

THE IMPACT OF FUNDAMENTAL RESEARCH ON THE FOREST INDUSTRY IN THE MARITIME PROVINCES

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Selected research topics in several scientific disciplines are reviewed in the context of their potential or demonstrated value to the forest industry in the Maritime Provinces.

Introduction

I should like in the course of this paper to discuss several studies which might be described as fundamental or basic research, and which have had or potentially could have directly or indirectly an important impact on forest-based industry in this region. My selection of topics is personal and subjective. It reflects my association with the Canadian Forestry Service (indeed much of the research which I shall discuss has been carried out by C.F.S. scientists); it also bears the stamp of my own research interests as an organic chemist. I only hope that it does not reflect too strongly my ignorance of the forest itself!

Having flaunted the term "fundamental research" in my title, I feel obliged to add something of a disclaimer! The distinction between fundamental research and applied research is not, I think, an important one. It is clear that much research relating to forestry, or other disciplines for that matter, does not fit neatly into classifications such as "fundamental" or "applied". One can of course recognize differences in objectives and motivation that give rise to these labels. If, however, we accept that *all* true research is concerned with generation of new knowledge, and surely is motivated at least in part by curiosity, then the "applied/fundamental" dichotomy loses much of its significance.

The distinction, nevertheless, is made and used by research administrators, funding agencies, and many scientists, and in recent years it often seems that the scales have been tipped in favor of "applied" (presumably equated with "useful") research, undoubtedly as a result of political expediency. History ought to teach us that both basic and applied research are essential to our development, and that neither alone can prosper for long. Nobel Laureate, Dorothy Hodgkin, in her presidential address to the British Association for the Advancement of Science in 1978, recognized the difficulty in deciding how to classify research. I quote from an account of her talk (L.K.H. 1978): "She described a recent study by Comroe and Dripps which evaluated the scientific papers that have contributed to advances in clinical medicine. Of 529 papers, 41% were not clinically oriented at all and 62% could be described as basic research. They concluded that, in general, basic research paid off twice as handsomely as all other kinds of research and development combined. Hodgkin cited insulin as a case in point. 'If we knew in all fundamental detail how insulin acts to control our metabolism we might be able to devise far better methods for treating the different disorders associated with diabetes.'"

I would like to paraphrase Professor Hodgkin's reference to insulin in a forestry context and suggest that if we knew in all fundamental detail how the forest ecosystem works, we would be able to devise far better methods for managing our forests.

Forestry in the Atlantic Region

Let us try to put the importance of the forest resource in perspective by examining some figures (Fraser 1979).

Thirty-five percent of Canada's land area is covered by forest, of which over two-thirds can be classified as productive and accessible forest land by today's standards.

The forest resource provides jobs either directly or indirectly for over 10% of the nation's labor force. Exports of forest products currently total \$9 billion and account for 18% of our exports: they make a greater net contribution to Canada's trade balance than agriculture, fishing, mining, and fuels combined.

Much of the importance of the forest, of course, lies in less tangible contributions, such as regulation of water flow, sheltering of wildlife, etc. but these are beyond the scope of the present discussion.

The economy of the Maritime Provinces is linked especially closely with the forest resource, as clearly illustrated in statistics compiled by Runyon (1979).

"Forest land area in the Maritime Provinces is about 110,000 km² or 85% of the total land area compared to 37% for all of Canada.

Privately owned forest land in the Region is about 62% of total forest land, compared to 6% for Canada.

There are 845 million m³ of merchantable wood in the Maritimes or enough to build over 30 million houses. Softwood species make up 81% of this volume.

Estimated direct revenue from nonconsumptive uses of forest and forest land (fishing, hunting, trapping, etc.) is over \$5 million.

Average primary forest production (1972-1976) is more than 11 million m³ most of which is pulpwood. This represents a pile of wood 1m high and 1m wide stretching from Fredericton to Vancouver and back.

There are some 662 logging and manufacturing establishments in the Region which purchase \$675 million worth of fuel, electricity, materials and supplies.

Direct employment in forest industries is over 21,000 with an estimated 40,000 jobs generated indirectly. Salaries and wages amount to more than \$250 million.

In New Brunswick, one of every five persons employed in goods-producing industries works in forestry or related manufacturing. Pulp and paper mills rank first and sawmills second in terms of wages paid within the manufacturing sector.

In Nova Scotia, one of every 13 persons employed in goods-producing industries works in forestry or related manufacturing. Pulp and paper mills rank second and sawmills fourth in terms of wages paid.

Value added from forestry and related manufacturing in the Region is over \$500 million.

In New Brunswick, value added from logging operations alone is higher than for agriculture, fishing, or mining.

Forestry and related industries account for about 10% of gross domestic product in New Brunswick, 4% in Nova Scotia and less than 1% in Prince Edward Island.

Value of shipments of forest products for this Region in 1976 was over \$1.1 billion.

Value of exports from New Brunswick and Nova Scotia outside Canada is over \$650 million or 50% of all exports.

In New Brunswick, forest product exports comprise over 60% of all exports compared to 5% for agriculture, 7% for fish products, and 14% for mining and mineral products.

In Nova Scotia, exports of forest products in 1976 were 31% of the value of all exports, while agriculture goods were 5% and fishing, 27%."

The Spruce Budworm

As the forest industry, particularly the fiber industry, has developed in eastern Canada, balsam fir, once considered a weed species, has become of central importance to the economy. This development has had the effect of putting us into direct competition with the spruce budworm, formerly, in its own way the undisputed chief harvester of fir, but not considered a major economic pest. As a result, during the last 3 or 4 decades, the spruce budworm, *Choristoneura fumiferana*, has received the lion's share of attention from the forest research community in this region.

It has become increasingly clear that forest management strategies to maximize our portion of the resource can only be devised on the basis of an in-depth understanding of the budworm, its population dynamics, and the forest ecosystem of which it is a part: this is fundamental research with very practical implications!

At endemic population levels, the insect constitutes no more of a problem than any of a dozen other defoliator species which share the same host trees. With a mere handful of larvae if any per tree, the impact of budworm feeding activity on the foliage is barely perceptible. The notoriety of the spruce budworm stems from its periodic outbreaks in fir-spruce stands over immense areas, when populations in the course of 5 or 6 years may jump from half-a-dozen larvae per tree to an estimated 20,000! Few trees can sustain feeding at this high level for long, and widespread mortality of balsam fir usually results within a few years of the peak of such an outbreak (Miller 1975).

Extensive studies conducted at Green River, New Brunswick between 1945 and 1970 have provided the basis for much of our present knowledge concerning the dynamics of spruce budworm populations (Morris 1963).

It is useful to look at the pattern of the insect's life cycle (Fig 1) as a prelude to considering its interaction with the forest ecosystem and its population dynamics.

Several features of the cycle are noteworthy in the context of budworm population dynamics: The number of eggs laid per female, for instance, indicates that only about a 1% generation survival rate is required to maintain a constant population. Dispersal is a major cause of mortality of small larvae, and adult dispersal can bring about massive shifts of populations. The survival of large larvae has been identified as one of the most significant factors determining population trends. The stage at which major feeding occurs, and, in general, synchronization of the cycle with host-tree phenology have an important bearing on the interaction. A major factor determining the occurrence and severity of outbreaks is the distribution, age, and condition of balsam fir, the most favorable host for the budworm (Fig 2).

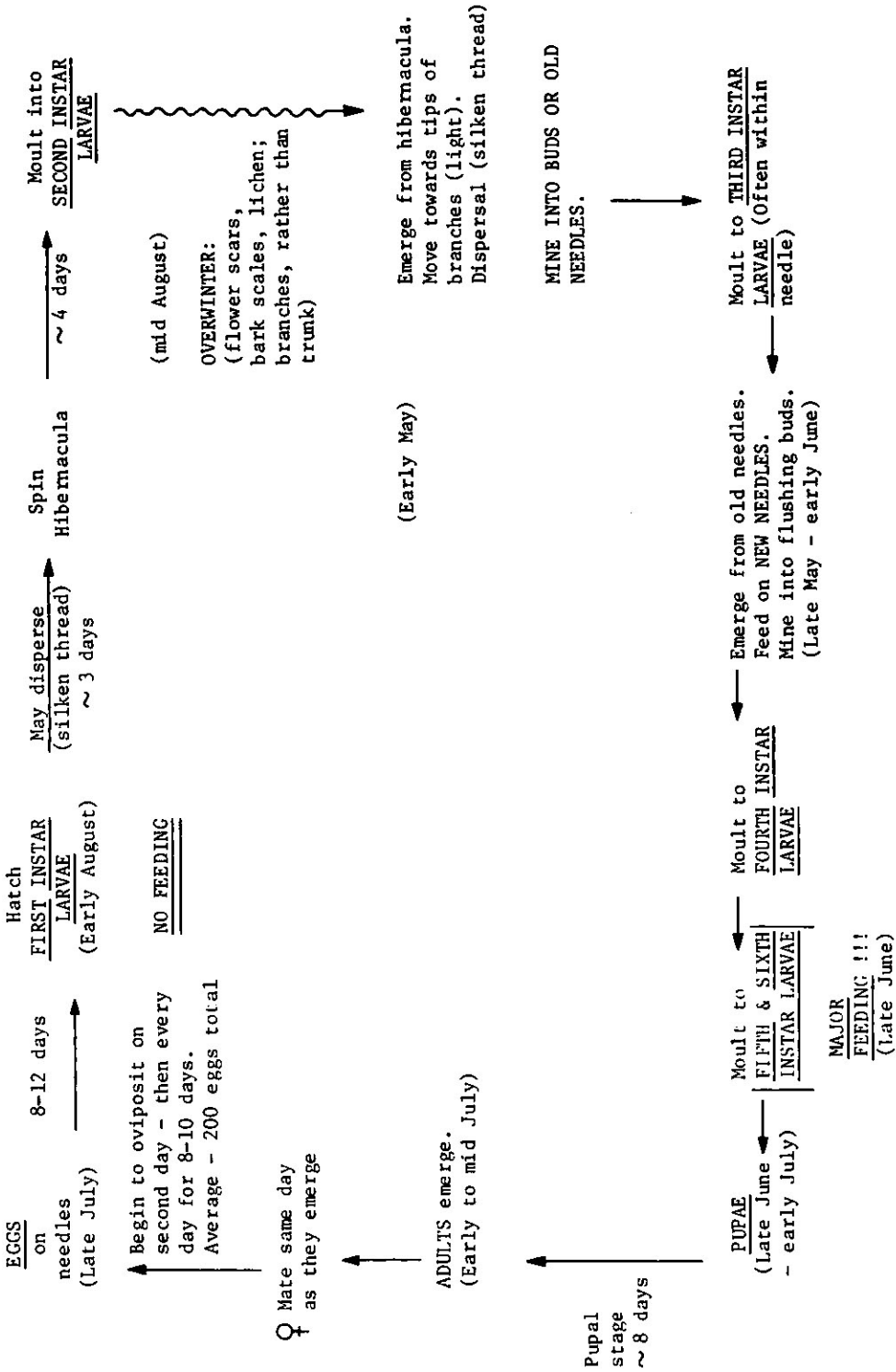


Fig 1 Life history of the spruce budworm.

PROCESS CYCLE
OF THE BUDWORM FOREST SYSTEM

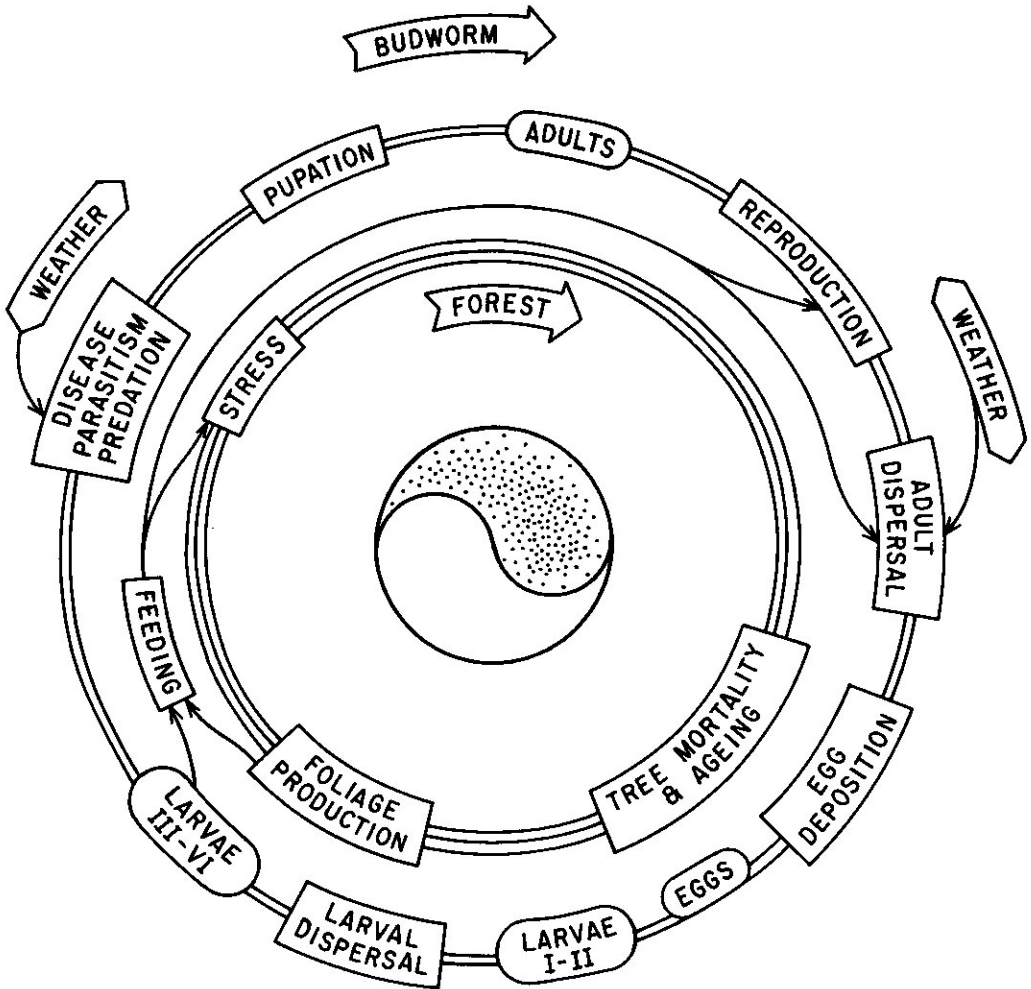


Fig 2 The process cycle for the budworm forest system. The inner ring represents the forest cycle, the outer ring the budworm cycle. Ellipses indicate insect life stages; arrows show causal relationships among processes and insect life stage densities. After Jones (1977).
Reproduced from W.C. Clark (1979) PhD thesis. University of British Columbia.

The overall picture which emerges, when we take into account the effects of biocontrol agents, weather, the composition and condition of the forest, etc. is a highly complex one with so many variables that it becomes virtually intractable. For this reason, mathematical models are currently finding favor as a method for organizing information on the dynamic behavior of the budworm-forest system (Baskerville 1976). Simulation models may prove to have great value in predicting in advance the consequences of various possible management strategies.

New Approaches to Forest Protection

While improved forest management and silvicultural practices may help to reduce our dependence on conventional chemical pesticides, it seems certain that some kind of insect control agents will remain a component of future integrated management approaches. Yielding to my chemical proclivities, I shall not elaborate in greater detail on the highly important areas of population dynamics, forest management and silviculture, but shall single out 3 recent "chemical-biological" approaches to insect control which might potentially supplement, if not supplant, the broad spectrum insecticides currently used against the budworm. I shall refer to the sex pheromone, insect growth regulators, and anti-feedants.

The Sex Pheromone

Let us consider first the sex pheromone of the spruce budworm, which has been identified (Weatherston et al. 1971; Sanders & Weatherston 1976) as a mixture of the E and Z isomers of 11-tetradecenal (Fig 3), in the approximate ratio 96:4. Several chemical synthetic routes have been developed, and the material is available in quantity for biological studies, including testing for mating disruption of the budworm in the field.

What is the function of the pheromone from the insect's point of view, and how can it be exploited for controlling budworm populations?

Investigations by Seabrook and collaborators at the University of New Brunswick, and by Sanders at the Great Lakes Forest Research Centre, indicate that in addition to its role as a long-range attractant, enabling male moths to locate their mates over a distance, the pheromone is also involved in initiating mating behavior patterns in the male. Thus, moths already in close proximity (for instance in high density populations) are most likely to mate during the evening while the female is releasing her pheromone.

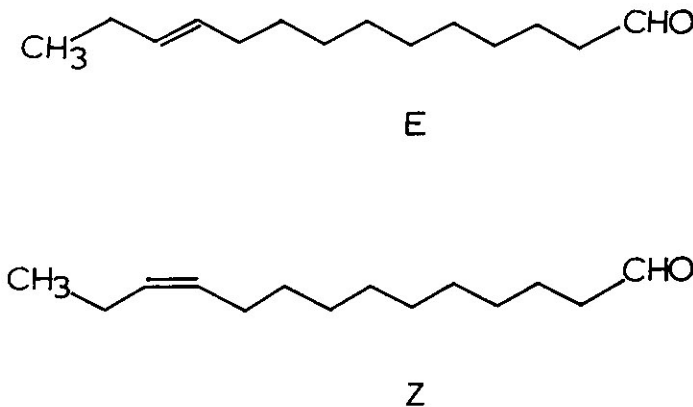


Fig 3 E and Z isomers of 11-tetradecenal

It is possible that the budworm's chemical communication system may be more complex than it appears on the basis of our present knowledge. Studies by P. Silk and C. Wiesner at the New Brunswick Research and Productivity Council could help to shed further light on the pheromonal interaction. These researchers are examining trace components in the effluvia and abdominal tips of virgin female moths, using inter alia, the powerful technique of combined gas chromatography—mass spectrometry. They and Seabrook's group are also seeking to determine whether or not a male pheromone is involved in the communication system.

The question which concerns the forest manager is: what is the potential of the sex pheromone for controlling budworm populations? At low to medium population densities where the male moth clearly depends on the pheromone to locate his mate, there are precedents to suggest that distribution of a large number of point sources of pheromone throughout the insects' range will confuse the males, and cause a reduction in male-female encounters. Suppression of budworm mating is indeed observed under such conditions in small-scale experiments with the pheromone.

Even more interesting, however, from the operational point of view, is the observation by the University of New Brunswick research group that when high density populations of budworm moths in cages (simulating outbreak conditions) are exposed to appropriate levels of pheromone, substantial reduction in mating also occurs. In such high population densities it seems unlikely that the male moth would experience difficulty in locating a mate, even without the guidance of pheromone, so mechanisms other than simple confusion must be responsible for the mating suppression. One such mechanism may be indicated by Seabrook's finding that exposure to concentrations of pheromone higher than the natural levels causes adaptation or blocking of the male's sensory system. There is also evidence which suggests that the female's own response to such levels of pheromone might contribute to a reduction in mating.

The true potential of the pheromone for controlling budworm populations can only be evaluated on the basis of a series of carefully conducted field trials, designed so that the effects of immigrating gravid female moths can be eliminated. Research along these lines is in progress.

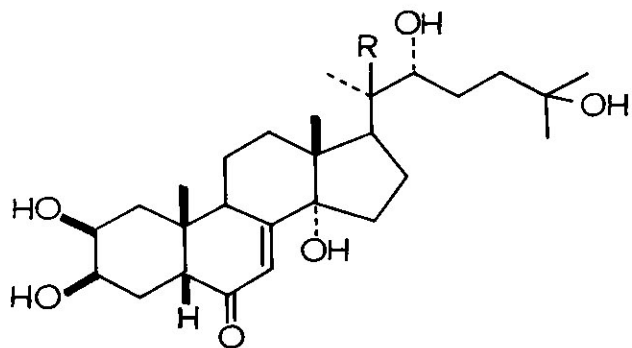
The problem of developing a suitable formulation which will ensure continuous release of the pheromone at appropriate levels for the required period (ca 3 wk) remains a formidable one, and calls for further extensive experimentation.

Another dimension has been added to the pheromone studies by University of New Brunswick chemists Z. Valenta, J.A. Findlay, and G. Lonergan who have prepared synthetic analogues of the natural material. Some of these compounds have displayed interesting biological properties in preliminary tests, and one shows a significant synergistic effect when used in traps with the natural pheromone.

From the foregoing discussion one may conclude that it is still too early to predict the extent to which pheromone-induced suppression of budworm reproduction will find use as a forest management tool.

My personal guess is that while pheromone will not be a panacea (there are no panaceas in forest management!), it could well play a significant role as a component of future integrated pest management programs.

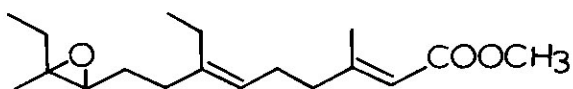
My discussion of the other "chemical-biological" approaches to which I alluded earlier, will be brief, and will be confined to description of the principles involved, as in contrast with the pheromone work, these have not yet progressed beyond the conceptual stage. I mention them mainly because of our own interest and involvement in this research.



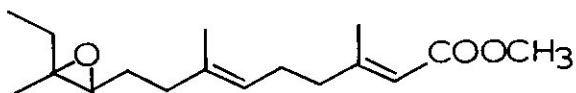
α - Ecdysone R = H

β - Ecdysone R = OH

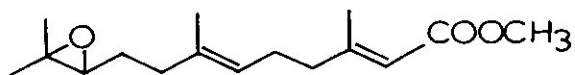
Fig 4 Alpha and beta ecdysone



JH I



JH II



JH III

Fig 5 Juvenile hormones

Insect Growth Regulators

While the development and metamorphosis of insects has fascinated philosophers and naturalists from time immemorial, by far the most dramatic progress towards our understanding of these phenomena has resulted from the research of contemporary insect physiologists such as V.B. Wigglesworth and C.M. Williams, not forgetting the important contributions of their biochemical and chemical colleagues. Their research has demonstrated that moulting and metamorphosis are under endocrine control, and that 3 major classes of hormones are involved (Rees 1977).

The brain hormone, characterized as a peptide, is produced by neurosecretory cells in response to various stimuli. The action of brain hormone on the prothoracic gland triggers the synthesis and release of the moulting hormone, ecdysone (Fig 4).

A third hormone (Fig 5), the juvenile hormone (JH) is produced by a gland known as the corpus allatum, which is under direct neural control from the brain.

Ecdysone induces the events associated with each moult. JH determines the morphological outcome of the moult by suppressing adult differentiation in favor of larval structures.

"High" levels of JH therefore favor a larval-larval moult, while a drop in JH titre results in a larval-pupal moult. JH is virtually absent at the pupal-adult moult (Rees 1977). Juvenile hormone or JH analogues applied exogenously at these last stages disrupt the normal development pattern of the insect, and thus have potential as insecticides. Indeed, JH mimics constitute one of the interesting groups of insecticides known collectively as insect growth regulators, some of which have already been used operationally.

Such insecticides, however, hold little promise for controlling insects like the budworm, which inflict their major damage at larval stages before they are vulnerable to exogenous JH.

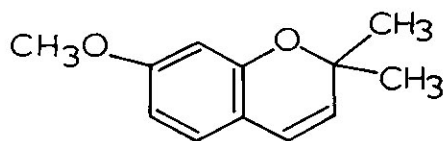
An alternative approach is suggested by the observation of Williams (1961) that surgical removal of the JH-producing corpora allata of the *Cecropia* silkworm during the last larval instar, led to pupae which possessed, prematurely, a number of adult characteristics.

Bowers et al. (1976) subsequently isolated 2 simple chromene derivatives, the precocenes (Fig 6), from the common bedding plant *Ageratum houstonianum*, which had the remarkable property of apparently disrupting JH production by the corpora allata of several hemipteran insect species.

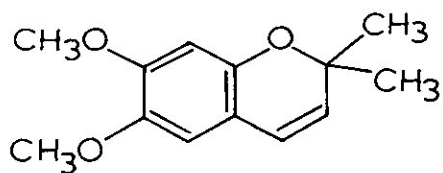
Precocenes, added at an early instar stage resulted in precocious moulting to unviable adults! Unfortunately the precocenes, simple natural products readily amenable to chemical synthesis, do not display this activity against the lepidoptera tested so far, including the budworm.

An approach differing from the extensive screening which showed up the precocenes is being pursued in our laboratory. We focus our attention on the JH I molecule. The biochemical pathway through which JH I is built up is understood in some detail, largely as a result of independent studies at the Zoecon Corporation and Texas A and M University (Rees 1977). The pathway appears to be unique to insects. The objective which we have set ourselves is to discover a compound which will specifically inhibit an enzyme catalyzing one of the biosynthetic steps to JH I. The consequent suppression of the JH level might then be expected to lead to precocious metamorphosis, as produced in hemiptera by the precocenes.

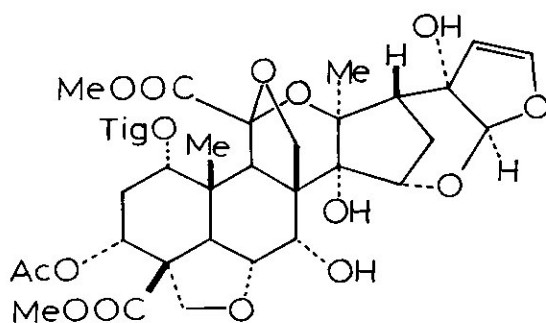
Several candidate compounds have been synthesized in our laboratory, and although none to date has shown the desired effect, this study is continuing, not without optimism.



Precocene 1



Precocene 2

Fig 6. Precocenes from *Ageratum houstonianum*

Azadirachtin

Fig 7 Azadirachtin

Antifeedants

The third of the "chemical-biological" approaches to budworm management which I want to mention is the possible exploitation of natural antifeedants.

For many insects, the selection of a particular host plant for feeding is influenced, at least in part, by the presence in the foliage of phago-stimulants or -deterrents. Included among the latter are antifeedants, defined by Kubo and Nakanishi of Columbia University as substances which when tasted can result in cessation of feeding, either temporarily or permanently depending on potency (Kubo & Nakanishi 1977).

One of the most powerful insect antifeedants known is azadirachtin (Fig 7), isolated from leaves and berries of *Azadirachta indica*, the Indian neem tree. This compound, investigated by E.D. Morgan and co-workers in England, and by K. Nakanishi and his group in the United States, exhibits strong antifeedant activity against a number of insect species, but most notably *Schistocerca gregaria*, the desert locust. These insects will starve to death rather than feed on leaves which have been surface-treated with only a few nanograms of azadirachtin per square centimeter! The compound does not appear to be generally toxic: neem sticks are chewed in parts of Africa and India to clean the teeth, and are also used as a remedy against malaria. Furthermore, berries of the tree constitute the favorite diet of several bird species (Kubo & Nakanishi 1977).

Recent entomological and chemical literature contains a growing number of reports of compounds (usually though not necessarily of plant origin) possessing anti-feedant activity against various insects. The activity may be detected by direct or indirect observation of feeding inhibition or by electrophysiological methods.

Antifeedants display a wide diversity of molecular structures, and many appear to be specific to certain insects. Little is known so far concerning the details of their mode of action. The possibility of finding antifeedants specific to certain insects and devoid of toxic effects on other organisms presents an exciting prospect in the context of pest control. For instance, reduction of feeding by budworm larvae through the agency of such a compound would not only have the effect of saving foliage, but the resulting physiological stress would reduce the vigor of the population and enhance the effects of natural mortality factors.

A mutual interest in discovering antifeedants for the spruce budworm has evolved into a collaborative research program between our laboratory and scientists at the University of Maine. At Orono, entomologist D.E. Leonard and chemist M.D. Bentley have developed a convenient and reproducible assay for observing antifeedant effects on sixth instar budworm larvae. Given an adequate supply of larvae, the method is suitable for an extensive screening program of non-host plant extracts and other materials. The Maine scientists have already tested more than 100 extracts and have observed some encouraging results. Additional extracts are being prepared in our laboratory.

Extracts which show promising antifeedant activity in the initial screens are fractionated into their constituents using standard chemical separation techniques. The fractions are monitored by bioassay, and material possessing the desired activity is purified. The molecular structure of the pure antifeedant may then be elucidated through chemical and physical studies.

A compound which displays strong antifeedant activity against the budworm in the preliminary tests must satisfy additional criteria before it can be considered as a potential control agent: its toxicity must be low, and it needs to be readily and cheaply available in quantity, either by extraction from natural sources or by synthesis.

The discovery of such an agent would add an invaluable new dimension to current strategies aimed at reducing damage caused by the spruce budworm.

Biological Control and the Winter Moth

In my brief reference to the population dynamics of the budworm I mentioned the influence of biocontrol factors, namely the predators, parasites, and disease organisms which have co-evolved with the insect in the forest ecosystem. How effective are these factors in regulating populations, and how does population density influence their effectiveness? What happens when extraneous factors are introduced which disturb the natural complex?

The answers to such questions have important implications in the planning of forest management strategies.

In the budworm-forest system (free from the intervention of *Homo sapiens*) insect populations have periodically risen to outbreak proportions, notwithstanding the existence of many natural enemies. The evidence indicates that there have been 6 or 7 such major outbreaks in eastern Canada during the past 200 years, each extending over millions of acres. As an outbreak situation develops, the budworm population at some stage exceeds the capacity of predators and parasites to contain it, and it then rises explosively until other factors eventually cause the population to collapse.

Baskerville (1975) argues that periodic outbreaks of the budworm do not indicate ecological instability of the budworm-forest system as a whole, when measured on a time-scale appropriate to the system function (50-100 yr). Indeed, he has termed the insect "super silviculturist", as its activities result in the development of successive generations of forest favorable to future generations of budworm: together they constitute a self-regulating system.

In contrast with the budworm, many native insects which could potentially be pests are in fact maintained at moderate levels by the action of parasites, predators, and disease. On the other hand, introduced species may become serious problems just because the biological agents which control their populations in their native habitat have been left behind (Balch 1960).

Whereas attempts to promote either indigenous or exotic biological control agents for regulating populations of native insect pests, such as the budworm, have not been fruitful so far, the approach holds great promise in combatting introduced insects which have become pests for the reasons just cited.

I would like to discuss one example of the successful application of biological control of such an introduced insect pest, which has particular relevance to this Province. Cast in the role of villain in this story was the winter moth (*Operophtera brumata* L.), apparently introduced accidentally from Europe into the eastern seaboard of North America via Nova Scotia (Embree 1971).

The winter moth is a serious defoliator of hardwoods and during the decade following its introduction, it wreaked havoc, particularly in Nova Scotia oak forests, with associated economic losses at today's values reaching millions of dollars. Had the insects' depredations continued, most of the oak now standing in Nova Scotia, estimated to be more than 1.5 million cords, would probably have been lost.

Credit for checking the infestation is shared by two parasites of the winter moth, deliberately introduced from a generally endemic environment in Europe. Following initial experiments and observations by A.R. Graham, D.C. Embree, a native of the Province working with the Canadian Forestry Service, led the control project and conducted the associated research on population dynamics of the host and parasites.

More than 60 parasites of the winter moth are known and presumably contribute to the regulation of its populations in Europe. Six species were selected and introduced in Nova Scotia during the period 1955 to 1960. Four of these were not

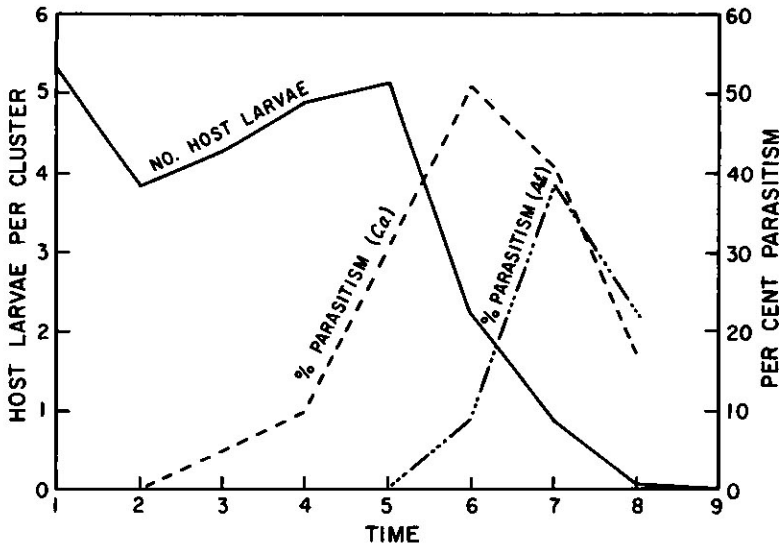


Fig 8 Typical history of a winter moth infestation and parasitism by *Cyzenis albicans* (Fall.) and *Agrypon flaveolatum* Grav.). Time refers to the number of years the outbreak persisted (Embree, 1966).

subsequently recovered, but 2, *Cyzenis albicans* (Fall.), a tachinid fly, and *Agrypon flaveolatum* (Grav.), an ichneumonid wasp became established, resulting within a few years in the collapse of the winter moth outbreak (Fig 8).

Embree (1971) points out that in 1954 when the study began, hardwoods were not extensively utilized in Nova Scotia, so that the winter moth was not deemed a major economic pest. Today on the other hand, paper and hardboard-producing mills designed to use hardwoods make an important contribution to the Province's economy. He adds wryly that if these mills had existed in the 1950's, it is likely that attempts to control the winter moth would have been based on the use of insecticides, because the success of the biological control program could not have been predicted.

The Nova Scotian experience has provided the stimulus for applying the same method in British Columbia, where an isolated population of the winter moth has appeared in the last 3 years. Parasites have been collected from surviving populations of winter moth in Nova Scotia and are now being released in Victoria, where the winter moth is feeding on Garry oak throughout the city.

Tree Improvement

Having devoted much of the foregoing discussion to insects which damage trees, I should like now to mention briefly some research which relates more directly to the trees themselves. Specifically, let us consider some aspects of the domestication of forest trees, an activity whose importance is being increasingly recognized in eastern Canada, as gloomy forecasts of wood shortages before the end of the century emphasize the finite nature of the forest resource.

Currently some 60 million trees, mainly spruces and jack-pine, are planted annually in the Atlantic region. This number is expected to rise to 100 million trees annually by 1985. By the time currently planted seedlings are 40 years old, it is projected that 20% of the region's forests will be of man-made origin (Fowler 1980 *in verb.*) Attempts to optimize productivity of this cultivated forest are based on application of

the art and science of tree breeding, nourished by research in genetics, tree physiology, ecology, and wood science.

The ultimate use of the crop will determine what characteristics are desirable in the cultivated tree: for example straight trees are obviously preferable for lumber production, but straightness is of little consequence to the paper industry, where fiber length is more important. Optimum growth performance, which is reflected in wood yield, is the single most important characteristic sought, regardless of the end use.

At first sight the domestication of trees might be expected to follow a pattern similar to that for other crops. Considering that for corn, genetics and intensive farming practices have increased yields perhaps 16-fold relative to the primitive wild corn plant (Rediske 1974), the implications for forestry are interesting indeed! However, problems of scale and long generation times present formidable difficulties to the tree breeder, not encountered by his counterparts in agriculture or horticulture.

How then does one go about large-scale production of genetically improved trees?

While hybridization experiments, usually intraspecific, sometimes yield interesting results, the most promising approach to tree improvement at present is the process of selection and breeding, based on the natural genetic variability of certain characteristics.

So-called plus-trees are first selected from the wild population. As part of this process the tree breeder attempts to sort out the relative contributions of genetics and environment in producing the desirable phenotype.

The trees of interest are those that in a given environment will be most productive, according to the criteria which have been set.

The usual method of mass producing genetically improved trees is by establishment of seed orchards on carefully chosen sites. In seedling seed orchards, progenies from the selected plus-trees are planted in an orchard pattern which is designed to maximize interpollination of the desired phenotypes. Alternatively, for many species, clonal seed orchards, based on grafts from the selected trees, have been found to be more effective in terms of genetic gain. In both kinds of orchards, culling procedures ensure that only the better genotypes are retained for final seed production.

The genetic gain which can be realized by propagation of selected plus trees is illustrated graphically in Figure 9 (courtesy of J.M. Bonga).

Plotting growth rate against frequency of occurrence of that rate, we obtain a normal distribution curve as shown (Rediske 1974). Plus trees are selected from the high growth end of the curve. The genetic gain from sexual reproduction of the plus trees is manifested as a displacement of the maximum, while decreased variability may lead to a narrowing of the base of the curve. Genetic gains of 10-20% are possible in the progeny, and these gains may be further enhanced in subsequent breeding phases (Rediske 1974).

While vegetative propagation in principle makes possible the retention of all the genetic components of the parental genotype, the method at present does not lend itself to mass production of most coniferous trees. The problem is that as a tree matures, it becomes increasingly difficult to propagate vegetatively, other than by the time-consuming process of grafting.

The use of tissue culture techniques might provide the key to vegetative propagation on a mass scale. However, although many plants have been successfully propagated by tissue culture, tissue from mature conifers has thus far proved obdurate. Callus from trees sufficiently mature to be recognizable as genetically superior has

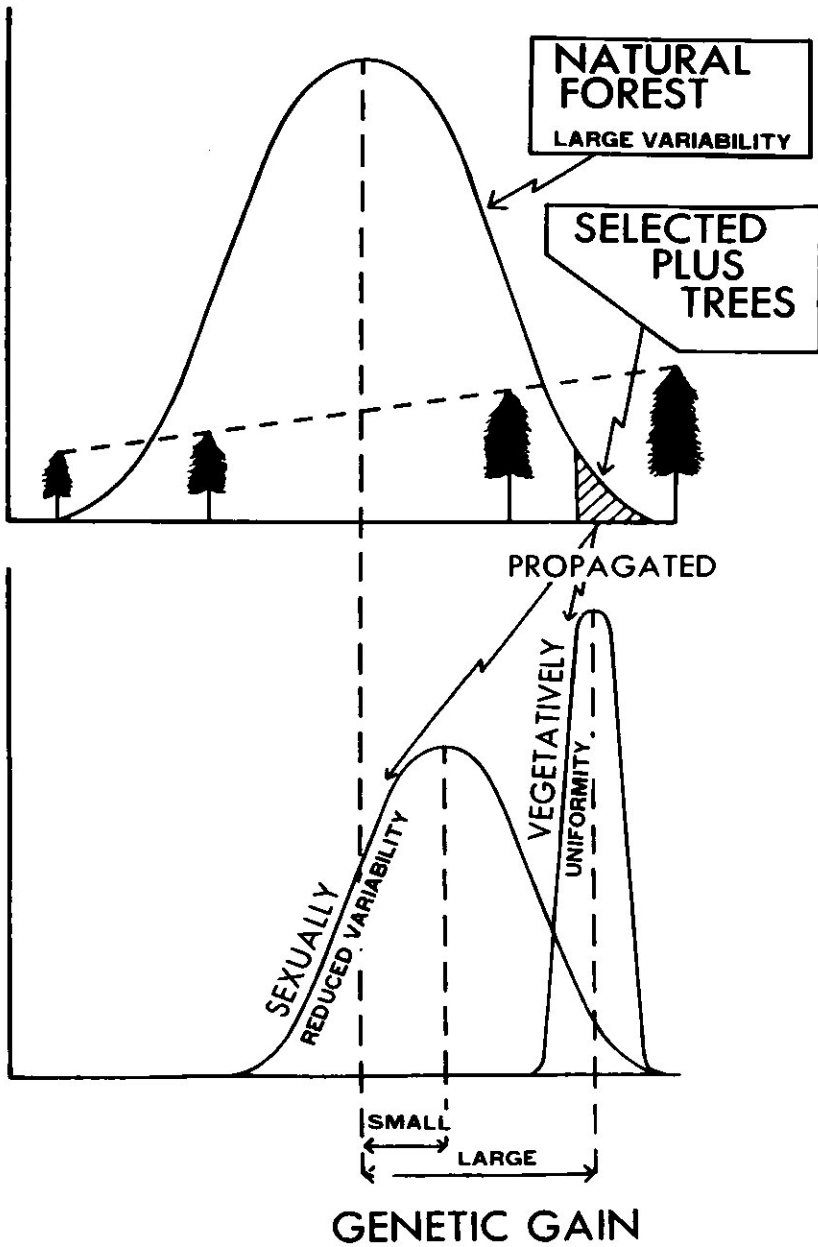


Fig 9 Genetic gain through propagation of selected plus trees.

yet to be induced to form differentiated structures. The search for a solution to this problem, which could make tissue culture an invaluable tool in tree improvement, remains a challenging research objective.

I alluded earlier to the problems posed to the tree breeder by the long generation time of his crop. Identification and evaluation of the best performers among parental or progeny generations may take 15 years or more. In attempts to develop methods to accelerate progeny ranking, several lines of research have been pursued. One interesting approach is based on studying correlations between photosynthesis and growth rate, in the hope that net photosynthesis, as measured by carbon dioxide uptake, could be used for early identification of growth potential.

Another time-related problem concerns flower initiation. As the juvenile tree is vegetative rather than reproductive in its growth pattern, the production of male and female strobili does not occur for several years. Research indicates that these patterns are regulated by hormones, and that exogenous application of appropriate compounds may in fact induce the reproductive stage in immature trees. Useful acceleration of sexual propagation could thus be effected.

The few examples which I have cited serve to illustrate that, as in other areas of forestry, basic research is an important component of the tree improvement process.

Fungitoxic Agents from Forest Fungi: Protection of Stored Wood Chips

In the final part of this paper, I should like to give a slightly more detailed account of some work from my own laboratory.

For a number of years it was my privilege to be associated with the late M.A. Stillwell in a joint study whose objective was to use modified biological control techniques in combatting microorganisms associated with tree diseases or with deterioration of stored wood or wood products. Besides *Ceratocystis ulmi*, the fungus causing Dutch elm disease, the target organisms of particular interest to us were those responsible for stain and decay of stored wood chips.

It is often necessary for pulpmills to maintain large inventories of wood chips in storage for periods of several months, because of fluctuating supplies and markets. During such extended storage the action of fungi and bacteria on the chips takes its toll, in the form of discoloration and reduced fiber quality.

The fate of stored wood chips is graphically described in a recent publication by Shields (Eastern Forest Products Laboratory Bulletin).

“Deterioration of chips begins as soon as a pile is built. Temperatures within a pile increase rapidly in the first few days. Initially this heat results from respiration of living wood cells and oxidation. This changes the chemical composition of wood extractives. In addition, fungi grow prolifically within a pile at this same time, creating more heat which contributes to the rapid increase in temperature in the large insulated chip pile mass. This warmer environment encourages growth of other “heat-loving” fungi which replace some of the earlier fungi that are restricted as the temperature rises above 40°C. Temperatures of 50 to 60°C and higher are found in large chip piles, and temperatures over 80°C, close to spontaneous combustion level, have been recorded.

The upward movement of hot moist air within a pile produces a “chimney-effect” and can result in steam appearing at the top of the pile on a cold day. Temperatures above 65°C kill virtually all fungi in a pile. However, many “heat-loving” fungi can grow profusely in warm moist chips at temperatures up to 65°C. Some loss of wood substance can occur under

the latter conditions, caused by those fungi which attack wood cellulose in the stored chips. An average weight loss of 1% per month of storage can be expected and losses up to 2% per month have occurred in some hardwood chips."

While several methods are available to ameliorate these problems including the use of small chip piles, first-on first-off processing, water sprinkling and chemical treatments, the pulp and paper industry still suffers substantial economic losses as a result of deterioration of stored wood chips, and the need for improved, environmentally acceptable methods to minimize these losses is recognized.

An attractive approach to the control of the wood-rotting and staining microorganisms affecting stored wood chips was conceived by Merlyn Stillwell, a tree pathologist at the Maritimes Forest Research Centre. In the course of studies on birch die-back disease, he had isolated from decayed yellow birch, *Betula alleghaniensis* Britt., an imperfect fungus, *Cryptosporiopsis* sp. which strongly inhibited the growth of other microorganisms (Stillwell 1966). Stillwell proposed that inoculation of a chip-pile with such a fungus, not itself a harmful wood-destroyer, could afford protection to the chips by inhibiting the establishment of the stain and decay organisms.

Studies conducted with a view to discovering the agent(s) responsible for the antagonism of *Cryptosporiopsis* sp. to other microorganisms led to the isolation of a crystalline fungitoxic metabolite from liquid culture medium which had supported growth of the fungus (Stillwell et al. 1969). This antibiotic, designated cryptosporiopsin, inhibited the growth of a broad spectrum of fungi, including many of those associated with stain and decay in wood-chip piles. On the basis of circumstantial evidence, it may be conjectured that cryptosporiopsin is at least partially responsible for the in vivo antagonism of *Cryptosporiopsis* sp. towards other organisms on a wood substrate (Stillwell 1966) but this has not been rigorously established.

In the context of the biological control strategy envisaged for protection of wood chips, pretreatment of the chips with a dilute solution of cryptosporiopsin prior to inoculation with *Cryptosporiopsis* sp. might assist the latter to become established on the substrate by reducing the population of competing microorganisms.

Chemical, spectroscopic, and X-ray methods were used in elucidating the molecular structure of cryptosporiopsin (McGahren et al. 1969; Strunz et al. 1969). The elemental composition of the substance was shown to be $C_{10}H_{10}O_4Cl_2$. Spectroscopic and other evidence suggested that the molecule could be represented by the partial structure I (Fig 10). Location of the point of attachment of the methyl ester group established the complete carbon framework of the molecule. This was accomplished by a two-stage reduction of cryptosporiopsin to the cyclopentanone derivative II, which was rigorously identified by comparison with a synthetic sample.

The remaining structural problem, that of placing the hydroxyl group and chlorine atom, was solved (after an initial erroneous assignment based on misleading evidence) by reductive removal of the latter atom through the agency of zinc in methanol. The product had spectroscopic properties which indicated structure III, proving that the zinc-labile chlorine was located on C-5, hence that cryptosporiopsin is represented by structure IV. The antibiotic was found to be identical with a metabolite of *Sporormia affinis*, investigated simultaneously in an independent study by researchers at Lederle Laboratories (McGahren et al. 1969). The Lederle group used chemical and spectroscopic evidence, and X-ray crystallography to assign the structure and absolute configuration V to the metabolite.

The potential value of cryptosporiopsin, not just in the context of wood-chip pro-

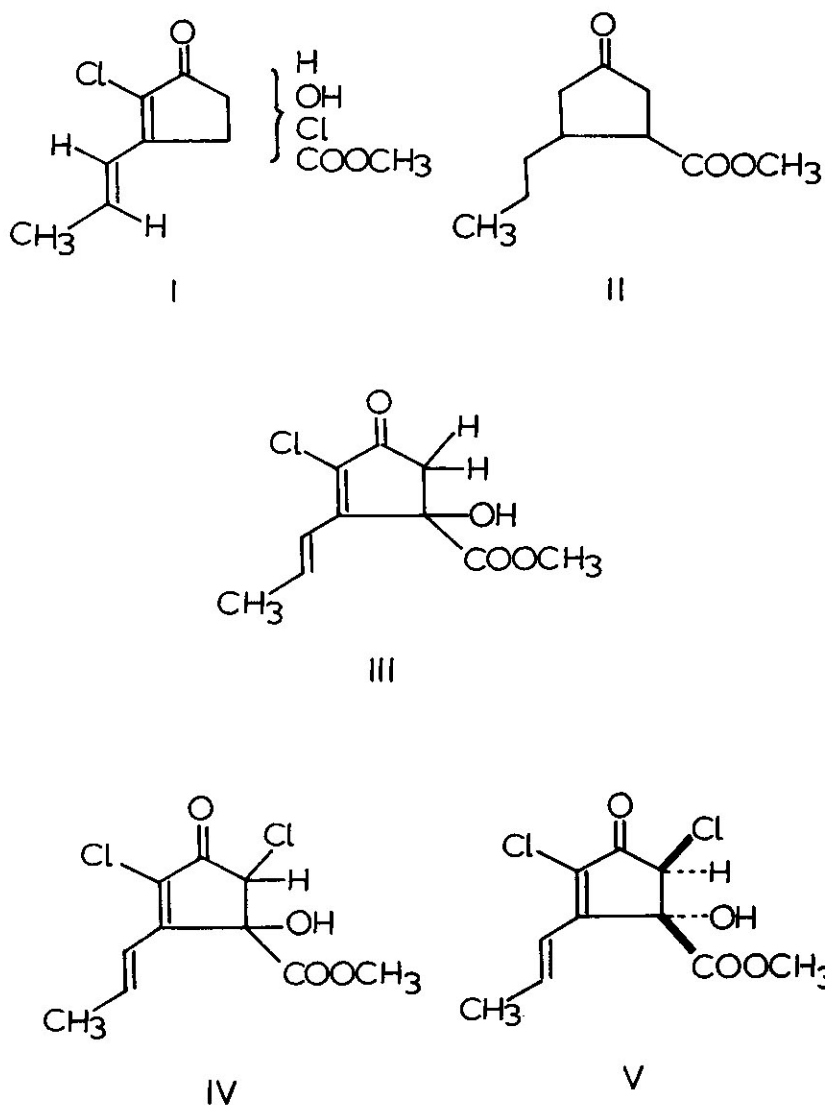


Fig 10 Structure of cryptosporiopsin (IV, V) and partial structures (I, II, III).

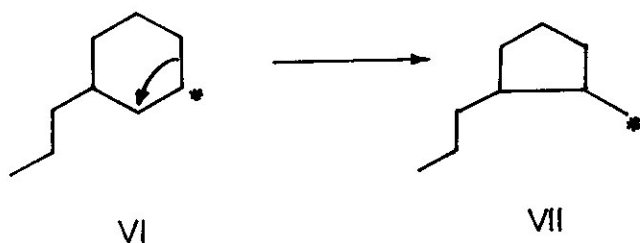


Fig 11 Contraction of a functionalized 6-membered ring to 5-carbon cyclic structure.

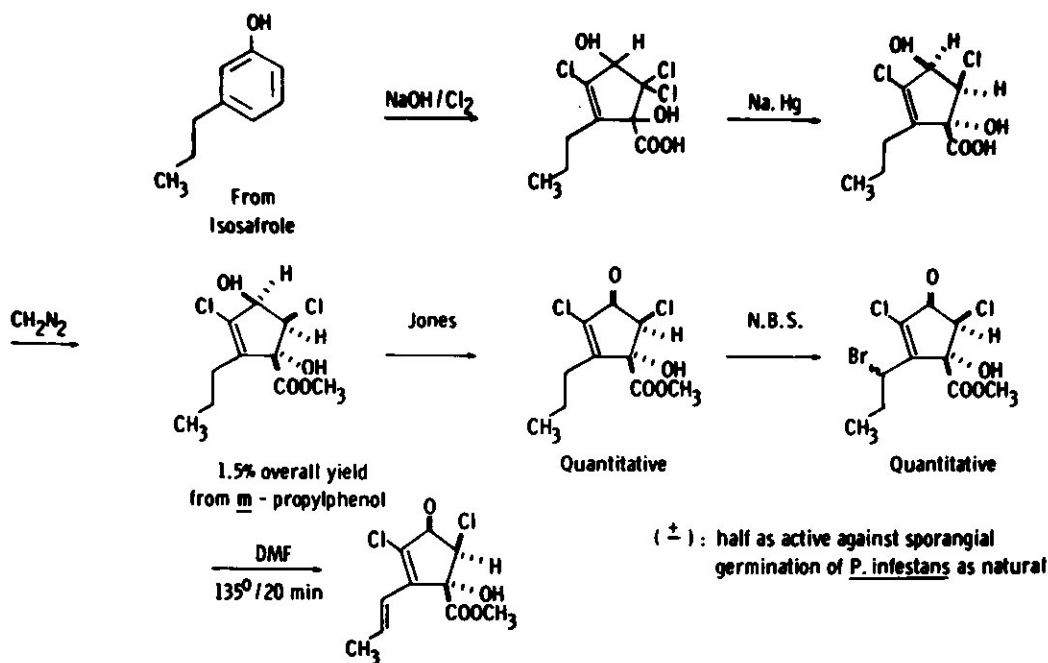


Fig 12 Synthesis of cryptosporiopsin.

tection, but in other areas including agriculture, possibly even medicine, provided impetus for research into production of the antibiotic by chemical synthesis. Development of a viable synthetic pathway to this small, richly functionalized molecule resulted (Strunz & Court 1973).

Central to the synthetic strategy was a reaction, discovered almost a century ago (Hantzsch 1887; 1889), which effected contraction of a suitably functionalized 6-membered ring to a 5-carbon cyclic structure, depicted schematically as the transformation VI to VII (Fig 11). This strategy parallels the biosynthetic pathway through which the metabolite is formed in nature. The synthesis is shown in detail in Fig 12.

The chemical synthesis, while less efficient and economical than the biological production of cryptosporiopsin, opens the way to studies on analogues of the antibiotic.

While the chemical work on cryptosporiopsin was proceeding, Stillwell was actively investigating the antifungal spectrum and biological properties of the organism and its interesting metabolite (Stillwell et al. 1969; Stillwell, unpubl.).

He did not neglect the potential practical application of *Cryptosporiopsis* sp. in protection of stored wood chips, and a collaborative study in this area was initiated with W.H. Whitney at the Fraser Company mill in Atholville, N.B.

In a typical pilot-plant scale experiment conducted by these investigators, chips contained in nylon mesh bags (ca 20 lb/bag green weight) were sprinkled with a suspension of *Cryptosporiopsis* sp. mycelium, or moistened with an aqueous solution of cryptosporiopsin (0.5-1.0 mg/ml). Control samples, stored under the same conditions were untreated. The temperature in the middle of the samples was periodically monitored. A second group of control samples was stored frozen.

At the end of the test period (6-12 mo) the chips were processed, and the properties of the chips and the pulp prepared from the 4 groups of samples were assessed. Early results were promising, apparently indicating that pulp from the antibiotic-treated chips was only slightly inferior to that from the frozen samples, and somewhat better than pulp from chips inoculated with *Cryptosporiopsis* sp. Both treatments afforded pulp of substantially higher quality than the untreated controls (Stillwell, unpubl.).

With these results as encouragement, additional tests were planned by Stillwell and Whitney, but these had to be abandoned because of a reorganization at the Fraser Company which resulted in the closing of the research facility and pilot plant at Atholville.

In 1970-71, further pilot-plant studies were organized with the enthusiastic cooperation of R.T. Thaxter and others at the Great Northern Paper Company. The initial experiments in Millinocket apparently substantiated by and large, the results obtained earlier in the Atholville study.

Subsequent attempts to reproduce these successful treatments failed however, and further research is necessary to determine and eliminate the source of these erratic results.

In the meanwhile, other fungi were screened by Stillwell for fungitoxic effects. Several promising organisms were selected on the basis of their antagonism to other microorganisms. Two of these fungi, *Scytalidium* sp. and *Hyalodendron* sp., received particular attention in our laboratory on account of their interesting fungitoxic metabolites scytalidin (Stillwell et al. 1973; Strunz et al. 1972) and hyalodendrin (Stillwell et al. 1974; Strunz et al. 1973).

On the chemical front, the structural puzzle presented by scytalidin was solved largely by careful analysis of its spectra, as well as by controlled oxidative degradations to the small readily identified fragments VIII, IX, and X. From the accumulated evidence the structure XI was deduced for scytalidin (Fig 13). It is a bis-anhydride, related structurally and biosynthetically to the nonadride metabolites glauconic, glaucanic, and byssochlamic acids, and the rubratoxins (references in Strunz et al. 1972). The biosynthesis of scytalidin appears to be closely analogous to that proposed for byssochlamic acid (references in Strunz et al. 1972), and accordingly can be considered to involve the coupling of 2-C₁₁ units possessing identical carbon skeletons at the appropriate oxidation level. The C₁₁ precursor may be derived from the condensation product of an octanoic acid derivative with oxaloacetate, a citric acid cycle intermediate (Fig 14).

The molecular structure of hyalodendrin XII (Fig 15), like those of the other metabolites was elucidated by a combination of spectroscopic and chemical methods (Strunz et al. 1973). It could be converted chemically to the tetrasulfide XIII or the di-methylthio ether XIV, both of which (Fig 15), were isolated as its co-metabolites from culture filtrates of *Hyalodendron* sp. (Strunz et al. 1975; Strunz et al. 1973). It is of interest to note that scientists at Lilly Research Laboratories in an independent study have isolated compounds enantiomeric with hyalodendrin and its congeners from fermentations of *Penicillium turbatum*. Similarly these compounds have been produced by an unidentified fungus NRRL 3888 at Abbott laboratories (references in Strunz et al. 1975).

Hyalodendrin is the simplest member so far isolated of the growing class of epidithiodioxopiperazine metabolites comprising, inter alia, gliotoxin, the sporidesmins, the aranotins, chaetocin, and verticillin A (references in Strunz et al. 1973).

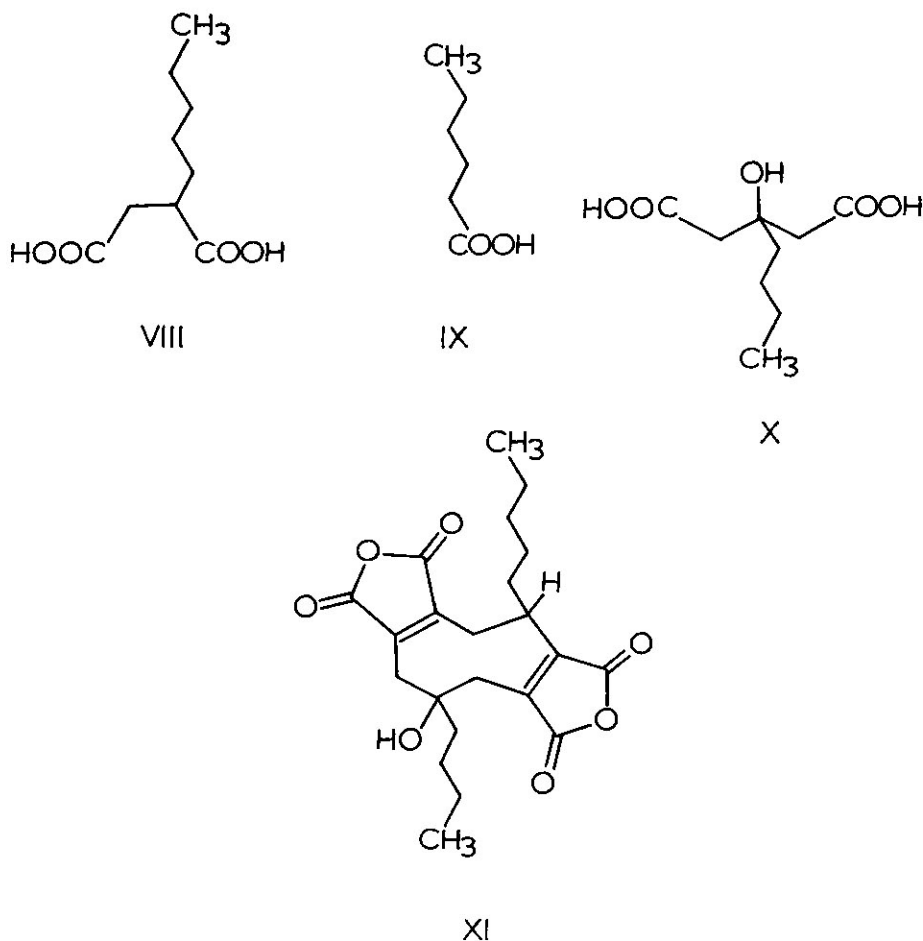


Fig 13 Structure of scytalidin (XI) and fragments (VIII, IX, X).

A chemical synthesis of hyalodendrin in our laboratory followed the ingenious strategy developed by Y. Kishi in his synthesis of dehydrogliotoxin and members of the sporidesmin family (Strunz & Kakushima 1974, and references therein). A key intermediate (Fig 15) in this approach is the thioacetal XV ($R = \text{CH}_3\text{OC}_6\text{H}_4$), a protected precursor of the epidithiodioxopiperazine ring. Sequential alkylation reactions are used to add the benzyl and protected hydroxymethyl functionality, and subsequent manipulations lead to racemic hyalodendrin. A simplified route to epidithiodioxopiperazines was later developed by Kishi and applied in a second synthesis of hyalodendrin (Fukuyama et al. 1976).

Interesting biological properties, including antifungal, antibacterial, antiviral, and cytotoxic effects have been found to be associated with various members of the class of epidithiodioxopiperazines (references in Strunz et al. 1973). While hyalodendrin was subjected to limited testing for protection of wood chips, its lack of solubility in water and its considerable mammalian toxicity will probably preclude its further development.

The untimely death of Merlyn Stillwell in 1977 has left the wood-chip study in our laboratory incomplete. The use of biological control methods to combat tree pathogens and organisms associated with wood decay remains an exciting possibility which continues to be explored in other laboratories.

Conclusions

The practical problems of forestry call for research in a number of scientific disciplines in order to develop all possible methods of dealing with them.

The ultimate objective is to integrate these methods into forest management plans, and their value should be assessed in terms of their long-term effects on the productivity of complex forest ecosystems.

Applied research and fundamental research are indivisible!

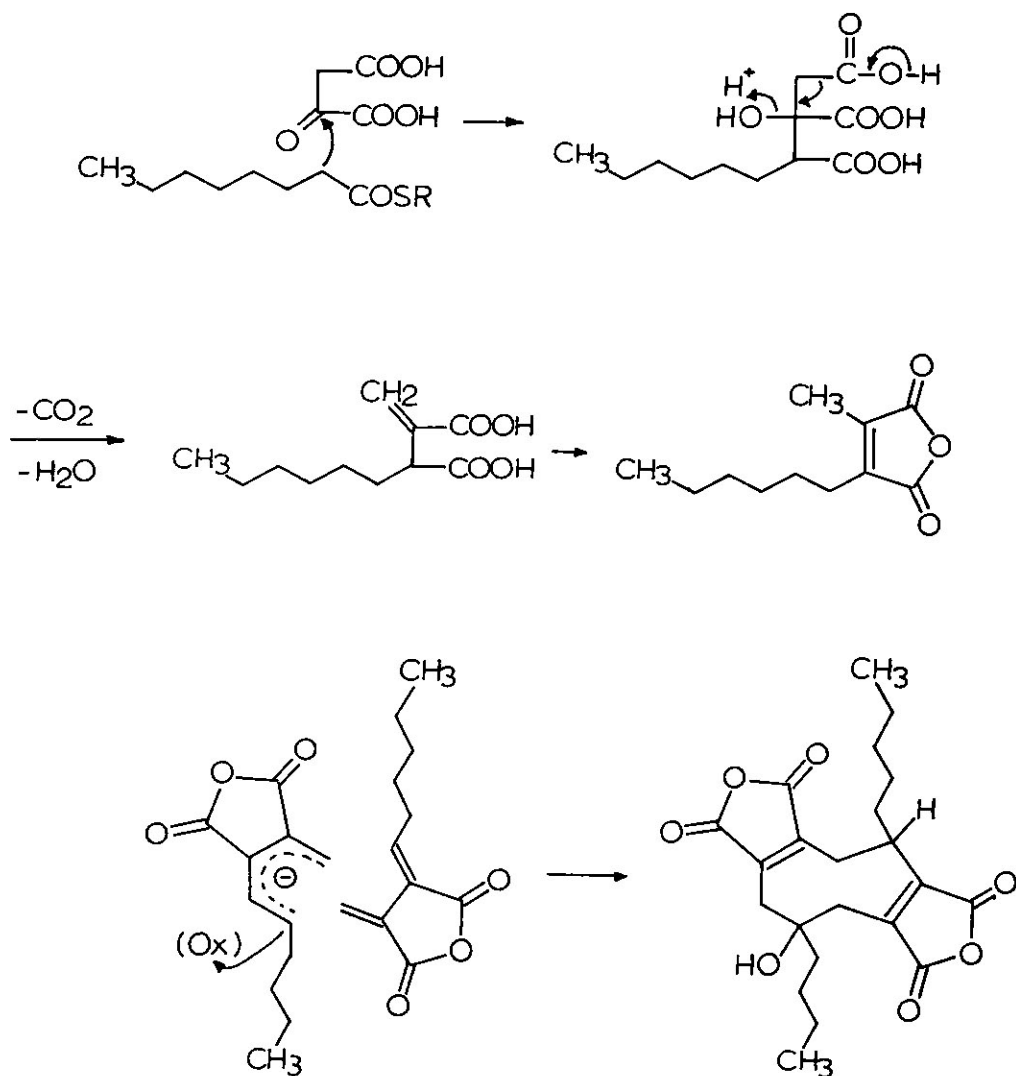


Fig 14 Biosynthesis of scytalidin

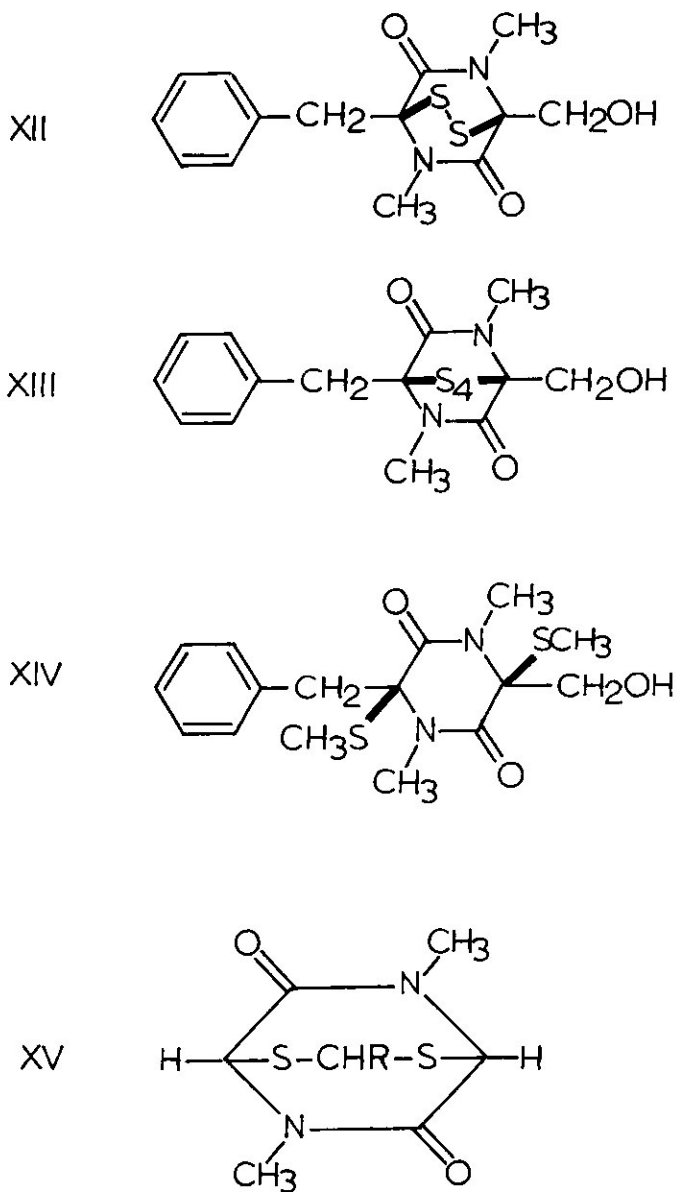


Fig 15 Structure of hyalodendrin and related compounds

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In the chemical work discussed in the latter part of the paper, I enjoyed the valuable collaboration of several co-workers whose names appear in the references.

Finally, I would like to pay tribute to the memory of Merlyn Stillwell, a stimulating colleague and good friend for more than 10 years.

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