mallik, A.U. 1994. Autecological response of *Kalmia angustifolia* to forest types and disturbance regimes. *Forest Ecology and Management* 65:231-249.
- Mallik, A.U. 1995. Conversion of temperate forests into heaths: role of ecosystem disturbance and ericaceous plants. *Environmental Management* 19: 675-684.
- Mallik, A.U. 1996b. Competitive ability and allelopathy of ericaceous plants as potential causes of conifer regeneration failures. *Journal of Korean Forestry Society* 84:394-406.
- Mallik, A.U. 1998. Allelopathy and competition in coniferous forests. In *Environmental Forest Science*, K.Sassa (ed.), Kluwer Academic Publishers, Boston. pp. 309-315.
- Mallik, A.U. 1999. Competition and allelopathy in tree-shrub-mycorrhizae interactions: problems and prospects of conifer regeneration with ericaceous understory. In *Advances on allelopathy*. Vol. I. A science for the future. F.A. Macias, J.M.G. Molinillo, J.C.G. Galindo and H.G. Cutler (eds.). pp. 79-90. University of Cadiz Press, Puerto Real, Spain.
- Mallik, A.U. and Gimingham, C.H. 1985. Ecological effects of heather burning II. Effects on seed germination and vegetative regeneration. *Journal of Ecology*, 73:633-644.
- Mallik, A.U. and Roberts, B.A. 1994. Natural regeneration of red pine on burned and unburned sites in Newfoundland. *Journal of Vegetation Science* 5:179-186.
- Mallik, A.U., Zhu, H. and Park, Y-G. 1998. Overcoming *Kalmia* induced growth inhibition of *Picea mariana* by mycorrhizal inoculation. *Journal of Korean Forestry Society* 87:429-444.
- McAfee, B.J. and Fortin, J.A., 1986. Competitive interactions of ectomycorrhizal mycobionts under field conditions. *Canadian Journal of Botany* 64: 848-852.
- Meades, W.J. 1983. The origin and successional status of anthropogenic dwarf shrub heath in Newfoundland. *Advanced Space Research* 2(8):97-101.
- Meades, W.J. 1986. Successional status of ericaceous dwarf-shrub heath in eastern Newfoundland. Ph.D. thesis, Univ. Connecticut.
- Messier, C. 1992. Effects of neutral shade and growing media on growth and, biomass allocation and competitive ability of *Gaultheria shallon*. *Canadian Journal of Botany* 70: 2271-2276.
- Messier, C. and Kimmins, J.P. 1990. Nutritional stress in *Picea sitchensis* plantations in coastal British Columbia; the effects of *Gaultheria shallon*. *Air, Water and Soil Pollutions* 54: 257-267.
- Messier, C. and Kimmins, J.P. 1991. Above and below-ground vegetation recovery in recently clear-cut and burned sites dominated by *Gaultheria shallon* in coastal British Columbia. *Forest Ecology and Management* 46: 275-294.
- Mole, S. and Waterman, P.G. 1987. A critical analysis of techniques for measuring tannins in ecological studies I. techniques for chemically defining tannins. *Oecologia* 72: 137-147.
- Nilsson, M.-C., Hogberg, P., Zackrisson, O and Fengyou, W. 1993. Allelopathic effects of *Empetrum hermaphroditum* on development and nitrogen uptake by roots and mycorrhizas of *Pinus silvestris*. *Canadian Journal of Botany* 71: 620-628.
- Nilsson, M.-C., & Zackrisson, O. 1992 Inhibition of Scots pine seedling establishment by *Empetrium hermaphroditum*. *Journal of Chemical Ecology* 51: 1857-1870. Page, G. 1970. The development of *Kalmia angustifolia* on black spruce cutover in central Newfoundland. Forest Research Laboratory, St John's, Newfoundland, Internal Report N-27, 7p.
- Pellissier, F. 1993. Allelopathic inhibition of spruce germination. *Acta Oecologica* 14: 211-218.
- Pellissier, F. 1994. Effect of phenolic compounds in humus on the natural regeneration of spruce. *Phytochemistry* 36: 865-867.
- Prescott, C.E. and Weetman, G.D. (Editors) 1996. Salal-Cedar Hemlock Integrated research Program: A synthesis. Faculty of Forestry, University of British Columbia, Vancouver. 85p.
- Prescott, C.E., Kumi, J.W. and Weetman, G.D. 1995. Long-term effects of repeated fertilization and straw application in a jack pine forest. 2. Changes in ericaceous ground vegetation *Canadian Journal of Forest Research* 25: 1984-1990.
- Prescott, C.E. Coward, L.P., Weetman, G.F. and Gessel, S.P. 1993. Effects of repeated nitrogen fertilization on the ericaceous shrub, salal (*Gaultheria shallon*), in two coastal Douglas fir forests. *Forest Ecology and Management* 61: 45-60.
- Preston, C.M. Trofymow, J.A. Sayer, B.G. and Niu, J. 1998. <sup>13</sup>C CPMAS NMR investigation of proximate analysis

- fractions used to assess litter quality in decomposition studies. *Canadian Journal of Botany* (in press)
- Read, D.J. 1982. The biology of mycorrhiza in the Ericales. *Canadian Journal of Botany* 61: 985-1004.
- Read, D.J. 1991. Mycorrhizas in ecosystems. *Experientia* 47: 31-36.
- Rice, E.L. 1984. Allelopathy, Academic Press, Orlando, FL.
- Sabhasri, S. 1961. An ecological study of salal (*Gaultheria shallon*). Ph.D. Thesis, University of Washington, Seattle.
- Sabhasri, S., and W.K. Ferrell. 1960. Invasion of brush species into small openings in the Douglas fir forests of the Willamette Foothills. *Northwest Science*, 34:77-80.
- Smith, N.J. 1991. Sun and shade leaves: clues to how salal (*Gaultheria shallon*) responds to overstory stand density. *Canadian Journal of Forest Research* 21: 300-305.
- Tackechi, M. and Tanaka, Y. 1987. Binding of 1,2,3,4,6-pentagalloylglucose to proteins, lipids, nucleic acids and sugars. *Phytochemistry* 26: 95-97.
- Thompson, I.D., and A.U. Mallik. 1989. Moose browsing and allelopathic effects of *Kalmia angustifolia* on balsam fir regeneration in central Newfoundland. *Canadian Journal of Forest Research* 19(4):524-526.
- Titus, B.D., Shidu, S.S. and Mallik, A.U. 1995. A summary of some studies on *Kalmia angustifolia* L.: a problem species in Newfoundland forestry. Canadian Forest Service, Newfoundland and Labrador Region, Information Report N-X-296. 68p.
- van Nostrand, R.S. 1971. Strip cutting black spruce in central Newfoundland to induce regeneration (Publication No. 1294). Natural Resources Canada, Can. For. Serv., St. John's Newfoundland, 68pp.
- Wallstedt, A. 1998. Temporal variation and phytotoxicity of Batatasin III produced by *Empetrium hermaphroditum*. Ph.D. thesis, Lund University, Lund, Sweden.
- Weatherell, J. 1953. The checking of forest trees by heather. *Forestry* 26(1): 37-41.
- Weetman, G.F., Fournier, R., Baker, J., Schnorbus-Panozzo, E. and Germain, A. 1989a. Foliar analysis and response of fertilized chlorotic Sitka spruce plantations on salal dominated cedar-hemlock cutovers on Vancouver Island. *Canadian Journal of Forest Research* 12: 1501-1511.
- Weetman, G.F., Fournier, R., Baker, J. and Schnorbus-Panozzo, E. 1989a. Foliar analysis and response of fertilized chlorotic Sitka spruce plantations on salal dominated cedar-hemlock cutovers on Vancouver Island. *Canadian Journal of Forest research* 12: 1512-1520.
- Weetman, G.F., Fournier, R., Schnorbus-Panozzo, E. and Baker, J. 1990. Post-burn nitrogen and phosphorous availability in coastal British Columbia cedar/hemlock forests and the use of fertilization and salal eradication to restore productivity. In S.P. Gessel, ed. Sustained Productivity of Forest Lands. Proceedings of 7<sup>th</sup> North American Forest Soils Conference. Friesen Press, Vancouver, BC.
- Wollenweber, E. and Kohorst, G. 1994. Novel epicuticular leaf flavonoids from *Kalmia* and *Gaultheria* (Ericaceae). *Z. Naturforsch* 39c: 710-713.
- Zackrisson O. and Nilsson, M-C. 1992. Allelopathic effects by *Empetrum hermaphroditum* on seed germination of two boreal tree species. *Canadian Journal of Forest Research* 22: 1310-1319.
- Zackrisson O. and Nilsson, M-C., Dahlberg, A. and Jaderlund, A. 1997. Interference mechanisms in conifer-Ericaceae feathermoss communities. *Oikos* 78: 209-220.
- Zhu, H. and A.U. Mallik. 1994. Interactions between *Kalmia* and black spruce: isolation and identification of allelopathic compounds. *Journal of Chemical Ecology* 20(2):407-421.

#### List of Figures

- Figure 1. Interacting factors affecting chemical ecology of a terrestrial habitat.
- Figure 2. High degree of ecosystem alteration occurs from natural forest to high-input agricultural system. New equilibria are reached and maintained by certain management regimes at each stage. Changes within each equilibrium state also occur such as, natural disturbance followed by micro-scale to large-scale secondary succession.
- Figure 3. With the increase in intensity of management complex chemical ecology of the habitat becomes simpler with a concomitant decline in buffering ability. The integrity of the ecosystem may break down by careless management practice.
- Figure 4. Allelopathically induced changes in soil chemical ecology.
- Figure 5. Role of resource competition, allelopathy and protein-phenol complex in conifer growth check in the presence of ericaceous plants (modified from Mallik 1998)

Table 1 Ericaceous plants causing growth check in conifers by competition, allelopathy and changing the chemical ecology of the habitats.

Ericaceous plant	Affected conifers	Geographic location	Interfering chemicals	References
<i>Kalmia angustifolia</i>	Black spruce	Eastern Canada	Phenols	Mallik 1987, 1995
<i>Ledum groenlandicum</i>	Black spruce	Central Canada	Phenols	Inderjit & Mallik 1996, 1997
<i>Gaultheria shallon</i>	Western hemlock, Western redcedar, Amabilis fir	Western Canada	Tannins	de Montigny 1992, Preston 1998; Mallik (pes. com.)
<i>Calluna vulgaris</i>	Sitka spruce	U.K. & Western Europe	Phenols & Fatty acids	Read & Jalal 1980
<i>Vaccinium myrtilloides</i>	Norway spruce	France, Switzerland, Norway	Phenols, caffeic acid Flavonoids	Pellissier 1993, 1994; Gallet 1994
<i>Eupetrum hermaphroditum</i>	Sitka spruce, Birch, Norway spruce	Fino-Scandinavia	Phenols, Batatasin	Zackrisson & Nilsson 1992, Nilsson 1994, 1998

## Antennal Arrays For Odor Detection and Discrimination

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Insect antennae offer a sensitive way to detect the presence of odor plumes and to monitor their fine structure, both in the laboratory and field, as first demonstrated by Baker and Haynes (1989). Interest in using antennae to quantify ambient concentrations of odor in the field has grown, as efforts to disrupt mating of moths with sex pheromones have met with increasing success in recent years (Sauer et al., 1992; Suckling et al., 1994; Suckling and Angerelli 1996). However, the usefulness and reliability of electroantennograms (EAGs) measured from such antennal preparations are limited due to their inability to discriminate both quality and quantity. A single EAG reading from a single antennal detector cannot discriminate a small amount of a compound to which the antenna is optimally tuned from a large amount of a compound to which it is sub-optimally tuned. Moreover, not only pheromone components, but plant volatiles as well can depolarize or adapt (or both) the antennal biosensor that is supposedly tuned to the pheromone, and prevent accurate quantitative EAG readings to be made. The EAG at present is useful in the field only for monitoring relative concentrations of a known pheromone compound dispensed for mating disruption or measuring concentration fluctuations within the fine structure of a plume of known composition. It has also been used to successfully measure plume fluctuations from a known source of host odor in the field from 60 meters away (Voskamp et al. 1998).

Recent interest by the U.S. Defense Department under the Controlled Biological Systems Program of the Defense Advanced Research Projects Agency (DARPA) in detecting and locating sources of anthropogenic compounds such as those emitted by unexploded landmines, has spurred efforts by our laboratory to increase the sensitivity and discrimination ability of insect antennal sensors. We have attempted to begin to overcome the fundamental limitations of single antennal biosensors by deploying arrays of differentially tuned insect antennae. Our initial results show that these arrays have the ability to at least crudely discriminate plumes or puffs of a single compound representing a single class of odor such as pheromone or host odor. These discriminatory assessments can be made in real time from split-second recordings of single puffs or plumes.

- Baker, T.C., and K.F. Haynes. 1989. Field and laboratory electroantennographic measurements of pheromone plume structure correlated with oriental fruit moth behaviour. *Physiol. Entomol.* 14: 1-12.
- Sauer, A.E., Karg, G., Koch, U.T., and J.J. De Kramer. 1992. A portable EAG system for the measurement of pheromone concentration in the field. *Chem. Senses* 17: 545-553.
- Suckling, D.M., and N.P.D. Angerelli. 1996. Point source distribution affects pheromone spike frequency and communication disruption of *Epiphyas postvittana* (Lepidoptera: Tortricidae). *Environ. Entomol.* 25: 101-108.
- Suckling, D.M., Karg, G., Bradley, S.J., and C.R. Howard. 1994. Field electroantennogram and behavioral responses of *Epiphyas postvittana* (Lepidoptera: Tortricidae) under low pheromone and inhibitor concentrations. *J. Econ. Entomol.* 87: 1477-1487.
- Voskamp, K.E., Den Otter, C.J., and N. Noorman. 1998. Electroantennogram responses of tsetse flies (*Glossina pallidipes*) to host odours in an open field and riverine woodland. *Physiol. Entomol.* 23: 176-183.

## Fruit, Flies and Chemoreception: What Have We learned?

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Tephritid fruit flies are one of the most serious pests of fruits and vegetables in the Asia-Pacific region. Because of man's cultivation of these crops, heavy use of insecticides to control fruit flies continues. Powerful semiochemical attractants have been developed to detect fruit flies in the field and even used in programs to suppress and eradicate fruit fly populations. However, what do we know about chemoreception and behavior of these pests? Can we improve on existing lures and traps and thus reduce the use of chemical insecticides by improving our basic understanding of fruit fly chemical ecology? In our laboratory we have been studying the chemical ecology and behavior of tephritid fruit fly pests. Our work has focused on basic mechanisms of chemoreception and behavior in the melon fly, oriental fruit fly and Mediterranean fruit fly. More recently we have investigated the physiological control of behavior in the Mediterranean fruit fly.

The chemical ecology of tephritids is complex and varies with species. Male flies of the genus *Bactrocera* appear to exhibit strong olfactory-mediated behavior to specific semiochemicals that are not clearly associated with their hosts. Electrophysiological studies (EAG's) have not explained the strong behavior of males and single cell recordings have not established specific receptors for the male attractants. In the oriental fruit fly, the attractive molecules elicit specific feeding behaviors that may provide an answer to the above question. In the Mediterranean fruit fly we have been studying the physiological basis for olfactory-mediated behavior. As a result of mating, females exhibit a clear switch in attraction from male pheromone to host-odors as a result of mating. The factors that are responsible for this change in behavior appear to be associated with the male accessory glands. We are currently trying to find out if other physiological changes such as alterations in the peripheral receptors change as a result of mating.

Although we can say that we understand some aspects of tephritid chemical ecology, there is still much more that we don't know about these pests. Continuing the spectrum of research studies from membranes to molecules to man will enhance efforts in control of these pests. Many other tephritid fruit flies have not been studied in any detail and thus promise to provide exciting research opportunities of scientists in the Asia-Pacific region.

## Chemical Ecology in Australian Cotton Agro-Ecosystems

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Intensive cotton production in Australia is less than 40 years old. In this short period Australia has become the world's third largest exporter of cotton, and regularly achieves the world's highest yields. The modern Australian cotton industry is capital-intensive, efficient and technologically sophisticated.

However, as in other countries there are challenges to the sustainability of the cotton industry. Cotton is grown on some of Australia's best agricultural land, but in regions which are ecologically sensitive. Insects, diseases and weeds are major threats to production, and intensive use of agricultural chemicals has been required to deal with these pests. This has resulted in the development of resistance to some chemicals and the contamination of soil, water and livestock products with pesticide residues. Consequences have included difficulties in international trade resulting from restrictions on access to markets for meat, and a widespread perception among the Australian public that the cotton industry lacks environmental responsibility.

In 1993, the Australian Cotton Cooperative Research Centre was formed to focus the resources of two universities and a three government research organisations on ways to improve the sustainability of the Australian cotton industry. Chemical ecology has been a central scientific discipline in the research programs of the Centre. In this paper I will provide a brief overview of the many ways in which chemical ecology has contributed to our efforts to avoid or ameliorate the problems associated with chemical use in the Australian cotton industry. These include:

1. Studies on the the fate of persistent compounds, especially endosulfan, in soil and water.
2. Research on the effects of these persistent pesticides on soil biota, and on the ways in which certain soil organisms metabolise pesticides.
3. Attempts to develop technologies which remove pesticides from irrigation water, and to contain them on farms, thus minimising the contamination of nearby waterways.
4. Research on the use of pheromones in reducing pesticides, including studies on the potential for attract-and-kill and mating disruption for key pests, especially *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae).
5. Studies on the development of blends of volatile chemicals derived from plants which attract adult *H. armigera*, particularly females. A more detailed description of this research will be presented by my colleague, Dr. Alice Del Socorro.
6. Research on the ways in which an understanding of chemical ecology can provide information about the mating behaviour, host-plant selection and survival of immature stages of the key pests, contributing to diverse aspects of integrated pest management such as the use of resistant varieties (including transgenics) and to the development of cultural control techniques including trap cropping.

This case study of the role of chemical ecology in providing the basic scientific knowledge required to underpin the development of ecologically acceptable pest management systems will illustrate its importance as a key scientific discipline in our attempts to develop sustainable agricultural systems for Australian cotton production, and by extension for other industries in Australia and throughout the world.