Anti-Insectan Compounds from the Tropical Tree Family Dipterocarpaceae

Final Report

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Executive Summary

The PSTC project "Anti-Insectan Compounds from the Tropical Tree Family Dipterocarpaceae" was a collaborative biology and chemistry research project between the Southeast Asian Ministers of Education Organization Research Center for Tropical Biology (BIOTROP), located in Bogor, Indonesia, and Cornell University, Ithaca, New York. Concentrated on the search for naturally occurring insecticides, and other potentially useful chemicals, from tropical tree resins, the project also had training and institution-building components.

Tree resins were collected in forest stands throughout Indonesia, and subjected to preliminary bioassay in the BIOTROP laboratories in. Crude resins which killed termites were subjected to further fractionation and bioassay, in order to isolate insecticidal chemicals. Samples of these chemicals were sent to the collaborating lab at Cornell for further purification and structural analysis.

The research established that some of the toxic dipterocarp resins possessed known insecticidal chemicals, the most active of which were alloaromadendrene, humulene, and caryophyllene.

Field trials were undertaken with a compound used to stimulate latex rubber flow to determine if it might also increase resin yields. Studies conducted in South Sumatera established that this material, a commercial formulation containing 2-chloroethylphosphonic acid, doubled resin yields.

Studies of a defoliating forest caterpillar, a tussock moth, were carried out in South Sumatera, and in experimental forests in West Java. In addition to gathering baseline data on a potential pest species, this research resulted in the discovery of parasitic

wasps which may be useful for biological control.

The scientific results have implications for international development and tropical forest conservation. Tree resins may be harvested continuously with little damage to the tree; in any case the tree continues to set seed. It is possible that potentially useful chemicals harvested from tropical forest trees may have more long term value than the wood extracted from the trees. Sustainable development, based on exploitation of the resins, would thus promote tropical forest conservation. Together with the prospect of identifying useful natural products from plants in developing countries, this idea formed the basis for an agenda for scientific action endorsed by The International Society for Chemical Ecology at its 1989 Annual Meeting in Gothenberg, Sweden.

The project contributed to BIOTROP's institutional development in several ways. Training opportunities were provided for BIOTROP staff members working with the project. Technical skills relating to the laboratory work were taught, and formal instruction in computer use was provided. Two Indonesian students performed Master's Degree research on topics related to the project. Commodities purchased for BIOTROP included laboratory instrumentation and equipment, a field vehicle, and computer equipment. Experience in participation in international collaborations was gained, which will be important as BIOTROP seeks to expand its research activities.

Finally, the project demonstrated that novel, topical scientific research can be conducted at a developing country institution working in collaboration with an American university research lab. It also proved that basic scientific research in chemical ecology may stimulate tropical forest conservation.

Scientific, Historical and Cultural Background

The vast botanical diversity of Indonesia would make an unfocused search for insecticidal chemicals very complex. The decision was taken to streamline the search by concentrating on a group of plants for which there was both anecdotal and scientific evidence of insecticidal properties, and which had the possibility of being exploited in a sustainable, environmentally sound, manner should commercial possibilities arise. The resins of the plant family Dipterocarpaceae met these criteria. A review of the relevant literature indicated that resins might have some insecticidal properties. Other recorded uses suggested that the resins might possess additional useful chemicals, as well. Rather than examine a large number of plant species, we chose to look at those for which traditional or cultural uses implied biologically active chemicals.

Besides their scientific interest, the resins considered in this project have a long history, but one which is sometimes obscure. Because many traditional uses of resins were in remote villages, they were not documented. References to traditional resin use are thus scattered throughout the literature of Southeast Asia. Modern uses, mostly those involving production of varnishes and related materials, are better recorded.

Known generically as damar in the Indonesian and Malay languages, resins obtained from tapping trees of the Dipterocarpaceae have long been exported from Asia (Wolters, 1967). At or near the production site, resins have been used traditionally in varnishes and caulks, as fuel for illumination, in manufacture of handicrafts, and to seal burial jars. Though trade in resins was prevalent enough to find mention in popular verse (Kipling, 1894), the exact botanical sources of the resins are often

obscured by vernacular names (Endert, 1935). On the basis of historical and field research, however, it is possible to determine what species have been (or are) exported from production zones.

Of prime importance for varnishes have been the resins known generically as damar mata kucing, or "cat's eye damar." Judged from the extent of past and current tree cultivation and resin gathering activities, two Indonesian dipterocarp species appear to have accounted for most of this resin. These are Shorea javanica K. & V. (Torquebiau, 1984), and Hopea dryobalanoides Miq. (Rappard, 1937). As part of an agroforestry ecosystem Shorea javanica is widely cultivated by smallholders in areas of Lampung Province, Sumatera. Similar agroforestry ecosystems are not known for H. dryobalanoides.

Several other dipterocarp species have also been included in the damar mata kucing classification (S. lamellata Foxw., S. virescens Parijs., S. retinoides Sloot., and H. celebica Burk.), but because these resins were collected from existing forest stands, and not produced on man-made plantations, they likely contributed little to the bulk of the trade (Endert, 1935; Jafarsidik, 1987).

Tapping of wild stands of the Malaysian dipterocarp Balanocarpus heimii King for a second type of damar, damar penak provided resins for varnish production, but economic conditions prevailing at the time (1920's) prevented this resin industry from flourishing (Watson, 1927). Compared to the dimensions of the damar mata kucing trade, the production of damar penak was rather limited. The damar output of the entire Federated Malay States was less than that of a single province of Sumatera at about the same time (Watson, 1927; Endert, 1935; Rappard, 1937).

Resins can also be harvested from trees which are not in the Dipterocarpaceae. Resin collection from Indonesian conifers of the genus Agathis (Araucariaceae) results in a product marketed as kopal damar, a trade category based on the older name "Manila copal." Use of this trade category persists, though the conifer resins so named are entirely distinct from copal resins collected from the New World legumes of the genus Hymenaea. In addition, it is unclear exactly which Agathis species are grown for resin harvest. The trees are cultivated on government plantations which cover more than 60,000 hectares (Soenarno and Idris, 1987).

Dipterocarp resins employed locally in varnish formulations may not always be called damar. Liquid resins from the Indian Dipterocarpus tuberculatus Roxb., D. turbinatus Gaertn., and D. indicus Bedd. have been used as wood varnishes and in formulations for printing inks. The regional names for these, respectively, are engini, garjan, and velliani. Use of these resins in household items and putative ethnographic artifacts (carved wooden deities) has been described (Dutt, 1961). Similarly, resins of the Malaysian D. kerrii King are used in varnish manufacture, but are known as minyak keruing (Gianno and Kochummen, 1981) or gurjun balsam (Gianno, 1986).

Taken as a verb, the word damar glosses to "illuminate an area with a torch" (Echols and Shadily, 1989). As a noun, damar may translate variously as "resin," "torch," or "oil lamp." Damar kurung, referring to an almost extinct form of Javanese folk art resembling Japanese paper lanterns, translates as "encircled oil lamp" (Sabdono, 1987).

Tapping procedures for various dipterocarps are remarkably consistent given that resin-producing trees range from India to the Philippines (Clover, 1906; Watson, 1927;

Tschirch and Stock, 1933; Rappard, 1937). Four or more vertical rows of triangular holes are opened in the bole, and these holes may extend to a height of 10 meters or more. The shape of the holes allows them to be used as footholds for climbing the trees, which is done with the aid of rattan slings. In general, at about monthly intervals, the hardened resins are scraped into a basket from the sides of the holes with a special iron hatchet.

In Indonesia, processing of S. javanica resin at the production site is limited to sorting (Torquebiau, 1984). Based on color, size of lumps, and presence of inclusions and dirt, hardened resins are manually graded and bagged. While small amounts of inferior grades of resin are retained for production of crude boat varnishes, most of the damar is shipped out. Domestic uses in the Indonesian batik, incense and paint industries account for about 1/3 of the 2000-4000 tons of S. javanica damar harvested annually; the remainder is exported to Singapore (Mary and Michon, 1987).

Export of dipterocarp resins to South Asia from Sumatera may have begun as early as the 11th century C.E. (McKinnon, 1985). Camphor, gathered from fallen trunks of Dryobalanops aromatica Gaertn., was valued by Tamil traders for its medicinal properties. In addition to collection for export, the local uses of dipterocarp resins in production areas included fuel for illumination, and varnish and caulking for boats and handicrafts (Ma-Huan, 1451; Marsden, 1783). Because kerosene lanterns, and to a lesser extent electric lamps, now provide lighting in traditional production areas, damar production is maintained almost exclusively as an income-producing activity (Mary and Michon, 1987).

Objectives

Scientific Objectives

The overall goal of the research program was to determine the nature of any defensive chemicals produced by dipterocarp trees. This family of trees often dominates lowland primary forests in Southeast Asia. Several anecdotal reports, as well as preliminary research we conducted prior to this project, suggested that chemicals involved in protecting dipterocarp trees against biological attack might be contained in the trunk resins.

In a number of other experimental systems, tree resins had been shown to be important in plant defense against insect attack. Perhaps the dipterocarps used the same sort of defenses, mediated by similar chemicals.

Aside from the inherent scientific interest, the chance of discovering new insecticidal or fungicidal compounds was an important factor in the research program. There was also the possibility of finding chemicals which might have other applications. Chemical products derived from raw materials extracted from sustainable forest resources might thus establish an economic foundation for tropical forest conservation.

To this end, efforts were also made to study traditional agroforestry systems in South Sumatera. For hundreds of years, villagers in these areas have cultivated dipterocarp trees and harvested resin from them. The hardened resins are exported, and are sold for use in varnish and paint manufacture. Demand for these natural tree resins has remained essentially stable, because despite considerable advances in synthetic formulations, it is not possible to duplicate the properties of the natural damar

resins. The traditional agroforestry systems thus are likely to persist.

Research was conducted on issues relating to resin production, as well. Commercially-available stimulants of latex flow, normally used to increase yields from rubber trees, were tested for their ability to amplify resin flow from dipterocarps.

Development Objectives

The project had several objectives related to international development. These included training, institution-building, and technology transfer. The overall strategy was to provide Indonesian scientists with the intellectual and technological tools necessary to conduct investigations in chemical ecology and natural products chemistry relevant to national needs.

Indonesia has a wealth of plant resources. Screening these plants for potentially useful natural products is valuable from both scientific and commercial perspectives. It is appropriate for these surveys to be conducted by Indonesian institutions and scientists. Besides the convenience of carrying out such survey programs close to sources of plant material, implementing the programs in this manner insures that maximal benefits flow to Indonesia.

Completion of Specified Technical Objectives

The contract document described nine specific research objectives. These are listed here, with a brief comments on each objective.

1. *Collection of resins from dipterocarp trees.*

   This objective was successfully completed. Techniques established for this procedure are described briefly in Methodology (see page 17), and fully documented in the scientific publications in Appendix 4, page 46. Procedures for stimulating resin flow were invented, and subjected to field trials, as shown below (see Figure 2, page 18).

2. *Isolation and chemical characterization of resin components.*

   This objective was successfully completed. As described in detail in the scientific papers prepared for the Journal of Chemical Ecology (Appendix 4, page 46), several biologically active components of resins were identified and characterized.

3. *Field observations of termite attack in Indonesia.*

   This objective was completed with respect to dipterocarp plantations in South Sumatera, Lampung province. Termite damage was not detected on intact Shorea javanica dipterocarps; trees which had been subjected to resin harvesting sometimes showed termite damage to regions where the resin flow had been cut off. This essentially allowed the wood to dry out, and lose the protective components of the resins.

4. *Resistance of resin treated wood to termite attack.*

   Technical difficulties prevented us from achieving this objective. The plan was to treat pieces of wood with resins, place the pieces in a dishes with termites, and

measure the amount of wood which was consumed by the group of termites. At the end of a specified time period both the wood block, and the frass (the normally dry and compacted insect fecal materials) produced would be weighed to determine to what extent the resins changed the palatability of the woods. These experiments failed because: 1) Resin treatments changed the nature of termite frass. Normally termite frass is dry and compact, almost like large grains of sand. Under resin treatments, however, the termites deposited wet slurries of frass which adhered to the wood, so it was not possible to determine the mass of either the wood or fecal material. 2) In control groups, the amount of wood consumed was so small (5-15 mg) compared to the 2-4 g mass of the wood block that it was not possible to determine accurately the wood consumption rates.

5. *Toxicity tests.*

   This objective was achieved, as described in the Methodology section (see page 17), and in the Journal of Chemical Ecology papers provided in Appendix 4.

6. *Repellency tests.*

   Direct tests of repellency described in the contract were not carried out. We were not able to duplicate our earlier repellency tests, possibly because of behavioral differences between the termites species used for bioassays. In Bogor, termites offered a choice between treated and untreated filter papers semicircles presented in a petri dish repeatedly distributed themselves at the edge of the dish, and were not in contact with either paper. However, in other bioassays for toxicity, repellent effects were seen. They are described in the Journal of Chemical Ecology papers

in Appendix 4. In addition, independent tests conducted by colleagues at Rohm and Haas Co. on some chemicals purified from dipterocarp resins indicated that while resins did have some repellent characteristics, they were not as repellent as commercially-available compounds.

7. *Fumigation tests.*

   Pilot tests showed that resin vapors did not kill termites; these experiments were not repeated.

8. *Effects of resin extracts on insect development.*

   This objective was achieved on a limited scale. The original proposal called for milkweed bug assays to be conducted on resin chemicals. These were completed with resins of Dipterocarpus kerrii in Dr. Hagedorn's lab at Cornell. Resins of D. kerrii showed no hormonal activity in the milkweed bug assays. Milkweed bug assays were not conducted in Indonesia. After low dosage and long exposure (2 weeks) to crude resins, termites used in bioassays showed no morphological changes which might be expected if resins possessed hormone-like activity.

9. *Effects of resin extracts on termite gut flora.*

   This objective was achieved. Experiments in which resins or their chemical components were fed to termites repeatedly demonstrated that these compounds were toxic to the symbiotic protozoa responsible for cellulose digestion in termites. These experiments are summarized in detail in the paper "Defensive role of tropical tree resins: anti-termitic sesquiterpenes from Asian Dipterocarpaceae," included in Appendix 4.

Institutional Collaborators and Working Arrangements

Structure of the Collaboration.

The project was split between the institutional collaborators listed below. A schematic diagram of the project is given in Figure 1, page 16.

Adam Messer, a Cornell Ph.D. candidate fluent in Indonesian and with research experience in Indonesia, was based at the BIOTROP campus for 2 1/2 years, beginning in January, 1987. Messer managed the Indonesian side of the project, and acted as a liaison between the participating institutions, departments, and individuals. In addition to scientific duties, he arranged for the necessary work permits and visas, security clearances, and coordinated other administrative aspects of the project.

SEAMEO-BIOTROP, Bogor, Indonesia. An institute under the Southeast Asian Ministers of Education Organization, BIOTROP is devoted to basic and applied studies of tropical biology, and training. BIOTROP has research programs in Tropical Agricultural Pest Biology, Tropical Aquatic Biology, and Tropical Forest Biology. The PSTC project was administered under the Tropical Forest Biology program.

BIOTROP provided facilities for field and laboratory research. Host country counterparts and technical staff were also part of BIOTROP's contributions to the project (Refer to Personnel, page 14). BIOTROP assisted with approaches to Indonesian government authorities, including the Immigration and Forestry Departments, as well as the Indonesian Institute of Sciences (LIPI).

Cornell University, Department of Chemistry Ithaca, NY. Chemical studies of biologically active natural products were conducted in the laboratory of Prof. J. Meinwald. Postdoctoral and graduate chemists carried out structural analyses of materials sent from the BIOTROP lab. The Meinwald group also provided technical assistance to the field, sending materials as well as suggesting research methodologies.

Cornell University Department of Entomology, Ithaca NY. Experimental methods for bioassay of potential insecticidal and fungicidal compounds, determination of modes of action, and related techniques were developed in the laboratory of Prof. H. Hagedorn. The Business Manager of the Entomology Department administered the overseas portion of the PSTC grant.

Personnel

The following were direct participants in the PSTC project described in this report. Educational level and field of expertise are listed.

BIOTROP

Drs. Sunjaya[1], Counterpart Scientist, Entomology

Mr. Koko Iskandar, High School Graduate, Senior Technician, Laboratory Operations

Mr. Prihat Ramdhani, Technical School Graduate, Chemistry Technician

Universitas Sam Ratulangi, Manado, Sulawesi Utara

Ms. Ferny M. Tumbel, B.S., Student Researcher visiting BIOTROP, Entomology

Ms. Noni N. Wantah, B.S., Student Researcher visiting BIOTROP, Entomology

Cornell University

Dr. Jerrold Meinwald, Principal Investigator, Chemistry

Dr. Henry Hagedorn[2], Co-Principal Investigator, Entomology

Dr. David Richardson, Postdoctoral Research Associate, Chemistry

Mr. Kevin McCormick, B.S., Graduate Research Assistant, Chemistry

Mr. Adam Messer[3], M.S., Graduate Research Assistant, Entomology

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1. Drs. is the abbreviation for *doctorandus*, a Dutch title corresponding roughly to a level between American Bachelor's and Master's degrees. The corresponding title for women is Dra., for *doctoranda*.

2. Hagedorn moved to the University of Arizona, Tucson, in 1988. He continued his participation in this research project in his role as Director of the Center for Insect Science.

3. Messer was awarded the Ph.D. degree in January, 1990.

Research Implementation and Procedures

Division of Research Responsibilities

Research tasks were divided between the collaborating institutions to take maximal advantage of their resources. Resin collection, bioassay, and initial fractionation were based at the BIOTROP labs in Bogor, Indonesia. Here participating staff, working under the direction of Mr. Messer or Drs. Sunjaya, had ready access to tropical forest trees and insects for bioassay. Partially purified chemical fractions of biologically active resins, identified in the BIOTROP labs, were then sent to the laboratory of Dr. Jerrold Meinwald at Cornell. There procedures which required more sophisticated experimental techniques, especially those involving analytical organic chemistry, were carried out under Meinwald's direction. After analytical work had been completed on a compound, an authentic sample was sent back to the BIOTROP labs for further bioassay to confirm the assignment of toxicity to a specified chemical. These bioassays were conducted in a blind fashion to remove the possibility of experimental bias.

Figure 1, page 16, shows how the major components of the research plan were divided among the participating institutions. Except for the usual delays introduced by international mails and customs officials, the research scheme itself worked well. In only one instance were samples damaged in transit.

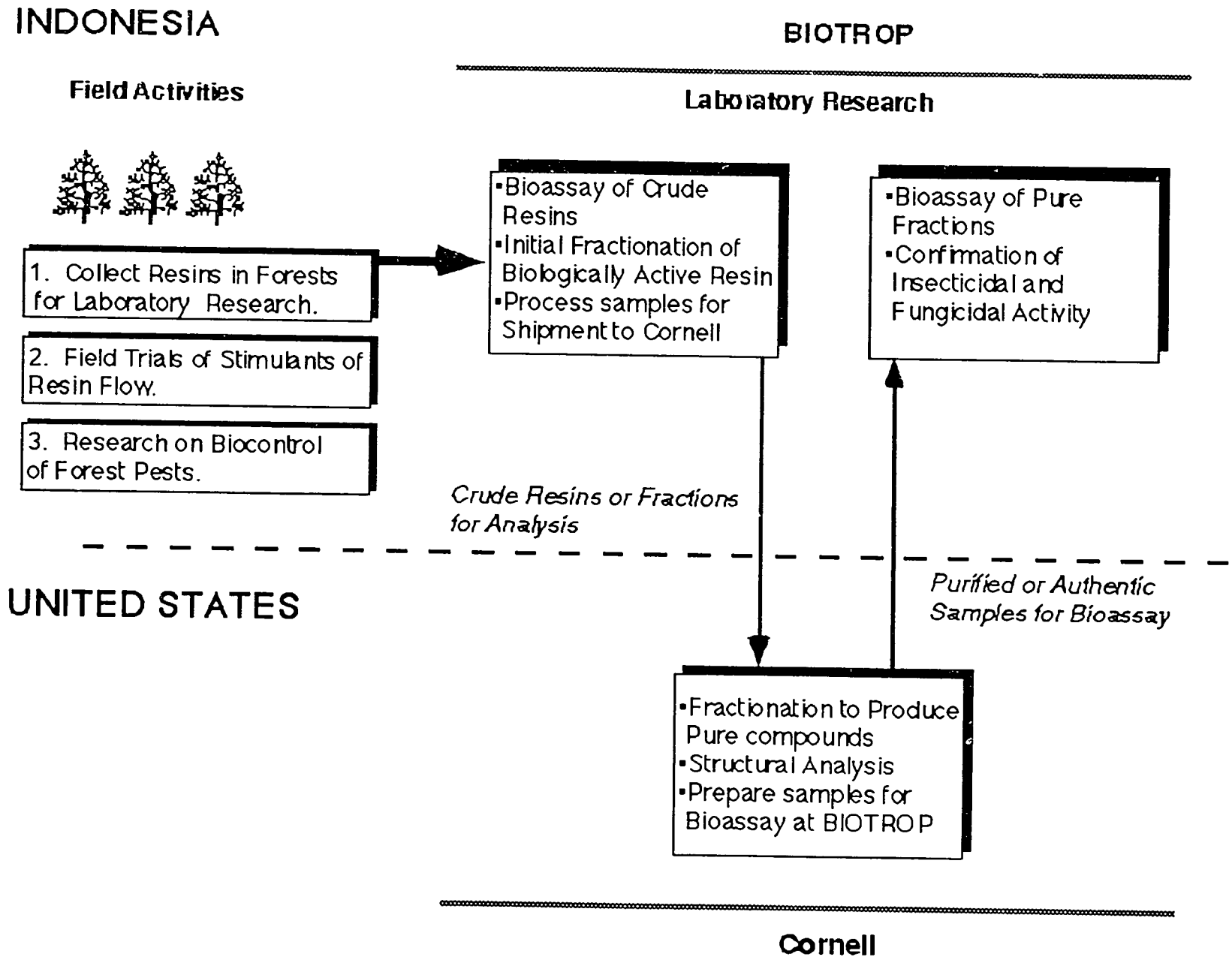


Figure 1. Diagram of Research Plan and Location of Research Activities.

Methodology

Experimental Procedures for Study of Tree Resins

Detailed protocols of experimental procedures and results are included in Appendix 4. Trees, either in forest plantations or in forest concession areas, were slashed with a machete to produce a 2 x 15 cm slash. At irregular intervals these slashes were checked for resin accumulation, and if resin was present it was scraped into a glass screw-cap jar for transport to the laboratory. The resin collection procedure did not appear to harm the trees in any manner. If the sample had to be transported a long distance then a small amount of dichloromethane was added to preserve the resin in its liquid state.

Crude resins were applied in dichloromethane solutions to deliver 25 mg of resins to 4.5 cm diameter filter papers. After the solvent had evaporated, the papers were placed in 5 cm petri dishes, and 25 termites were added. At daily intervals mortality was checked, and dead termites removed.

Crude resins which displayed biological activity in these initial bioassays were subjected to fractionation to isolate biologically active chemical components. Initial fractionations were carried out using low pressure flash column chromatography, which was performed at low temperature. Fractions resulting from these columns were subjected to bioassay, and any biologically active materials were then purified in sufficient quantity to send to Cornell scientists for structural analysis.

Samples were purified to homogeneity as needed, and analyzed via mass spectroscopy and nuclear magnetic resonance spectroscopy. Data from these

procedures was used to determine molecular structures. As far as possible structures were confirmed by comparison to authentic standards prepared in the laboratory, or those which were available commercially. Standards were also sent back to Bogor for bioassay, to confirm the identity of toxic compounds.

Field Trials for Stimulation of Resin Flow

Field trials were conducted with 2-chloroethylphosphonic acid, a commercially

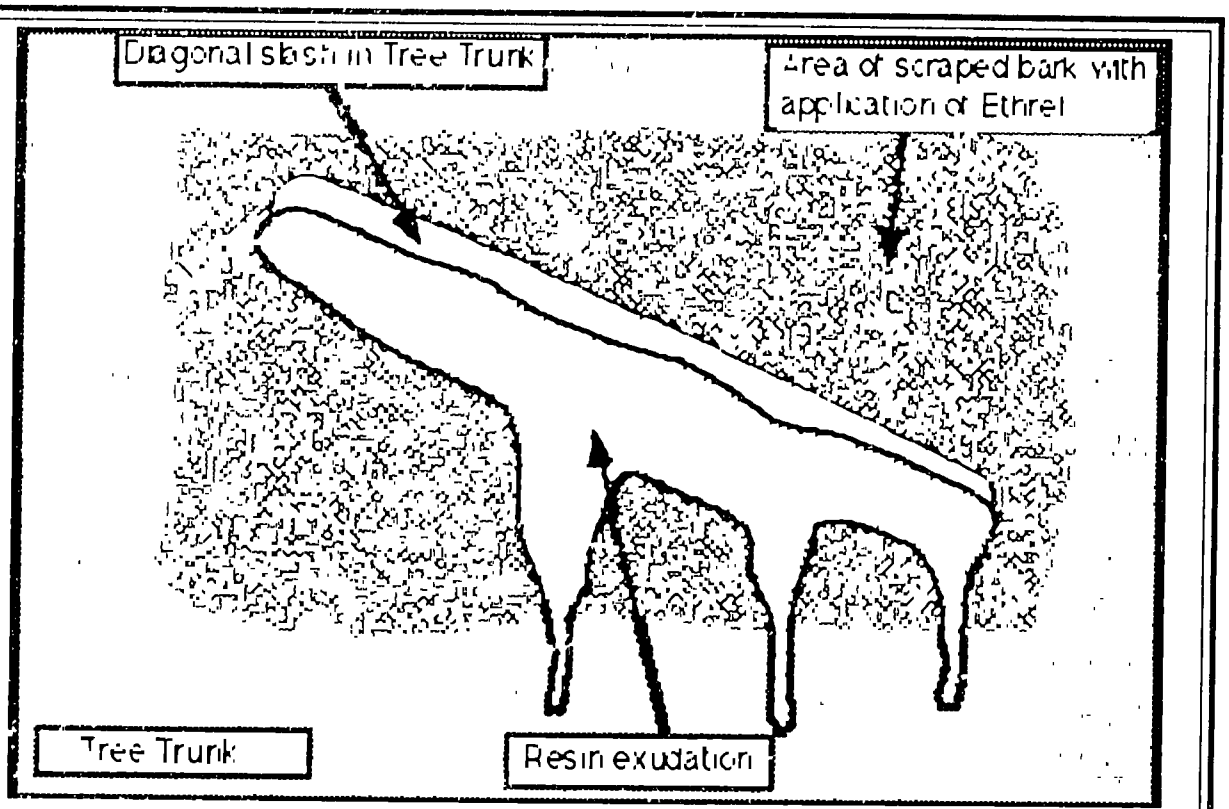


Figure 2. Stimulation of Resin Flow by Ethrel 10LS

available stimulant of latex flow, to determine if this chemical would stimulate resin flow. As shown in Figure 2, an area of a tree trunk was cleaned of bark, a diagonal slash cut into the bark, and Ethrel 10LS latex stimulant was applied to the bark-free area. After 72 hours resins were collected from control and treatment trees, and weighed.

These field trials, conducted in *Shorea javanica* agroforests in South Sumatera, indicated that the Ethrel treatment doubled resin exudation in the short term experiments conducted. The effect was seen only on trees routinely tapped for production; trees which had never been tapped did not respond in any manner to Ethrel stimulation.

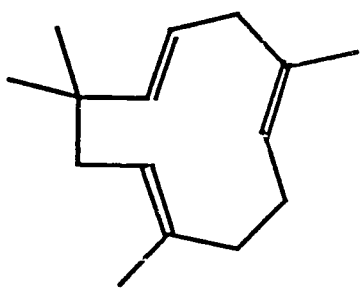
Studies of Herbivore Biology

We were fortunate to encounter a defoliating caterpillar which fed on dipterocarps both in agroforests and in experimental forests In areas of South Sumatera where resin is produced, these moths substantially reduce resin flow, and hence income to resin harvesters. We were able to rear these larvae to the adult stadium, when they were identified as *Calliteara cerigoides,* a tussock moth with several biological parallels to the gypsy moth. Feeding experiments indicated that the moths fed preferentially on dipterocarps, though they would feed on other species.

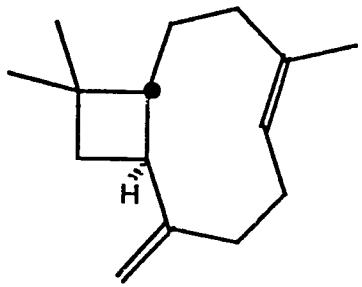
Egg masses of the moth held in the laboratory for rearing purposes often were parasitized heavily by wasps. Field studies of these wasps showed that they parasitized over 70% of all egg masses, suggesting that these minute wasps may be valuable in biological control efforts. The exceptionally high rate of parasitism was possibly due to the fact that studies were conducted in a experimental forest, which had a relatively open profile.

Chemical Structures

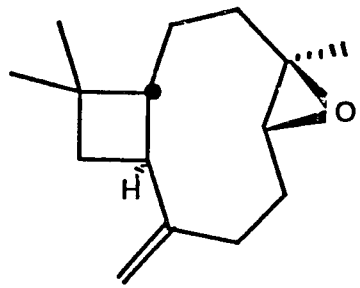
Structural analysis of the biologically active fractions of dipterocarp resins were completed. As shown in Figure 3, page 20, these compounds are all sesquiterpenes.



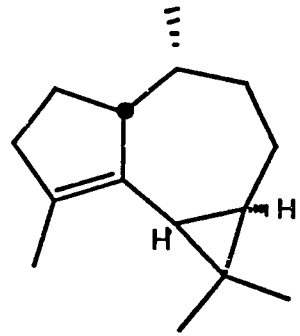
Humulene



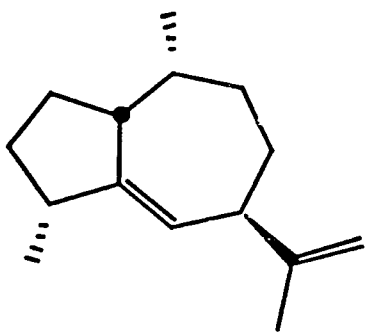
Caryophyllene



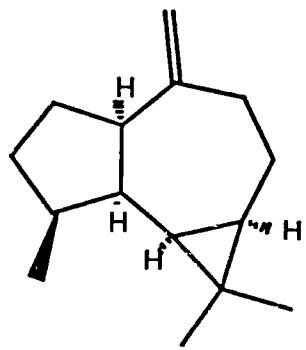
Caryophyllene Oxide



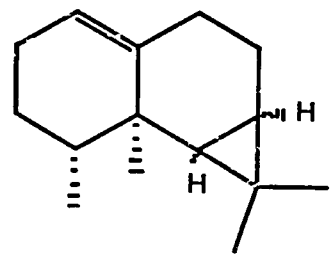
α-Gurjunene



γ-Gurjunene



Albaromadendrene



Calarene

Figure 3. Structure of biologically active chemicals isolated from dipterocarp resins.

All of the sesquiterpene molecules described in this study have been isolated previously, and some are commercially available. We were thus able to confirm our identifications by comparing materials isolated from trees to authentic standards which we had purchased, or which were prepared in the laboratory.

Institution-Building

Intellectual contributions.

Training programs, described in a section beginning on page 27 in this report, enhanced the research potential of BIOTROP. Specific technical knowledge required for the execution of the project may be of use in similar research programs BIOTROP may decide to implement. In particular, the techniques of bioassay of natural products for insecticidal activity, and fractionation, isolation and subsequent identification of biologically active natural products from crude preparations may be especially timely. During a visit to BIOTROP in 1988, Dr. Federico Mayor, the Director-General of UNESCO, was given a briefing of the research activities being conducted under the aegis of the PSTC project described here. Dr. Mayor was very supportive of the search for new, biorational pesticides, because at the time of his visit African Desert Locusts were a serious problem in Africa.

But much of the knowledge and expertise gained by counterparts is useful for any sort of scientific investigation. In particular, the introduction of computer methodology, for keeping and maintaining experimental records, deserves mention. At the time of Messer's departure from Indonesia in May, 1989, BIOTROP personnel who had received computer training in conjunction with the project were helping other staff members to set up spreadsheets, perform statistical analysis, and create graphs.

The presence of an American graduate student in Bogor provided BIOTROP staff members with a resource person who could correct and clarify proposals and scientific papers, suggest funding possibilities, and participate in planning and development of new research projects. Messer worked closely with Tropical Forest Biology program

staff at BIOTROP to write a proposal for a UNESCO-funded training course on non-timber forest products.

The value of an international collaboration which benefits from the unique strengths of each participating institution is another intellectual contribution of the PSTC program. As BIOTROP's foreign contacts expand, the experience gained through this PSTC-funded research project will be a valuable guide in structuring other collaborations. In particular, the demonstration that it is possible to purchase locally virtually all of the instrumentation and supplies needed for research, and construct what is not available off the shelf, may result in more sophisticated laboratory research being located at BIOTROP.

Influence on Scientific Progress.

In addition to adding to BIOTROP's research capabilities, the project also provided several Cornell scientists with direct experience in tropical natural products chemistry. Some of these scientists (Richardson and McCormick) are at early stages in their careers, and will be able to contribute to similar projects in the future. As comprehensive screening programs devoted to uncovering potentially useful chemicals from natural sources will undoubtedly multiply, this expertise will be essential. Further, because these individuals have an understanding of what working situations are like in developing country research institutes, they are better able to advise researchers on appropriate methodologies for processing, fractionating or shipping samples.

During the course of the project, the concept of "chemical prospecting" was advanced by members of the scientific community. One proponent of this concept

proposed a scheme for exploitation of tropical natural products that has some similarities to the PSTC project described in this report (Eisner, 1990). The topic was energetically debated at the 1989 Annual Meeting of the International Society of Chemical Ecology in Gothenberg, Sweden, which was attended by Messer and Meinwald. Noting the rapid disappearance of plant species, the membership of the Society unanimously passed a resolution calling for increased efforts to identify and characterize natural products of use to man. The text of this resolution is presented in Appendix 3, page 45.

Publication of research results in international, peer-reviewed scientific journals will add to BIOTROP's stature as a research institution. Possibly this will inspire other staff members to take the effort necessary to prepare their research for publication in similar journals.

Aside from the direct participation of counterparts, the project was important in providing an opportunity for one BIOTROP staff member to matriculate into a Ph.D. program in forestry at Michigan State University. The USAID/Jakarta Women in Development Officer (Dr. D. Putman) informed Messer of the existence of the Forestry/Fuelwood Research Development (F/FRED) Program fellowships for graduate study in the US. BIOTROP staff scientist Lilian Gadrinab applied for one of the fellowships, was accepted, and is now finishing course work at Michigan State University prior to returning to Indonesia to do her Ph.D. fieldwork. In addition, two women Master's Degree candidates (see page 28) conducted research projects under Messer's supervision.

In summary, this PSTC Project furthered a number of USAID objectives, including those of institution-building, biodiversity, and Women in Development.

Technical Contributions.

From the PSTC project, BIOTROP now has a small, but technically sophisticated setup for basic research in organic chemistry. The equipment can be used for several types of investigations in addition to those of natural products chemistry. Supplying an integrating chart recorder will enable BIOTROP scientists to perform quantitative chemical investigations. The fractionation columns can be used for analysis of fungal metabolites, or pesticide residues, for example. In addition to the instrumentation which was bought by the project, a 1-2 year supply of spares was purchased.

The project also provided a high-quality field vehicle to BIOTROP. In light of decreasing Government of Indonesia contributions to BIOTROP's operating budget, the addition of a reliable four-wheel-drive vehicle to the fleet represents an important contribution to research efforts. Even in nearby experimental forests, access during the rainy season requires the use of four wheel drive vehicles because of poor road conditions.

The computer equipment purchased has been integrated into BIOTROP's Tropical Forest Biology Program. This IBM-compatible equipment was configured with the needs of experimental analysis in mind. To speed statistical operations, the computer was supplied with a math-coprocessor. Color graphics capabilities also enhance the utility of the computer. The computer is used also for word processing, so that reports, requests for funding, and correspondence can be prepared more efficiently.

A complete list of the most important commodities purchased for and provided to BIOTROP is presented in Appendix 1, page 40.

Training

The Work Plan submitted in 1986 called for an Indonesian chemist to visit the Cornell Chemistry Laboratories of Dr. Meinwald. At the time the proposal was written it was expected that Dr. Haryanto, a BIOTROP staff scientist who received his PhD in analysis of pesticide residues in France, would be the counterpart chemist on the project. Unfortunately Dr. Haryanto left BIOTROP late in 1986, and was not replaced. Despite repeated attempts to locate staff members who would benefit from training in the US, it was not possible to identify one who had appropriate expertise and English language skills. Thus all training was provided in Indonesia.

Training components of the project are divisible into four parts: formal training of participants, on-the-job training, language assistance and student research participation.

Formal Training. The project provided approximately 18 person-months of computer and mathematics training for BIOTROP staff. Computer training was provided for two participants in private schools in Bogor during the evening. At the end of this training, the BIOTROP participants (Sunjaya and Iskandar) were fully capable of basic computer operation, including use of spreadsheets for tabulating and analyzing experimental results. Opportunities were provided for Iskandar to upgrade mathematics skills in order that he could be a more effective participant in the research program.

On-the-job training. Extensive training of 5 project personnel, and other BIOTROP staff members, took place in the laboratory. In all situations there was considerable emphasis on laboratory safety. Participants were instructed in techniques of column,

thin-layer, and gas chromatography to the point where they had a thorough understanding of these techniques and could apply them independently. Instruction in microscopy, specimen preparation and photomicrography were given, and methods of insect and fungal bioassay were taught. Microcomputer use was encouraged in all appropriate laboratory situations.

Language Assistance. For approximately the last nine months Messer was at BIOTROP, he conducted a weekly English language session for 4 junior scientists at BIOTROP who had been selected for overseas training. Participants were given a short reading assignment of topical scientific or environmental interest (usually an article from the local daily newspaper Jakarta Post) and class discussion focussed on this article.

Student Research Participation. Two M.Sc. candidates (Wantah and Tumbel) from the Universitas Sam Ratulangi, Manado, North Sulawesi, conducted research projects under Messer's supervision. One student investigated biology and parasites of an economically important moth in forestry plantations; the other assessed insecticidal effects of plant natural products. Publications have resulted from each of the students' work. These research projects will form part of their Master's theses. Both students are expected to continue in scientific research, so their participation in the project was a valuable addition to their careers.

Publications

Publications produced or currently in preparation as a result of this project are listed below. Other papers on related biological topics are under discussion between Sunjaya, Messer, Richardson and other collaborators.

Complete copies of these publications are provided in Appendix 4, following page 46. As stipulated, all publications acknowledge the support of USAID, and provide the contract number.

Richardson, D. P., A.C. Messer, S. Greenberg, H.H. Hagedorn and J. Meinwald. 1989. Defensive sesquiterpenoids from a dipterocarp (Dipterocarpus kerrii). J. Chem. Ecol. 15: 731-747

Messer, A.C., Wantah, N.N. and Sunjaya. 1990. Notes on polyphagy and parasitism of Calliteara cerigoides (Lepidoptera: Lymantriidae), a defoliator of Southeast Asian dipterocarps. submitted to Ecological Entomology.

Messer, A.C. 1990. Traditional and chemical methods for stimulation of Shorea javanica (Dipterocarpaceae) resin exudation in Sumatra. Economic Botany 44: in the press.

Messer, A.C., McCormick, K., Sunjaya, Tumbel, F.T., Hagedorn, H.H. and Meinwald, J. In preparation. Defensive role of tropical tree resins: anti-termitic sesquiterpenes from Asian Dipterocarpaceae. for submission to: Journal of Chemical Ecology.

Messer, A.C. 1990. Chemical Ecology in an Indonesian Context. Cornell University Doctoral Dissertation. Ann Arbor, MI, University Microfilms.

Constraints to Project Progress

When this project was initially designed, it was assumed that the major obstacles to efficient progress would be largely technological. Though proven methods for identifying and isolating biologically active components of dipterocarp resins had been developed by the Cornell Chemistry labs, there was some question about the availability in Indonesia of the technology, specifically chemicals and instrumentation, necessary to duplicate these procedures.

In general there were no technological barriers to progress. While there were occasional difficulties in obtaining some replacement parts (rubber septa for the gas chromatograph), the quality of instrumentation service was equivalent to that found in the U.S. The service technician would gladly come to BIOTROP on weekends if he had no time available during the week. Almost all chemicals, lab equipment and glassware were available off the shelf in Bogor; those which were not available from stock could be sent from Jakarta in 24 hours. Industrial gases could be purchased in Jakarta on a walk-in basis. Scientific glassblowers were easily available. Finally, if technical assistance was needed, personnel working with one of the several international research projects in Bogor could be of help.

Administrative obstacles often severely hampered the project. Possibly because BIOTROP's role as a collaborator on a United States Government-funded research project was not well defined, the institute did not seek an official ("Dinas") visa for Messer. For future projects of this type, the USAID Mission to Indonesia might be asked to assist in clarifying such matters to collaborating Government of Indonesia

agencies. As a result of his visa status, Messer estimates that 20% of his time in Indonesia was consumed by matters related to visa applications or renewals. A renewal of the first 12-month visa required 10 months to obtain, and Messer was forced to leave Indonesia twice in order to obtain to new tourist or business visas in neighboring countries. Further, BIOTROP was not able to arrange customs clearances for commodities, requiring local sourcing when those of US origin would have been preferred.

The first Business Manager of Cornell's Entomology Department associated with the project was not familiar with USAID procedures, and had little experience with international collaborations on the scale of the PSTC project described in this report. This resulted in an unnecessary 7 month delay in purchase of a critical instrument, and complicated the process of filing vouchers for project expenses. Fortunately the second Business Manager had substantial overseas experience, both as a Peace Corps Volunteer and USAID Personal Services Contractor. Her expertise and familiarity with USAID procedures greatly facilitated the project during the final year.

Legacies of earlier research projects were a problem. Access to a key experimental forest was at first denied by the Forestry Department, ostensibly because an earlier foreign researcher did not give proper credit to the Forestry Department for use of the forest. The assistance and advice of Prof. Dr. Ishemat Soeniagara, who accompanied Messer on several visits to the Forestry Research Institute in Bogor, ultimately resulted in the grant of permission to use this experimental forest.

Finally, the chaotic Government of Indonesia budget situation impinged directly on BIOTROP. Several times during the project the BIOTROP Director was forced to

make serious staff cuts, and at one point remaining staff members had to accept 80% reductions in their pay. Maintenance had to be deferred. These events, which were beyond the control of BIOTROP, sometimes made it difficult to work efficiently.

Implications for Development and Conservation

Basic research into the chemical ecology of dipterocarp resins provides new information about uses and sources of natural products. For example, nine triterpenes isolated from dipterocarp resins exhibited *in vitro* antiviral activity when tested against *Herpes simplex* I and II viruses (Poehland et al., 1987). Synthesis of these chemicals and other biologically active natural products, while possible, may not be economically feasible, so that the only viable sources of the chemicals could be the trees themselves. Establishing that the tree resins offer greater long term value than timber harvests made of the same trees, could encourage conservation of primary forests, and be consistent with USAID Biodiversity Program Objectives. Resins are obtainable only from mature trees, around thirty years of age. Virtually all such trees are found only in primary forests. Correctly tapped, dipterocarps have a productive lifetime of fifteen or more years, during which time they continue to set fruit. Thus the trees would represent a sustainable resource. Harvest of resins could provide additional income for villagers, and it might be possible to add some value to product through local processing. This model is shown schematically in Figure 4, page 34.

Given current emphasis on economic approaches to tropical forest conservation, social forestry, and sustainable development, the topic of dipterocarp resins is especially timely. This research program has shown that there are biologically active compounds in the dipterocarp resins, and that these materials probably have a defensive function. Research conducted on improving resin yields, and on pests of dipterocarps is of potential value to resin producers. Effecting

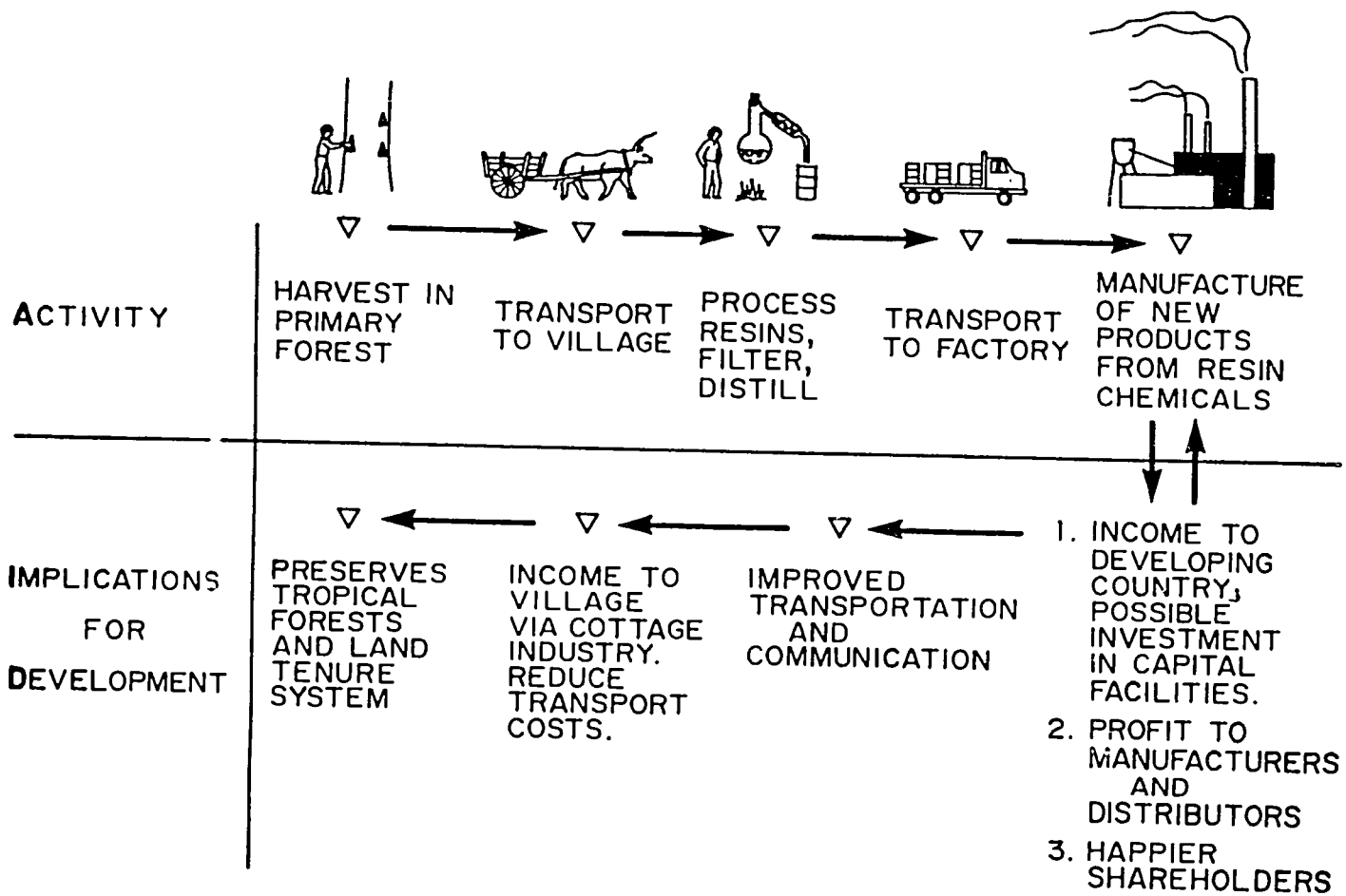


Figure 4. Schematic diagram showing a potential scheme for sustainable development and tropical forest conservation based on harvest of tree resins

forest conservation by replacing tree cutting with resin harvests still requires the demonstration that natural products grant more income than timber. As stands of suitable trees in relatively accessible areas become depleted, timber operations move to more remote areas. As this happens, the cost of extracting natural products from these remote areas increases. This results from the costs of the labor and transportation inputs. A sufficiently large labor force may have to be brought to a remote collection zone, and maintained there. The products harvested will carry these additional costs, as well as the cost of moving the products to distant markets, so the products will have to command higher prices in the marketplace. Thus the scheme offered in Figure 4, based on harvest of products from existing forest stands, becomes progressively harder to realize. However the cost of timber operations in remote areas also increases with distance from processing and population centers. Although in the short term the economics of timber harvesting may appear more attractive than conservation, it is likelyt that over the longer term that timber will not be a viable economic activity, nor an environmentally sound choice.

This scheme relates directly to long-term development issues in Indonesia, specifically the transmigration program. Current transmigration programs have been hampered by the failure of transmigrants to farm successfully in regions newly opened for habitation. A mixture of edaphic and cultural factors are often to blame: tropical soils are poor in nutrients, and the transmigrants themselves have to adapt to unfamiliar farming methods. Installing resin harvesting and processing as a component in some transmigration projects would be a prudent step for several reasons: 1) Tree-tapping is relatively straightforward, and the large number of workers

involved in latex harvesting suggests that the technology could be easily learned; 2) Tapping extant stands of primary forest would encourage their conservation; 3) There would be long term incentives to plant more dipterocarps; 4) Resin harvests generate cash income, and processing in the village would add value to the products.

Recent advances in biotechnology, notably those made by the TROPENBOS team led by Willi Smits, allow mass propagation of dipterocarps from hedges. This team has cracked the problem of inducing root formation in plagiotrophic branches--in other words they have succeeded in getting shoots to develop roots, which go on to develop the appropriate mycorrhizal associations critical for tree growth. Smits' methodology would allow planting of seedlings on a vast scale. Vegetative propagation of dipterocarps would also allow rapid expansion of varieties selected for desirable characteristics, for example a particular chemical composition. Plant genetic engineering may also have a role to play in selection or development of useful varieties.

The research presented in this report has shown that work in chemical ecology has, in addition to its intrinsic scientific value, potentially useful linkages to tropical forest conservation.

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APPENDICES

1. Commodities Purchased

The following commodities were purchased for, and are located at BIOTROP, in Bogor Indonesia. In accordance with existing regulations, commodities were labelled to indicate that they were USAID contributions. It was not possible to label laboratory glassware and related items.

- Toyota Land Cruiser, V-6 gasoline engine, short wheelbase, tools and spare parts.
- Hewlett-Packard 3390A Integrating Chart Recorder, approximately 1 year supply of consumables (thermal paper, fuses, etc.)
- Laboratory Air Compressor, valves and fittings.
- IBM compatible microcomputer with 8088 microprocessor and 8087 math co-processor, color graphics adapter and color monitor, 30Mb hard disk drive, color monitor, statistical, spreadsheet and wordprocessing software, voltage stabliser and related equipment.
- Hewlett-Packard Thinkjet printer.
- Chromatography columns, Ace Glass Co., frits, valves, plugs, and packing materials for liquid chromatography.
- Chromatography columns, Shimadzu, with Porapak packings for gas chromatography; silicon rubber septa for gas chromatography, precison syringes (5, 10, 25 and 50 µl), analytical gas standards, gas regulators for oxygen and nitrogen tanks.
- Laboratory glassware and hardware, including glass petri plates (± 300), pipettes, clamps, high quality screw cap bottles, test tubes and beakers,

thin-layer chromatography supplies, etc.

- Safety equipment, including goggles, safety glasses, gauntlet gloves, face shields, dust masks, respirators and replacement cartridges, rubber aprons and booties.
- In addition to these commodities, the contract provided for total overhaul and refurbishment of the BIOTROP gas chromatograph. The chart recorder and printer were furnished with one-year service contracts.

2. List of Relevant Contacts

BIOTROP
Dr. T. Binarko Suselo, Manager, Tropical Forest Biology Program
Ms. Claire Elouard, phytopathologist, University of Toulouse
Ms. Lilian Gadrinab, Tropical Forest Biology Program
Mr. Koko Iskandar, Head Technician, PSTC project
Dr. A. Kostermans, Botanist
Dr. Y. Laumonier, Chief of French Team (from 6/87)
Dr. Genevieve Michon, phytogeographer, French Team
Ir. Rafael Pranata, Head, Stored Products Pest Laboratory
Dr. Djoko Purwanto, Deputy Director for Finance and Administration
Drs. Iwan Setiawan, Tropical Forest Biology Program
Prof. Dr. S. Soetarmi Tjitrosomo, Director
Drs. Sunjaya, Tropical Agricultural Pest Biology Program (Counterpart)
Dr. E. Torquebiau, Chief of French Team (departed 6/87)
Dr. Ruben Umaly, Deputy Director for Programs
Dr. Irene Umboh, Manager, Tropical Forest Biology Program

Indonesian Forestry Department
Mr. Djunedi, Superintendent, Cikarawang Research Forest
Ir. Komar, Director, Forestry Research Institute
Ir. Masano, Head of Silviculture, Forestry Research Institute
Drs. Djatnika Natawira, Entomologist, Forestry Research Insitute
Ir. Panjaitan, Chief, North Sumatera Branch
Ir. S. Riyadi Martoyo, Chief of Branch VI (Dairi, North Sumatera)
Dr. Toga Silitonga, Forest Products
Ir. Sugiono H.S., Chief of Estimates and Mapping (North Sumatera)

Other Bogor Institutions
Ms. Janet Cochrane, Green Indonesia Foundation
Dr. Clifford Hoelscher, Fulbright Visiting Professor, Bogor Agricultural University.
Dr. Chris Lomer, Entomologist, Overseas Development Administration
Dr. Aunu Rauf, Entomologist, Bogor Agricultural University
Mr. Hank Reichart, Resident Director, World Wildlife Fund
Mr. Marcel Silvius, Asian Wetland Bureau
Drs. Fred Smiet, School of Environmental Conservation and Management
Prof. Dr. Ishemat Soeniagara, Department of Forestry, Bogor Agricultural University.
Mr. Rodney Sterne, Green Indonesian Foundation
Dr. Douglas Stoltz, Toxicologist, Bogor Veterinary Research Institute
Dra. Suharyati Purnomo, P.T. Politani Khatulistiwa Nusantara
Mr. Walter Tappan, International Rice Research Institute Liaison Scientist
Ir. Bruno de Wilde, Belgian Biogas Project

United States Government Agencies in Indonesia

Dr. Barry Annis, Head, Entomology Department, Naval Medical Research Unit
Ms. Margaret Brown, USAID
Ms. Lynn Cassell, USIS
Mr. Richard Cobb, USAID
Mr. William H. Douglass, USAID
Dr. Eugene Galbraith, USAID
Mr. Ronald J. Greenberg, USAID
Ms. Joanne Hale, USAID
Ms. Virginia Kurapka, American Consulate, Medan
Dr. Jeffrey T. Lutz, Science Counselor, American Embassy
Dr. David Macauley, USAID
Ms. Isna Marifa, USAID
Mr. Gregg Marshall, USIS
Dr. E. Edwards McKinnon, USAID
Mr. David Merrill, Director, USAID Jakarta Mission
Mr. Desmond O'Riordan, USAID
Dr. Diana Putman, USAID
Ms. Suzanne Siskel, USAID

Other Agencies and Institutions in Indonesia

Mr. Titus Bekkering, forester, Kali Konto Project
Mr. Gordon Bishop, Export Development Consult
Mr. Richard Borsuk, Asian Wall Street Journal, Jakarta
Ms. Henny Buftheim, Program Officer, CIDA Jakarta
Mr. Mohamad Cholid, Journalist, Tempo
Mr. Tb. U Chusaen, Head, Commercial Export Section, West Sumatera
Mr. A. Dahana, Journalist, Tempo
Ms. Clara van Eijk-Bos, Tropenbos Project, East Kalimantan
Mr. Larry Fisher, Ford Foundation
Ms. Lucy Fisher, World Neighbors
Ms. Sally Gelston, United Press International, Jakarta
Mr. James K. Harlan, Business Advisory Indonesia
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Mr. Gael Charveriat, Agronomist, Roussel-Uclaf, Singapore
Dr. James Collins, University of Hawaii, Department of Indo-Pacific Languages
Dr. N. Mark Collins. World Conservation Monitoring Center, Cambridge, England
Dr. Peter Delp, USAID/ANE/TR
Dr. Joel Erwin, Associate Editor, National Geographic Research, Washington, D.C.
Dr. Kevin Gallagher, Entomologist, IRRI, Manila
Mr. Carl Goldstein, Journalist, Far Eastern Economic Review, Hong Kong
Dr. Mason Hoadley, Associate Professor, Lund University, Sweden
Dr. Robert Hughes, President, Associated Universities, Inc., Washington, D.C.
Dr. Jean Langenheim, University of California, Santa Cruz.
Dr. Stephen Lintner, Environmental Coordinator USAID/ANE
Dr. Federico Major, Director-General, UNESCO
Ms. Judith Mayer, Insitute for Current World Affairs
Dr. Charles Mehl, Winrock F/FRED Project, Kasetsart University, Thailand
Dr. Janet Rice, AAAS Fellow, USAID ANE/TR
Dr. Donald Roberts, Boyce Thompson Institute for Plant Research, Cornell University
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Dr. Eizi Suzuki, Kagoshima University, Japan
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Ms. Jane Towson, Tropical Weeds Unit, Long Ashton Research Station, England
Ms. Janice Waller, Business Manager, Cornell Department of Entomology
Dr. W. Paul Weatherly, American Farmland Trust
Dr. J. Wolff, Cornell University

3. Gothenburg Resolution

Presented here is a brief summary of the International Society of Chemical Ecology (ISCE) Annual Meeting held in Gothenburg, Sweden, in August, 1989. The summary includes text of the Gothenberg Resolution adopted by the Society. The Principal Investigator for the PSTC grant, Dr. Jerrold Meinwald, served as President of the ISCE 1988-1989.

Gothenburg Meeting Resounding Success

One-hundred forty four people from 23 countries attended ISCE's sixth annual meeting in Sweden, making it the society's largest, with the attendees representing our most geographically diverse group to date. Gunnar Bergström hosted the meeting at the Nordic School of Public Health, University of Gothenburg. An introductory lecture by Dr. Thomas Eisner titled "The insect as druggist—the utilization of plant secondary metabolites by insects" got us thinking about the impact of the current rate of species extinction on the future of natural products and chemical ecology. During his plenary address Dr. Douglas Futuyma addressed the major theme of the Gothenburg meeting with his presentation titled "Ecological chemistry in evolutionary focus: What are the questions?" The invited papers, minisymposia, and contributed papers represented the diverse interests of society members. In addition to the scientific discussions considerable attention was directed to proposals suggesting that the society make a statement regarding conservation of species not only for their biological value but also for their potential chemical value. At the conclusion of the meeting, attendees unanimously adopted the following resolution:

"Natural Products constitute a treasury of immense value to humankind. The current alarming rate of species extinction is rapidly depleting this treasury, with potentially disastrous consequences. The International Society of Chemical Ecology urges that conservation measures be mounted worldwide to stem the tide of species extinction, and that vastly increased biorational chemical studies be undertaken aimed at discovering new chemicals of use to medicine, agriculture, and industry. These efforts should be undertaken by a partnership of developing and developed nations in such a way that the benefits flow to the developing nation as well as to all humankind."

Comments regarding this Gothenburg resolution are most welcome. Please address them to Dr. Jerrold Meinwald, Cornell University, Baker Laboratory, Ithaca, NY 14853-1301.

Following a sumptuous banquet, President Jerrold Meinwald passed on the Swedish cedar gavel to incoming President Wittko Francke. Society Medals were awarded to Drs. Miriam Rothschild and Murray Blum. Certificates of appreciation were presented to meeting host Gunnar Bergström, treasurer James Nation, outgoing president Jerrold Meinwald and outgoing ISCE councilors Ana Luisa Anaya, Gunnar Bergström, Wittko Francke, Clive G. Jones, and Martine Rowell-Rahier.

4. Scientific Publications

Following are photocopies of scientific publications which were produced by personnel working under this PSTC grant. The publications included are:

Richardson, D. P., A.C. Messer, S. Greenberg, H.H. Hagedorn and J. Meinwald. 1989. Defensive sesquiterpenoids from a dipterocarp (Dipterocarpus kerrii). J. Chem. Ecol. 15: 731-747

Messer, A.C., Wantah, N.N. and Sunjaya. 1990. Notes on polyphagy and parasitism of Calliteara cerigoides (Lepidoptera: Lymantriidae), a defoliator of Southeast Asian dipterocarps. submitted to Ecological Entomology.

Messer, A.C. 1990. Traditional and chemical methods for stimulation of Shorea javanica (Dipterocarpaceae) resin exudation in Sumatra. Economic Botany 44: in the press.

Messer, A.C., McCormick, K., Sunjaya, Tumbel, F.T., Hagedorn, H.H. and Meinwald, J. In preparation. Defensive role of tropical tree resins: anti-termitic sesquiterpenes from Asian Dipterocarpaceae. for submission to: Journal of Chemical Ecology.

Journal of Chemical Ecology, Vol. 15, No. 2, 1989

DEFENSIVE SESQUITERPENOIDS FROM A DIPTEROCARP (*Dipterocarpus kerrii*)[1]

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(Received December 11, 1987, accepted February 23, 1988)

Abstract—Four sesquiterpenoids (**2**, **4**, **7**, and **9**) have been isolated and characterized from the termiticidal fraction of *Dipterocarpus kerrii* resin. The major constituent of this resin is α-gurjunene (**1**)

Key Words—Resins, *Dipterocarpus kerrii*, termite, sesquiterpenes, α-gurjunene, epicyclocolorenone, fungicidal, termiticidal, bioassay.

INTRODUCTION

Trees of the plant family Dipterocarpaceae dominate many lowland primary forests of Southeast Asia. Often attaining a height of 70 m or more, these hardwoods can comprise 80% of the emergent vegetation in some areas (Ashton, 1982). With species richness at a maximum on the Indonesian island Kalimantan, Dipterocarpaceae range from Africa to Papua New Guinea. Because of their abundance and durability, these timbers have become important economic commodities.

Southeast Asian dipterocarps produce copious amounts of resins (Ashton, 1982; Torquebiau, 1984). Exuded from natural or artificially induced trunk wounds, these viscous and sticky resins have long been items of commerce

[1] Presented at the Third Annual Meeting of the International Society of Chemical Ecology, June 21-24 1985, University of California, Berkeley
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(Marsden, 1783). Dipterocarp resins contain a large and complex array of chemical constituents (Hegnauer, 1966; Bisset et al., 1971; Ashton, 1982), many of which are terpenes. The diversity of terpene and other fractions indicates that dipterocarps dedicate a large number of biosynthetic pathways to the production of resin chemicals. These biosynthetic pathways must be metabolically costly, but in the absence of data on biological roles of dipterocarp resins, the adaptive benefits (if any) of maintaining these pathways remain obscure.

Several lines of evidence suggested that dipterocarp resins might contain biologically active molecules. Considering evidence scattered throughout the literature of Asian forestry, we postulated that some of the chemicals made by dipterocarps might play a defensive role. Dipterocarp timbers are well known to resist biological attack from many sources. *Shorea robusta* was shown highly resistant to the termites *Microcerotermes beesoni* and *Heterotermes indicola* (Sen-Sarma, 1963; Sen-Sarma and Chatterjee, 1968). Particle boards constructed from *Shorea* species are also protected against *Cryptotermes cynocephalus* (Moi, 1980). Fresh resins of *Anisoptera thurifera* appear to protect bee nests from termites (Messer, 1984).

Dipterocarp woods cause substantial mortality to insects feeding on them. Over a three-month test period, termites feeding on *Shorea* species suffered 99% and 86% mortality, while termites feeding on the nondipterocarp *Dyera costulata* showed only a 13% death rate (Moi, 1980). Tested as a possible substrate for insect culture, dipterocarp sawdust killed stable fly larvae in the first instar, while other woods did not (Sutherland, 1978).

Chemical factors in resins may also protect dipterocarps from microbial attack. Untreated dipterocarp timbers are reported to be highly resistant to fungal invasion (Bakshi et al., 1967), and volatile components of *Hopea papuana* were shown to inhibit fungal growth (Messer, 1985). Bacterial growth inhibitors of dipterocarp origin include essential oils of *Vateria indica* (Bhargava and Chauan, 1968) and stemnoporol and alpha-copalliferol from Sri Lankan dipterocarps (Sootheeswaran et al., 1983).

The research described here tested the hypothesis that dipterocarp resins contain biologically active components that protect the trees against insects and fungi. Antifungal and termiticidal properties of whole resins and resin fractions were evaluated, and novel compounds possibly mediating the observed biocidal properties were isolated and characterized.

METHODS AND MATERIALS

Tree Resins

Fresh resins of *Dipterocarpus kerrii* King were collected by tapping trees cultivated at the Forest Research Institute, Kepong, Malaysia. Resins were stored in sealed glass ampoules for transport to the United States.

Termite Bioassays

Zootermopsis angusticollus (Hagen) termites were taken from a permanent laboratory culture. Termites selected for bioassay experiments were undifferentiated larvae ("workers") beyond the third instar, and weighed 10–15 mg. Ten termites were placed in a 5-cm plastic Petri dish containing a 4.5-cm-diameter filter paper treated with compounds of interest. Treatment of filter papers involved uniform application of a methylene chloride solution of the chromatographic fractions of interest, followed by evaporation of the solvent. Three to four duplicate dishes were used for each condition. Mortality was checked at 24-hr intervals, and dead termites were removed.

Pilot experiments established that termite mortality was dose dependent. All filter papers were thus treated with the amount of a compound normally found in 10 mg of crude *D. kerrii* resin.

Fungal Bioassays

Ten milligrams of crude *D. kerrii* resin dissolved in methylene chloride was applied to a 10 × 20-cm polyester TLC plate and eluted with 50% ether–hexane. After the solvents had evaporated, the plate was sprayed with a suspension of *Cladosporium cucumerinum* spores. The suspension was prepared by scraping spores from three confluent plates into ≈5 ml of sterile water, mixing this solution with 50 ml of 0 35% green bean juice agar at 37°C, and sonicating the solution for 3 min prior to spraying the TLC plates. Plates were examined for regions of antifungal activity after incubating for 48 hr at room temperature in TLC tanks lined with moist paper towels. Zones of fungal inhibition were apparent as white bands against the background of grey-green fungal growth.

Isolation

Crude *D. kerrii* resin was dissolved in methylene chloride and applied to 1000 μm preparative TLC plates (silica gel GF, Analtech, Inc., Newark, Delaware), which were eluted with 100% pentane or 50% ether–hexane. Component bands were visualized by means of a UV light and/or anisaldehyde spray reagent (90% of a 5% solution of anisaldehyde in 95% ethanol–5% acetic acid–5% conc. H_2SO_4). Bands of interest were removed from the plates, and the silica gel was repeatedly washed with methylene chloride, which was removed by evaporation in vacuo.

Further purification was effected by high-performance liquid chromatography (HPLC) using a Waters Associates M6000A solvent delivery system and a preparative scale column (20 mm ID × 250 mm) packed with 5-μm silica. The HPLC column was eluted with 5% ether–hexane at a flow rate of 1.5 ml/min. Ultraviolet detection was performed at 254 nm with a Perkin-Elmer model

LC-65T variable wavelength UV detector. Eluent from HPLC peaks of interest was collected and concentrated at ambient pressure using Kaderna-Danish (Ace Glass, Inc.) microscale concentrators.

Final purification of resin components was effected by semipreparative gas–liquid chromatography (GLC) with a Varian model 2100 gas chromatograph equipped with a flame ionization detector and a glass column (3 mm × 1 m) packed with 3% OV-1 on 100/120 Supelcoport (Supelco, Inc.). Nitrogen was used as the carrier gas at a flow rate of 30 ml/min, and the column temperature was held at 150°C for 6.5 min, and then programmed to 200°C at 20°/min. The effluent from the column was split in a 1:9 ratio between the detector and 25-cm glass capillary collection tubes (Brownlee and Silverstein, 1968) that were cooled in a Dry Ice–acetone bath. Analytical GLC was performed with the aforementioned Varian system or a Shimadzu Mini-2 equipped with a fused silica capillary column (007-methylsilicone, 0.25 mm × 15 m, Quadrex Corp., oven temperature 200°C isothermal, helium as carrier gas at a linear flow of 2 kg/cm), a capillary split injection system with a 100:1 split, and a flame ionization detector.

Identification

The components of interest were identified using a combination of gas chromatography–mass spectrometry [GC-MS, using both electron impact (EI) and chemical ionization (CI)], gas chromatography–Fourier transform infrared spectroscopy (GC-FTIR), ultraviolet (UV) spectroscopy, and Fourier transform–nuclear magnetic resonance (FT-NMR) spectroscopy. Both proton [^{1}H]- and carbon [^{13}C]FT-NMR were utilized.

Mass spectra were obtained on a Finnigan model 3300 mass spectrometer equipped with a Systems Industries model 150 data reduction system, or a Finnigan model 4500 GCMS system. EI mass spectra were obtained at 70 eV; CI mass spectra utilized methane as the reagent gas. High-resolution mass spectra (HRMS) were measured on an Associated Electronics Industries MS-902 spectrometer interfaced to a VG Micromass 2040 data reduction system.

Microscale trimethylsilylation of purified resin components was performed in methylene chloride solution with excess Sylon-BTZ (Supelco, Inc.) at room temperature (10 min). Portions of these solutions were analyzed by GC-MS (EI).

Infrared spectra were obtained in $CHCl_3$ solution using a Perkin-Elmer model 299B infrared spectrophotometer; GC-FTIR spectra were obtained on an IBM model 98 GC-FTIR system coupled to a Hewlett-Packard model 5790A capillary gas chromatograph (007-methylsilicone column, 0.25 mm × 25 m, Quadrex Corp.).

[^{1}H]NMR spectra were obtained using either a Bruker model WM-300

(300 MHz) or a Varian model XL 400 (400 MHz) NMR spectrometer. [^{13}C]FT-NMR spectra were obtained using either the aforementioned Varian system (at 100 MHz), or a Jeol model FX-90Q (at 22.5 MHz) NMR spectrometer. NMR spectra were measured in $CDCl_3$ or deuteriobenzene, which were stored over anhydrous K_2CO_3 and passed over a column of neutral alumina immediately prior to use.

Ultraviolet spectra were measured in cyclohexane or 95% ethanol using a Hewlett-Packard model 8450A UV-visible spectrophotometer. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter.

RESULTS AND DISCUSSION

Fungal Bioassays

Several components of *D. kerrii* resin inhibited fungal growth, as shown in Figure 1. Strong inhibition was shown by compounds with $R_f = 0.04$ and $R_f = 0.11$, as well as by a broad band at $R_f = 0.45$. Compounds with $R_f = 0.22$ and $R_f = 0.35$ produced less intense fungal growth inhibition.

Termite Bioassays

Guided by the termite bioassay, initial fractionation of crude resin was performed by TLC on 1000 μm preparative plates which were eluted with 50% ether-hexane. Resolved components were bioassayed at levels that mimicked a 10-mg dose of crude resin. After three sequential fractionations, termiticidal activity was found in a single TLC band that was UV active and that stained intensely with anisaldehyde. This TLC band, comprising ca. 12% of the crude resin, had the same R_f (0.45) as that which displayed strongest inhibition in the fungal bioassays. Toxicity results are shown in Figure 2A.

Further fractionation of the TLC active zone using preparative HPLC yielded one minor peak (1) and two major peaks (2 and 3, Figure 3). The major peaks were isolated and each was found to represent $\approx 4\%$ of the crude resin. As shown in Figure 2B, peaks 2 and 3 displayed essentially equivalent toxicity when bioassayed at levels corresponding to the 10-mg crude resin dose level.

Final fractionation of HPLC peaks 2 and 3 was accomplished using semi-preparative GLC. HPLC peak 2 was found to contain two major components (A and B), as was HPLC peak 3 (C and D, see Figure 4). The major GLC peaks were isolated in the following quantities: A: 2 mg; B: 0.2 mg; C: 2 mg; D: 0.8 mg. All isolated GLC peaks were found to be $\geq 95\%$ pure upon reinjection by analytical GLC. These materials were extremely labile and exhibited significant decomposition, even when stored at $-10°C$. Decomposition was

FIG. 1. Fungicidal bioautograph of crude *Dipterocarpus kerrii* resin against *Cladosporium cucumerinum*. Details in text.

exceptionally rapid in NMR solvents containing traces of acid (e g., $CDCl_3$). As a result, these solvents were carefully pretreated with neutral alumina immediately prior to use. Due to these stability restrictions, and the quantities of materials needed for structure elucidation, bioassays of the individual components A, B, C, and D have not yet been completed.

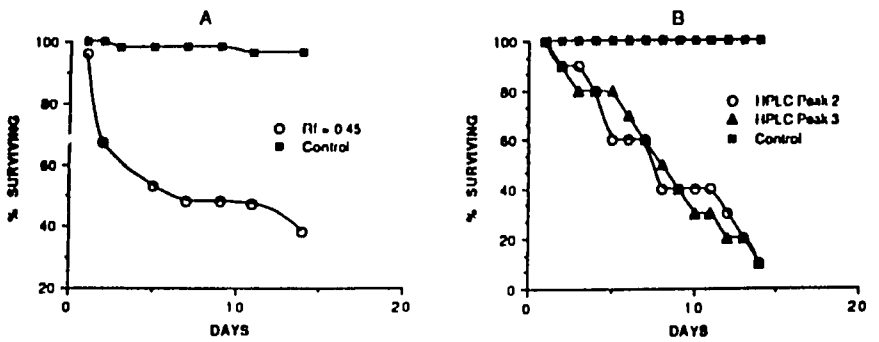


FIG. 2. Toxicity of *D. kerrii* resin components towards *Z. angusticollis*. (A) Preparative TLC band (R_f = 0.45); (B) preparative HPLC peaks 2 and 3. Details in Methods and Materials.

Isolation of Major Resin Component

In the preliminary stages of this study, examination of crude *D. kerrii* resin by GLC had shown the presence of a single major volatile component. Fractionation of resin by preparative TLC showed that the major volatile component corresponded to the most mobile TLC band, which was UV active and which

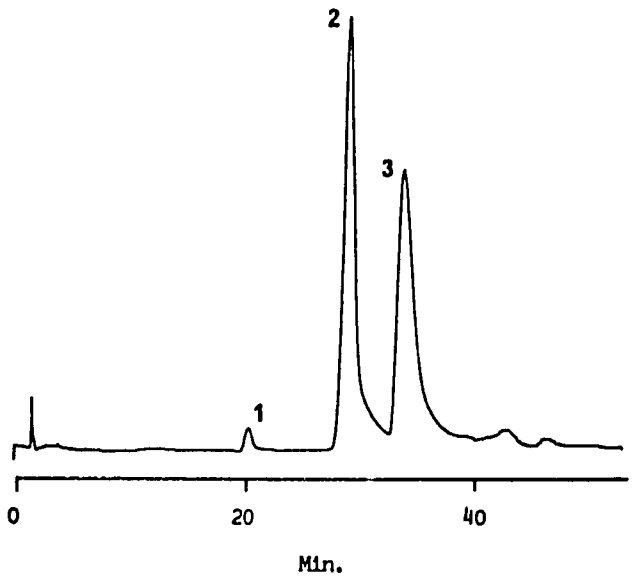


FIG. 3. High-performance liquid chromatogram of preparative TLC band (R_f = 0.45).

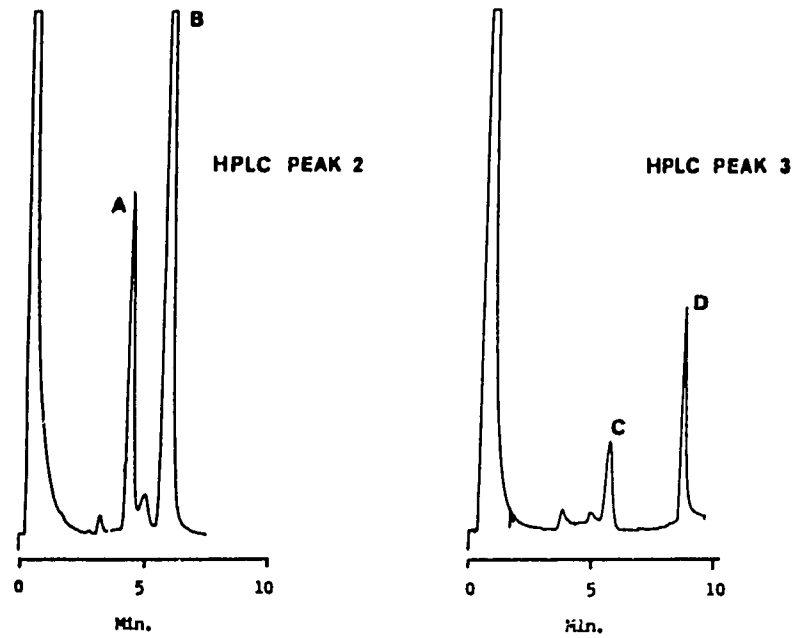


FIG. 4. Capillary GLC analysis of HPLC peaks 2 and 3.

stained intensely with anisaldehyde. Isolation of this component was accomplished by preparative TLC (R_f = 0.95, 1000 μm silica plate) using 100% pentane as eluent. This component, a colorless oil, comprised $\approx$24% of the crude resin and was shown to be $\geq$95% pure by analytical GLC (R_t = 3.6 min, 3% OV-1, 150°C). This material exhibited the same degree of lability as did the individual components A–D. Naturally, since this component did not elute with the TLC active zone, it did not display any toxicity in the bioassay.

Structural Analysis

Major Volatile Component. Analysis of the major volatile component by CI-MS established that the molecular weight of this material was 204. From HRMS analysis of the ion at m/z = 204, its molecular formula was found to be $C_{15}H_{24}$, indicating that this component was a sesquiterpene with four sites of unsaturation. The [^{13}C]NMR spectrum of this material displayed a single pair of olefinic carbons (δ 137.26, 136.04) implying the presence of one double bond and three rings. Further, the [^{1}H]NMR of this material exhibited three prominent methyl group signals [δ 0.84 (s), 0.91 (d), 1.66 (br.d)], and no olefin proton signals. Comparison of these data with information recorded in compilations of sesquiterpene data (Devon and Scott, 1972; Ourisson, 1966) allowed

the major volatile component to be identified as α-gurjunene, **1** (Figure 5). This compound, a member of the aromadendrane family of sesquiterpenes, was originally isolated from *Dipterocarpus dyeri* (Palmade et al., 1963) and has since also been found in resin from *Shorea flava* (Bisset et al., 1971). Our measured values for other physical properties of **1** (IR, UV, $[\alpha]_D$) were also in good agreement with the literature values (Palmade et al., 1963).

Peak A. Low-resolution EI-MS analysis of component A, a colorless oil, showed a very weak peak at m/z = 220 with diagnostic peaks at m/z = 205 [M-15 (CH_3)] and m/z = 202 [M-18(H_2O)], suggesting that A was a labile alcohol. The CI-MS spectrum of A also displayed a weak molecular ion signal at m/z = 220. HRMS analysis of this ion suggested the molecular formula of $C_{15}H_{24}O$. Finally, treatment of A with Sylon-BTZ followed by low-resolution GC-MS (EI) analysis showed quantitative conversion to a more volatile product with a molecular ion at m/z = 292. A gain of 72 mass units implied that A had been trimethylsilylated and strengthened the conclusion that A was an alcohol. This conclusion was also supported by observation of a weak OH band at 3637 cm^{-1} in the GC-FTIR. The [^{13}C]NMR spectrum of this material showed a single pair of olefinic carbons (δ 153.46, 106.25). Taken together with the HRMS data, this suggested that A was a tricyclic sesquiterpene alcohol with one double bond.

Comparison of this information, and extensive [^{1}H]NMR data, with liter-

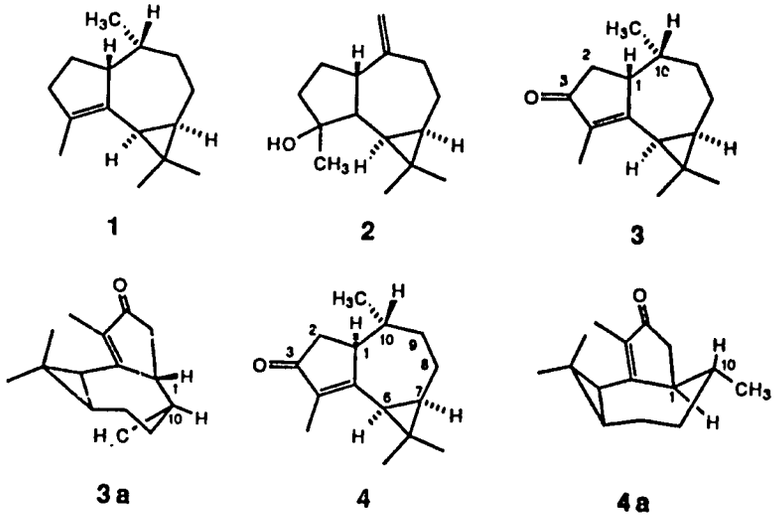


FIG. 5. Structures of previously identified sesquiterpenes isolated from *D. kerrii* resin, with the exception of cyclocolorenone, **3**, which has been isolated from *P. colorata*. Details in text.

ature data allowed identification of A as spathulenol, **2** (Figure 5). Like α-gurjunene, this material is an aromadendranoid sesquiterpene and was first isolated from *Eucalyptus spathulata* (Bowyer and Jefferies, 1963). In subsequent studies, spathulenol has been isolated from several other plant sources (cf. cotton plant, Elzen et al., 1984; *Citrus junos*, Shinoda et al., 1970). An especially extensive NMR/structural study of **2** was recently published (Inagaki and Abe, 1985). Our measured NMR data (conventional [^{1}H]- and [^{13}C]NMR spectra as well as two-dimensional [^{1}H]- and [^{13}C]NMR spectra) completely match these data.

Peak D. Analysis of D, also a colorless oil, by low-resolution CI-MS showed a molecular ion at m/z = 218. HRMS analysis of the m/z = 218 ion gave a molecular formula of $C_{15}H_{22}O$, implying that D was a sesquiterpene with five sites of unsaturation. GC-FTIR analysis showed an intense band at 1690 cm^{-1} and a moderately intense band at 1627 cm^{-1}, suggesting the presence of a conjugated enone; no OH band was observed. This was supported by the [^{13}C]NMR spectrum, which showed distinctive carbon signals at δ 200.96 (carbonyl), 141.60, and 99.47 (double bond).

Comparison of this information with tabulated sesquiterpene data (Devon and Scott, 1972; Ourisson, 1966) suggested that D was the known enone **3**, cyclocolorenone (Figure 5), first isolated from *Pseudowintera colorata* (Corbett and Speden, 1958). However, the UV data (λ_{max} = 264 nm; e = 13,000; EtOH) and optical rotation ($[\alpha]_D$ = −400°) reported for cyclocolorenone did not match our measured values: UV: λ_{max} = 252 nm (e = 4400, EtOH); $[\alpha]_D$ = −200° ($c \approx$ 0.8 mg in 5.0 ml, EtOH). These results lead us to conclude that D was actually **4** (Figure 5), the known C-1 epimer of cyclocolorenone. This compound, referred as epicyclocolorenone, has been obtained from **3** upon treatment with KOH (Corbett and Young, 1963) or upon chromatography on basic alumina (Büchi and Lowenthal, 1962). To the best of our knowledge, it would appear that our isolation of epicyclocolorenone from *D. kerrii* represents the first report of this material as a naturally occurring substance.

Our data for D correlated well with data reported for **4** (Büchi and Lowenthal, 1962; Corbett and Young, 1963; Büchi et al., 1966); UV: λ_{max} = 253 (e = 9300, EtOH); $[\alpha]_D$ = −167° (EtOH). Especially diagnostic was the signal for the C-10 methyl group [δ 0.99 (d)] in the [^{1}H]NMR spectrum of D. As originally discussed by Büchi in his synthesis of epicyclocolorenone (Büchi and Lowenthal, 1962; Büchi et al., 1966), the C-10 methyl doublet in **4** is observed at δ 1.02, while in naturally derived cyclocolorenone this signal appears at δ 0.78. These chemical shift differences can be accounted for by consideration of the most stable conformations of cyclocolorenone and epicyclocolorenone (Büchi and Lowenthal, 1962; Büchi et al., 1966), as depicted in structures **3a** and **4a**, respectively (Figure 5). In **3a**, the seven-membered ring is in a pseudoboat form and the C-10 methyl group is in the shielding region of the extended

chromophore. In **4a**, however, the more stable conformer is the pseudochair form, which disrupts conjugation of the cyclopropyl group with the enone and places the C-10 methyl group in a pseudoaxial position. This accounts for the both the higher field position of the C-10 methyl in **4a** and for the large differences in the UV spectra of **3a** and **4a**.

Peak B. Low-resolution MS analysis of B, an extremely labile colorless oil, showed a very weak signal at m/z = 220 (both EI and CI), as well as moderately intense peaks at m/z = 205 [M-15(CH_3)] and 202 [M-18(H_2O)]. These data, like those for A, suggested that B contained a labile alcohol group. Also in common with A, GC-MS analysis of B after treatment with Sylon-BTZ showed quantitative transformation to a more volatile product with highest molecular weight ion at m/z = 277 [M(292)-15(CH_3)]. As before, this result suggested that B was an alcohol that had been trimethylsilylated. GC-FTIR analysis showed the presence of a very weak OH band at 3637 cm^{-1} and a moderately intense band at 1635 cm^{-1} (double bond). In analogy with data for A, the 3637 cm^{-1} IR signal implied that B was a tertiary alcohol. HRMS analysis of the B molecular ion gave a molecular formula of $C_{15}H_{24}O$. Taken together, these data suggested that B was a sesquiterpene alcohol with four sites of unsaturation.

The nature and number of the multiple bonds in B were deduced using several lines of information. First, because so little of B was isolated (0.2 mg), only a very poor [^{13}C]NMR spectrum of this material could be obtained (20,000 transients). However, this spectrum showed four olefinic carbon signals: δ 149.1, 140.95, 131.32, 116.53 (d_6-benzene), implying the presence of two double bonds. Secondly, the [^{1}H]NMR spectrum (Figure 6) of B displayed two, one-proton olefin signals (δ 5.67, 5.40), which were not coupled, implying that both double bonds were trisubstituted. Finally, the UV spectrum of B showed λ_{max} = 243 nm ($\varepsilon \approx 2400$; cyclohexane), implying the presence of a conjugated diene. Hence, it was concluded that B was a bicyclic sesquiterpene alcohol containing a conjugated diene chromophore.

Compound B displayed four methyl group signals in its [^{1}H]NMR spectrum characterized as follows: δ 1.71 (m, olefinic methyl group); 1.225, 1.220 [singlets, implying two nearly equivalent methyl groups α to the tertiary alcohol moiety: $C(CH_3)_2OH$]; 0.70 (d, implying a $CHCH_3$ group). Accounting for the five carbons in these groups left 10 carbons to be distributed in the bicyclic system. Of the various possibilities, similarities between the [^{1}H]NMR spectra of B and compound **4** led us to begin with partial structure **5** (Figure 7). Thus, in compound **4**, the protons at position 2 appeared as a geminally coupled set with relatively low field positions [δ 2.58 (dd, J = 6.4, 16.8 Hz); 2.08 (br. d, J = 16.8 Hz)] due to the presence of an sp^2 center (carbonyl) at C-3. In B, a similar pair of signals was observed [δ 2.45, H_d (dd, J = 7.8, 18 Hz); 2.12, H_f (br. d, J = 18 Hz)] implying a similar arrangement of groups, in this case

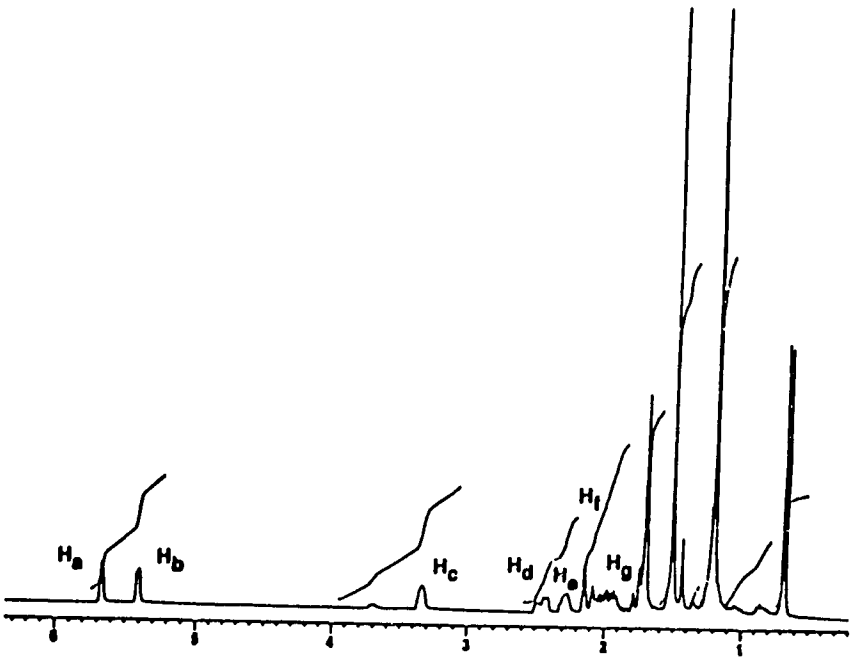
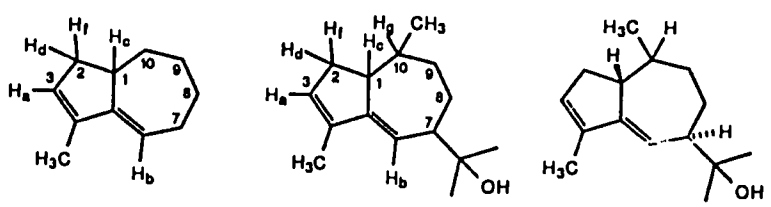


FIG. 6. 300-MHz PMR spectrum of compound B, identified as 7 (Figure 7).

with an olefinic carbon providing the sp^2 center at position 3. Very weak spin-spin coupling was observed between H_d and the olefin proton at δ 5.65 (H_a) and between H_a and the olefinic methyl group, indicating the double bond substitution pattern shown in 5. In addition, H_d was coupled (J = 7.8 Hz) to a methine signal at δ 3.34 (m, H_c) implying that the C-2 methylene was flanked by a bridgehead proton, as shown in 5.

Placing the remaining groups on the now required seven-membered ring in 5 was straightforward. Thus, methine proton H_c was coupled to a one-proton



5 6 7

FIG. 7. Partial structures (5 and 6) and final structure for compound B, identified as 7.

signal at δ 1.93 (H_g) which was, in turn, coupled to the methyl doublet at δ 0.70. This implied that H_c was flanked by the $CHCH_3$ group. Finally, the unassigned $C(CH_3)_2OH$ group was placed in the remaining allylic position (C-7) giving structure **6** (Figure 7). This connection was consistent for several reasons: (1) it allowed assignment of H_e (δ 2.28, e.g., allylic and α to OH); (2) it corresponded to a conventional substitution pattern for a sesquiterpene; and (3) it correlated well with the observed labilty of B (e.g., acid-catalyzed dehydration would produce a highly conjugated product). Hence, we assign structure **6** to B.

A computer search of the Chemical Abstracts On-Line data base for structure **6** produced a single entry, which has the stereochemistry shown in formula **7**. Ourisson reported the synthesis of **7** in the course of determining the structure of α-gurjunene, **1** (Streith and Ourisson, 1963). Oxidation of **1** with SeO_2/acetic anhydride, followed by $LiAlH_4$ reduction, produced an "extremely labile compound," which was assigned structure **7**. Our measured values for B matched Ourisson's physical data for 7 well: UV: λ_{max} = 243 nm (cyclohexane); IR; 1630 cm^{-1} ($CHCl_3$); [^{1}H]NMR: δ 0.70 (d, 3H), 1.15 (s, 6H), 1.7 (m, 3H), 5.38 (1H), 5.6 (1H) ($CDCl_3$, 60 MHz). Our observation of **7** in *D. kerrii* resin represents the first isolation of this sesquiterpene from nature.

Peak C. Much of the chemical behavior of C in the early stages of analysis matched that of A and B. Thus, this material, also a colorless oil, displayed a very weak molecular ion at m/z = 220 and moderately intense peaks at m/z = 205 [M-15(CH_3)] and 202 [M-18(H_2O)]. GC-FTIR analysis showed a very weak OH band at 3741 cm^{-1} and a moderately intense band at 1643 cm^{-1}, implying the presence of a tertiary alcohol group and C–C double bonds, respectively. Low-resolution GC-MS analysis, after treatment with Sylon-BTZ, showed quantitative transformation to a more volatile product with a molecular ion at m/z = 292. Finally, HRMS analysis of the molecular ion for C once more indicated a molecular formula of $C_{15}H_{24}O$. In addition, C exhibited four olefinic carbon resonances in its [^{13}C]NMR spectrum (δ 152.09, 145.83, 123.86, 110.96), and showed UV absorption (λ_{max} = 219 nm, e = 1400), implying the presence of a conjugated diene. Altogether, this information indicated that C was a bicyclic sesquiterpene containing a tertiary hydroxyl group and two conjugated double bonds.

Although rather complex, the [^{1}H]NMR spectrum of C (Figure 8) displayed the following salient features: (1) three one-proton olefin signals [δ 5.71, H_a, dd (J = 3.85, 6.7 Hz, no coupling with other olefin signals); 4.77, H_b, br. s; and 4.68, H_c, br; coupling between H_b and H_c ($J \approx$ 2 Hz), as well as the chemical shift and general appearance of these signals, implied that they represented a terminal methylene group]; (2) two downfield methine signals (δ 3.03, H_d, m; 2.85, H_e, m); and (3) three methyl group signals [δ 1.70, br. s (implying an olefinic CH_3 group); 1.27, s (implying a CH_3 α to the tertiary

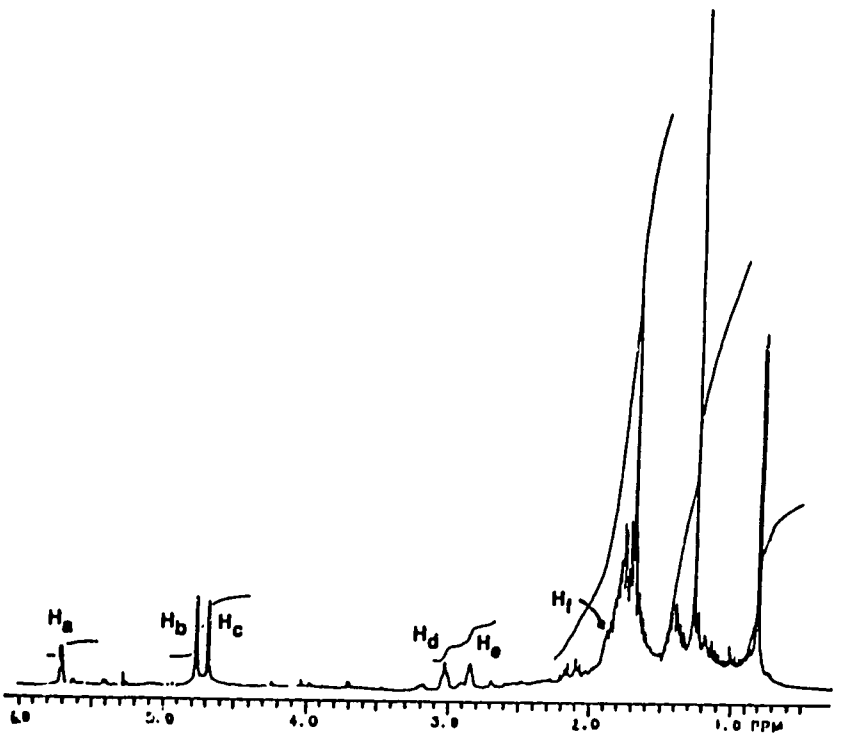


FIG. 8. 400-MHz PMR spectrum of compound C, identified as 9 (Figure 9).

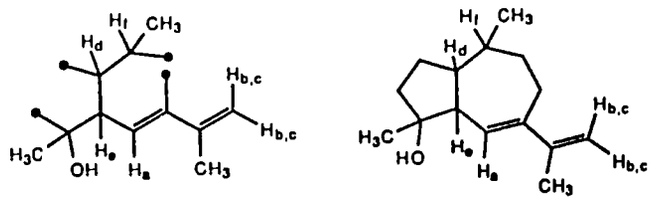
hydroxyl group, as in A); 0.81, d (implying a $CHCH_3$ group)]. The remaining proton absorbances were located in two broad, complicated bands centered roughly at δ 1.75 ($\approx$ 5H) and 1.4 ($\approx$ 4H). Finally, by [^{13}C]NMR analysis using an INEPT pulse sequence (Morris and Freeman, 1979; Doddrell and Pegg, 1980), C was found to contain three methyl (δ 27.27, 21.51, 14.76), five methylene (δ 110.96, 41.33, 32.86, 26.87, 25.33), four methine (δ 123.86, 46.65, 45.43, 32.86), and three quaternary (δ 152.09, 145.83, 80.74) carbon atoms.

Partial structure **8** (Figure 9) can be proposed for C on the basis of exhaustive decoupling studies (one- and two-dimensional), which yielded the following spin–spin coupling relationships. First, weak coupling between the olefin methyl group and the terminal methylene protons (H_b/H_c), together with a lack of coupling between H_a and H_b/H_c and the olefin methyl, suggested that the diene was composed of an isopropylidene group conjugated with a trisubstituted, endocyclic double bond. Secondly, C contained three nonolefinic methine carbons. One of these had to be present in the $-CHCH_3$ group. Indeed, irradiation at δ 1.89 (H_f, on the shoulder of the complicated band centered at δ

1.75) collapsed the methyl doublet at δ 0.70, establishing the chemical shift of this methine proton. Since C had been shown to be bicyclic, and since all of the quaternary carbon atoms had already been accounted for (olefins and tertiary alcohol), it was deduced that the remaining methines had to occupy bridgehead positions. The one-proton multiplets were obvious candidates for these methines. Thirdly, olefin proton H_a coupled to both of these methine signals (H_d, δ 3.03, J = 3.8 Hz; H_e, δ 2.85, J = 6.7), which were, in turn, coupled to each other. This implied that H_a, H_d, and H_e were on contiguous centers; the relative magnitudes of the H_a/H_d and H_a/H_e coupling constants indicated that H_e was the nearer to H_a. Finally, H_f coupled to H_d but not to H_e, while H_d was coupled to signals in the δ 1 85 region but not so for H_e. This implied that H_e was bordered by the remaining quaternary center, the tertiary alcohol, and that H_d was bordered by one of the unassigned methylene groups.

At this stage, only placement of the remaining four methylene groups was needed to complete a structure assignment for C. Of the various possibilities, a symmetrical distribution of the methylenes in the two rings of the bicyclic system was judged to best fit the [^{1}H]NMR, producing structure **9** (Figure 9). The nonsymmetrical distributions were ruled out as each would have produced highly distinctive features in the [^{1}H]NMR that were not observed. In structure **9**, extensive coupling interaction between the methylene groups in the two sets would be expected to produce complex and broad [^{1}H]NMR absorptions, just as is observed for C. To the best of our knowledge, this is the first report of structure **9** in the literature, constituting a new addition to the guiane family of sesquiterpenes. Studies to establish the stereochemical configuration of **9** are in progress.

What we can conclude from these studies is that the resin of *Dipterocarpus kerrii* contains small quantities of four labile sesquiterpenoids, closely related to α-gurjunene, which are responsible for the resin's termiticidal and antifungal activity. We hope to prepare each of these components in quantities sufficiently large to permit more detailed evaluation of their biological properties.



8 **9**

FIG. 9. Partial structure (**8**) and final structure for compound C, identified as **9**.

Experimental

Peak B. [^{1}H]NMR: δ 0.70 (d, 3H, J = 7.3), 1.22 (s, 3H), 1.225 (s, 3H), 1.24 (m, 2H), 1.71 (br. s), 1.76 (br. d, J = 11.2), 1.93 [m, 1H, J = 7 with C-10 CH_3 (δ 0.70)], 2.02 (m, 1H), 2.12 (br. d, 1H, J = 18), 2.29 (m, 1H), 2.45 (br. dd, 1H, J = 7.8, 18), 3.34 (m, 1H), 5.4 (br. d, 1H, J = 3.9), 5.67 (br. s, 1H). GC-FTIR: 3637 (v. weak), 1643 cm^{-1}. [^{13}C]NMR (d_6-benzene, incomplete): 149.10, 140.95, 131.32, 116.53, 72.74, 52.36, 40.26, 34.70, 33.49, 32.42, 23.28, 12.36. UV (cyclohexane): λ_{max} = 243 nm (e = 2400). $[\alpha]_D$ = +50° (c ≈ 0.2 mg in 1.0 ml CCl_4). EI-MS m/z (rel. intensity): 220 (M+, 0.6), 205 (3), 202 (4), 187 (3), 162 (100), 161 (37), 147 (53), 133 (42), 120 (25), 119 (45), 107 (22), 105 (71), 94 (62), 91 (54), 81 (40), 79 (20), 59 (80). EI-MS (peak B + Sylon-BTZ) m/z: 277 [M-15(CH_3)]. HRMS: calcd for $C_{15}H_{24}O$: 220.1827; found: 220.1848.

Peak C. [^{1}H]NMR: δ 0.81 (d, 3H, J = 7.2), 1.27 (s, 3H), 1.28–1.5 (br. m, 4H), 1.70 (br. s, 3H), 1.40–1.83 (br. m, 5H), 1.89 [m, 1H, J = 7 with C-10 CH_3 (δ 0.81)], 2.85 (m, 1H), 3.03 (m, 1H), 4.68 (br. s, 1H), 4.77 (br. s, 1H), 5.71 (dd, 1H, J = 3.85, 6.7). GC-FTIR: 3741 (v. weak), 1643 cm^{-1}. [^{13}C]NMR: δ 152.09 (s), 145.83 (s), 123.86 (d), 110.96 (t), 80.74 (s), 46.65 (d), 45.43 (d), 41.33 (t), 32.86 (overlapping d and t), 27.27 (q), 26.87 (t), 25.33 (t), 21.51 (q), 14.76 (q). UV (cyclohexane): λ_{max} = 219 (e = 1400). $[\alpha]_D$ = +63° (c ≈ 2 mg in 1.0 ml CCl_4). EIMS m/z (rel. intensity): 220 (M, 7), 205 (24), 202 (58), 187 (42), 173 (16), 162 (82), 159 (48), 149 (31), 147 (46), 145 (74), 131 (63), 120 (64), 119 (70), 117 (30), 107 (65), 105 (100), 95 (29), 93 (63), 91 (91), 81 (34), 79 (62), 77 (47), 67 (29), 55 (43), 43 (88), 41 (74). EI-MS (peak C + Sylon-BTZ) m/z (rel. intensity): 292 (M, 3), 277 [M-15(CH_3)]. HRMS: calcd for $C_{15}H_{24}O$: 220.1827; found: 220.1809.

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Biological and ecological studies of Calliteara cerigoides (Lepidoptera: Lymantriidae), a polyphagous defoliator of Southeast Asian Dipterocarpaceae

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Running Head: Biology of a tropical tussock moth

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ABSTRACT. 1. Larvae of Calliteara cerigoides (Walker) (Lepidoptera: Lymantriidae) were encountered as important herbivores of the dipterocarp trees Shorea javanica and Hopea odorata.

2. Feeding preference tests indicated that while C. cerigoides is polyphagous, it feeds preferentially on dipterocarps.

3. The urticating effects of larval setae appear to be caused by the structure of numerous apically-directed tines.

4. Eggs hatched in 10.4 ± 1 ($\bar{x}$ ± SD) days, and larval instars were 7-9 days in duration. Female C. cerigoides deposited masses of 283 ± 274 eggs on tree trunks in an experimental forest.

5. The parasitoid wasps Mescomys orientalis (Eupelmidae) and Tyndarichus ?navae (Encyrtidae) were reared from eggs. The rate of parasitism for eggs in a field study was 78%, suggesting that biological control of C. cerigoides is possible.

Key words: Lymantriidae, herbivory, tropical biology, Eupelmidae, Encyrtidae, Dipterocarpaceae, parasitism.

Introduction

Dipterocarp trees are a major component of lowland primary forests in Southeast Asia, and are important natural resources (Ashton, 1982). Though they contain defensive chemicals, which presumably protect against biological attack, (Richardson et al., 1989), massive defoliations of dipterocarp trees by unidentified Lepidoptera have been reported (Anderson, 1961).

Information on insect herbivores which feed on dipterocarps is important not only to an understanding of insect-plant interactions, but also for management of dipterocarp plantations established to aid reforestation in tropical areas. This report presents biological data on the dipterocarp defoliator Calliteara cerigoides (Walker) (Lepidoptera: Lymantriidae) gathered in the course of research on defensive chemicals of these trees.

Methods

Study localities

Moths were collected in cultivated Shorea javanica K.& V. (Dipterocarpaceae) agroforests (Torquebiau, 1984) in Krui, Lampung Province, Sumatera, Indonesia. Resins, scraped from man-made holes in tree trunks of S. javanica, are sold for formulation into varnishes, paints, and other materials. Extensive observations were made of C. cerigoides in the Cikarawang experimental forest, Bogor, West Java, Indonesia. This forest contains about fifteen dipterocarp and forty other species planted in blocks. In profile, the Cikarawang forest is essentially open, because lianas and extraneous tree species are removed by the forest caretakers. There is dominant canopy of mature trees above, with a horizon of 1-2 m high seedlings below. Scattered through the forest are trees of intermediate (approximately 5-15 m) height.

The observations and experiments below were carried out during two heavy C.

cerigoides infestations of the Cikarawang forest, during the periods June-August 1988, and March-May, 1989. Where possible, voucher specimens have been deposited in the Cornell University Insect Collection, Lot No. 1185, and at SEAMEO-BIOTROP.

Laboratory Methods

All insect materials were taken from the Cikarawang Forest. Eggs, larvae and pupae of C. cerigoides were collected from foliage and tree trunks. Eggs were held at ambient laboratory temperatures (28-32°) in 5 cm diameter petri dishes lined with moistened filter paper. Larvae and adults were maintained in 50 cm square nylon mesh cages. Larvae were offered freshly cut leaves of host plant seedlings taken from the Cikarawang forest, or from a nursery; dilute honey solutions were offered on cotton to adult Calliteara. Pupal cases were removed from the surrounding sheath of setae, and examined for parasitoids. After emergence of egg or pupal parasitoids, these parasitoids were maintained in 5 cm petri dishes and offered honey solutions in the manner described.

Reproductive potential

To determine reproductive potential of C. cerigoides, females and males which had emerged from field-collected pupae were paired in individual nylon mesh cages. The moths mated, and females oviposited in the cages. The number of eggs deposited was counted, and after the females had died, dissections were performed to determine the number of eggs remaining in the ovaries.

Measurements of eggs were made with a compound microscope equipped with an ocular micrometer; larvae and pupal dimensions were measured with calipers. All measurements are presented as $\bar{x} \pm$ S.D.

Herbivory

Leaf preference studies were conducted with naive 1st instar larvae within three days

after emergence. Freshly cut 2 cm x 6 cm strips of leaves were taped to the inside of a 30 cm glass arena. Twenty-five larvae were placed in the center of the arena. The amount of leaf damage was noted after 24 hours.

Studies of urticating setae

In this paper, the term setae is used sensu Peterson (1956). Approximately 50 simple setae of 2-3 cm length were plucked from the dorsal surface of larvae in their last instar. The setae were extracted overnight in 5 ml 100% dichloromethane prior to gas chromatography. Extracts were analyzed with a Shimadzu GC-7A gas chromatograph, fitted with a 3 m x 3 mm glass column of 3% OV-101 on GasChromQ, and a flame ionization detector. Samples were eluted with nitrogen, with the column oven temperature programmed to rise from 150°-250° at 4° per minute.

For examination with the scanning electron microscope (SEM), dried larval setae were mounted on stubs, coated with gold, and observed.

Parasitoid field studies

Tree trunks were systematically examined for C. cerigoides egg masses during March-April 1989. When an egg mass was located, its height on the trunk, and the diameter at breast height (DBH) of the tree were measured. The total number of eggs in each mass was counted, and the egg mass was examined in situ to determine the extent to which egg masses had been parasitized. Pilot observations made in the laboratory during the 1988 outbreak established that parasitoids made smaller, distinct, round exit holes with smooth edges at an angle to the micropyle, while C. cerigoides larvae partially consumed the egg shell, including the micropyle, resulting in a larger irregular hole or leaving only a partial shell (Fig. 1). Exit hole sizes were not measured in the field study.

It was not possible to determine when parasitized egg masses were deposited. Egg masses were counted after all emergence had ceased. By this time, virtually all of the eggs in the masses had some sort of exit hole. When encountered, intact masses were left undisturbed until emergence had taken place.

Results

Extent and Consequences of Defoliation Specimens of C. cerigoides were collected as larvae from trunks of S. javanica trees cultivated for resin production near the village of Gunung Kemala, Krui, in February, 1987. Resin harvesters stated that the larvae of C. cerigoides were responsible for massive defoliations of S. javanica agroforests, with a loss of resin flow resulting.

In the 1988 infestation of Cikarawang experimental forest, C. cerigoides larvae were concentrated on the canopy of Hopea odorata. Though it was not possible to measure larval density, the frass fall from the canopy was great enough to be clearly audible, and the entire ±1 ha block of H. odorata canopy was defoliated by the herbivore. However, seedlings of the same species remained largely undamaged. Examination with binoculars of canopy cover in adjacent blocks, including an adjoining block of Hopea mengerawan, did not reveal any detectable defoliation of other dipterocarp or non-dipterocarp species.

Feeding Preferences In feeding preference tests, naive first instar C. cerigoides showed a preference for leaf strips of H. odorata (Fig. 2). Though other species were eaten, none showed as much damage as H. odorata.

Urticating Effects and Structure of Setae

Resin harvesters in Sumatera noted that the setae of the larvae were extremely irritating, a fact confirmed during work with C. cerigoides in the Cikarawang forest. To

determine if organic chemical factors might cause the observed urticating effects, dichloromethane extracts of larval setae were analyzed with the gas chromatograph. Except for the solvent peak, no other chemicals were detected in the dichloromethane extract. Examination of the setae via SEM revealed that numerous, sharp, apically-directed tines project from the shaft along its entire length (Fig. 3).

Biological Observations of C. cerigoides

Eggs of the moth collected in the field were 1.54 ± .04 mm in diameter (n = 45); eggs deposited by females in the lab were 1.46 ± .022 mm (n = 66) in diameter. Eggs hatched in 10.4 ± 1 days (n = 18). Attempts to rear C. cerigoides, on freshly cut leaves of dipterocarp seedlings, past the 5th instar were not successful, but to this point the duration of each instar was 7-9 days.

In the experimental forest, small groups (usually no more than 10) of larvae were often seen in aggregations on boles. The larvae were observed actively feeding and moving up and down boles during daylight hours.

Pupae were enclosed in a tightly webbed sheath, constructed out of tightly woven urticating hairs of the last larval instar. Pupal sheathes were found in low foliage and seedlings. Pupae were dimorphic. Those developing into females were 43 ± 1.8 mm long (n = 8); pupae developing into males were 27 ± 1.3 mm long (n = 16). The duration of the pupal stadium was not determined.

Adult female C. cerigoides observed in the field were never seen in flight, even after specimens in the field were disturbed. Laboratory reared females did not achieve full wing expansion following eclosion, but mated nonetheless. Copulation was observed in progress in early morning hours in the field on two occasions (Fig. 4). In both cases apparently newly-emerged females hung in a near vertical position from the

webbed sheath surrounding the pupae, with the head up and the sternites and wings appressed to the sheath surface. Males were in a similar position, lower on the opposite side of the sheath, so that with the terminalia in contact the male was at right angles to the female (Fig. 4). Copulation was also observed in laboratory cages, with both insects on the same vertical surface, facing in opposite directions with the terminalia joined.

Deposition of eggs Eggs were deposited on tree trunks (n = 126) in masses (n = 236). It was not possible to reach egg masses more than 2.5 m above ground level so the results below do not describe all egg masses located. Below this 2.5 m level, eggs masses ranged from 4-222 cm above the ground, with the mean height of 132 ± 46 cm (n = 156). The DBH of trees (n = 89) with egg masses which could be counted ranged from 18-85 cm, with the mean DBH of 41 ± 12.7 cm (n = 156). Freshly deposited egg masses were off-white in color, and were not covered with any hairs. Egg masses contained 4-1174 eggs, with the mean mass size of 283 ± 274 eggs (n = 156). The distribution of egg mass size, is shown in Fig. 5.

The sum of the number of eggs deposited by females in individual cages and the number of eggs dissected from the same females after death, indicated that ovaries of adult females contained a mean of 391 ± 254 chorionated oocytes (n = 14).

Parasites and Associates

Unidentified parasitoids were observed ovipositing singly, and in groups, on intact egg masses. Two parasitoid Hymenoptera species emerged from egg masses of C. cerigoides. These were determined to be Mescomys orientalis Ferriere (Eupelmidae), and Tyndarichus ?navae Howard (Encyrtidae). Parasitoid exit holes (n = 88) in eggs held in the lab were divisible into two groups ($p < .001$, $t = -18.6$, 76 df). The smaller

holes were 0.48 ± .038 mm (n = 53) in diameter; the larger holes were 0.68 ± .06 mm (n = 25) in diameter. The larger holes resulted from emergence of M. orientalis.

Dipteran larvae and pupae were dissected from pupae of C. cerigoides. The larvae were referable to Sarcophagidae and Tachinidae. Adult dipterans were recognized as belonging to an undetermined phorid species, and the tachinid genus Carcelia. Wasps emerging from the Carcelia sp. pupal cases were identified as Brachymeria lugubris (Walker).

Parasitized pupae of C. cerigoides also contained other insects, which appeared to be be feeding on decaying tissues. These insects were identified as adult Aleochara postica Walker (Coleoptera, Staphylinidae), and adults and larvae of ?Eporus sp. (Coleoptera, Rhizophagidae).

Quantitative Aspects of Parasitism

The parasitism rate for all egg masses (n = 156) ranged from 0-100% (Fig. 6), with a mean of 78%. For the mean parasitism rate, the 95% confidence limits were 72.5% and 83.5%. Two-thirds of the egg masses (n = 100, 64%), were parasitized at a rate which placed them in the interval of highest parasitism rate (91-100%). For these 100 egg masses, the mean parasitism rate was 99.2%. There was no correlation (r = -0.038, Spearman Rank Correlation) between the number of eggs in a mass, and the parasitism rate of the mass.

Data relating to rates of parasitism of C. cerigoides pupae were not collected.

Discussion

A survey of the literature pertaining to C. cerigoides revealed no previous records of feeding on dipterocarps (Holloway, 1976). In the experiments reported here, C. cerigoides was shown to be polyphagous. Related lymantriid species are also

polyphagous, and have been shown to feed on a variety of cultivated and wild plant species (Dupont & Scheepmaker, 1936; Mehra & Sah, 1974; Mathew, 1978; Islam & Joarder, 1983; Zaman & Karimullah, 1987; Reddy et al., 1988). That C. cerigoides may feed preferentially on H. odorata parallels the finding that Dasychira mendosa Hubn. form fusiformis Walker is polyphagous, but shows a distinct preference for Mohgania macrophylla (Willd.) O.Ktze (Mehra & Sah, 1974).

Perhaps of more interest than the polyphagous behavior of C. cerigoides is that it eats dipterocarp species. Though chemical data is not available for the Shorea sp. and Hopea sp. studied here, dipterocarp leaves possess tannins (Waterman et al., 1988), and dipterocarp resins in general contain terpenoid chemicals known to be toxic and repellent to insects (Richardson et al., 1989). These resins circulate through the leaf laminae in canals which lie beside vascular bundles (Metcalfe & Chalk, 1979). Toxic and inhibitory effects of some of the chemicals found in dipterocarp resins (β-caryophyllene, and caryophyllene oxide for example) have been demonstrated for the generalist herbivores Spodoptera exigua (Lepidoptera: Noctuidae) (Langenheim et al., 1980) and Heliothis virescens (Gunasena et al., 1988), as well as for leafcutting ants (Hubbell et al., 1983; Howard et al., 1988). Such toxic chemicals might have been a factor in causing the mortality of C. cerigoides larvae in laboratory culture; other lymantriids cultured on toxic host plants (castor, Ricinus communis L.) in the laboratory showed substantial (>60%) mortality by the fifth instar (Islam & Joarder, 1983).

The sexual dimorphism and urticating setae are consistent with descriptions of other Indonesian Lymantriidae (Dupont & Scheepmaker, 1936). No organic molecules

soluble in dichloromethane were found in extracts of the larval setae. Though the chemical techniques used would not have have detected the presence of any proteins possibly responsible for urticating effects (Lamy et al., 1986), there is no evidence that this defensive function does not have the same physical basis described for other lymantriid larval setae.

The morphology of the setae (Fig. 3) is interesting because it suggests that a complex process may underlie their development. Tines do not bear a monotonic relationship to diameter of the setal shaft. The relationship between tine length and shaft diameter at a given point can be approximated with a second order polynomial which describes a parabola.

The number of eggs deposited by female C. cerigoides in egg masses overlaps that reported for other lymantriids (Mehra & Sah, 1974; Hérard, 1979; Bellinger et al., 1988). The mean number of chorionated eggs in gravid females, determined by adding the number of eggs deposited to the number of eggs dissected from the same dead female (891 eggs), is 3.15 times larger than the mean number of eggs per mass (283 eggs), indicating that female C. cerigoides may deposit more than one egg mass. Though direct field observations are necessary to confirm this, an indirect corroboration of this result is provided from examination of the mean number of egg masses per tree, 1.9 ± 1, and the fact that the females appear to be flightless. A single female moth might thus oviposit more than once on the same tree.

On the basis of exit holes, it was determined that a mean of 78% of the C. cerigoides eggs in masses located in the experimental forest had been parasitized. Holes originating from exit of parasitoids were distinguishable from exit holes of C. cerigoides by their shape, size and position on the egg. These criteria were

established on the basis of laboratory observations.

The high rate of parasitism seen in C. cerigoides eggs may result from the apparency of egg masses in the experimental forest. Compared to natural primary or second growth forest, the Cikarawang forest is much less heterogeneous, and much more open in profile, perhaps making it easier for parasitic wasps to locate egg masses.

It is possible that the restriction of our study to egg masses below 2.5 m above ground level influenced the rate of parasitism determined for C. cerigoides. However, L. dispar eggs masses above the 2.5 m level experienced greater mortality due to predators and parasites than those below (Hérard, 1979).

The exact number of species of egg parasites attacking C. cerigoides was not determined in our research, nor were we able to study the biology of M. orientalis and T. ?navae. Surveys of L. dispar egg parasites in Asia revealed that six primary parasites attacked eggs (Schaefer et al., 1988). Tyndarichus navae was recorded as a hyperparasite. Regardless of whether or not the Tyndarichus sp. reared from C. cerigoides is a hyperparasite, the fact remains that C. cerigoides eggs are subject to a high degree of parasitism.

The observations and experimental results presented here indicate that C. cerigoides may feed on at least eight dipterocarp species. This suggests that C. cerigoides has overcome chemical defenses of these trees. As such C. cerigoides is of potential economic importance as a defoliator of dipterocarps, both in naturally-occuring and cultivated environments. The discovery and identification of egg parasites, and the finding that eggs sustain high rates of parasitism, indicate that biological control alternatives may be valuable in controlling C. cerigoides infestations.

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FIGURE LEGENDS

FIG. 1 Portion of egg mass of Calliteara cerigoides. Parasitoid exit holes are visible as small, rounded holes with smooth edges, offset from the micropyle. Larvae of C. cerigoides consume the eggshell, including the micropylar region, leaving a larger, jagged exit hole, or eggshell fragment.

FIG. 2 Feeding preferences of C. cerigoides. Tests were conducted as described in Materials and Methods. The figure shows experimental results of two herbivory bioassays 24 h after larvae were placed into the arena. The leaves of each row represent a single bioassay conducted with five species. Top row: (1) Hopea mengerawan, (2) Shorea javanica, (3) Shorea selanica; (4) Shorea pinanga; (5) Shorea seminis. Bottom row: (6) Hopea odorata; (7) Vatica sp.; (8) Szygium sp.; (9) Altingia excelsa; (10) Shorea stenoptera.

FIG. 3 Sections of urticating setae of C. cerigoides viewed with the scanning electron microscope. Setae are oriented with their origin to the left, thus the spines point in the direction of the apex. Bar = 100 μ. A. Adjacent to base of seta, showing small spines 25-50 μ long; the shaft is cylindrical. B. At the midsection of the seta, spine length reaches approximately 150 μ, more than the length of the distinctly laminated shaft, which is cylindrical in form. C. Three-quarters of the distance to the apex, the shaft has assumed an angular aspect, and the spines are about 70 μ long. D. At the apex,

viewed in profile, the spines are about 10 μ long and the shaft maintains its angular shape.

FIG. 4 Copulation of C. cerigoides. The insects are resting on the pupal case of the newly emerged female.

FIG. 5 Histogram showing number of egg masses in each interval. The percent of all eggs accounted for by egg masses in each interval is indicated by the dark squares.

FIG. 6 Relative and cumulative frequency of parasitism of C. cerigoides egg masses. The histogram shows the relative frequency of percent parasitism of egg masses, the curve indicates the proportion of the total of all egg masses parasitized.

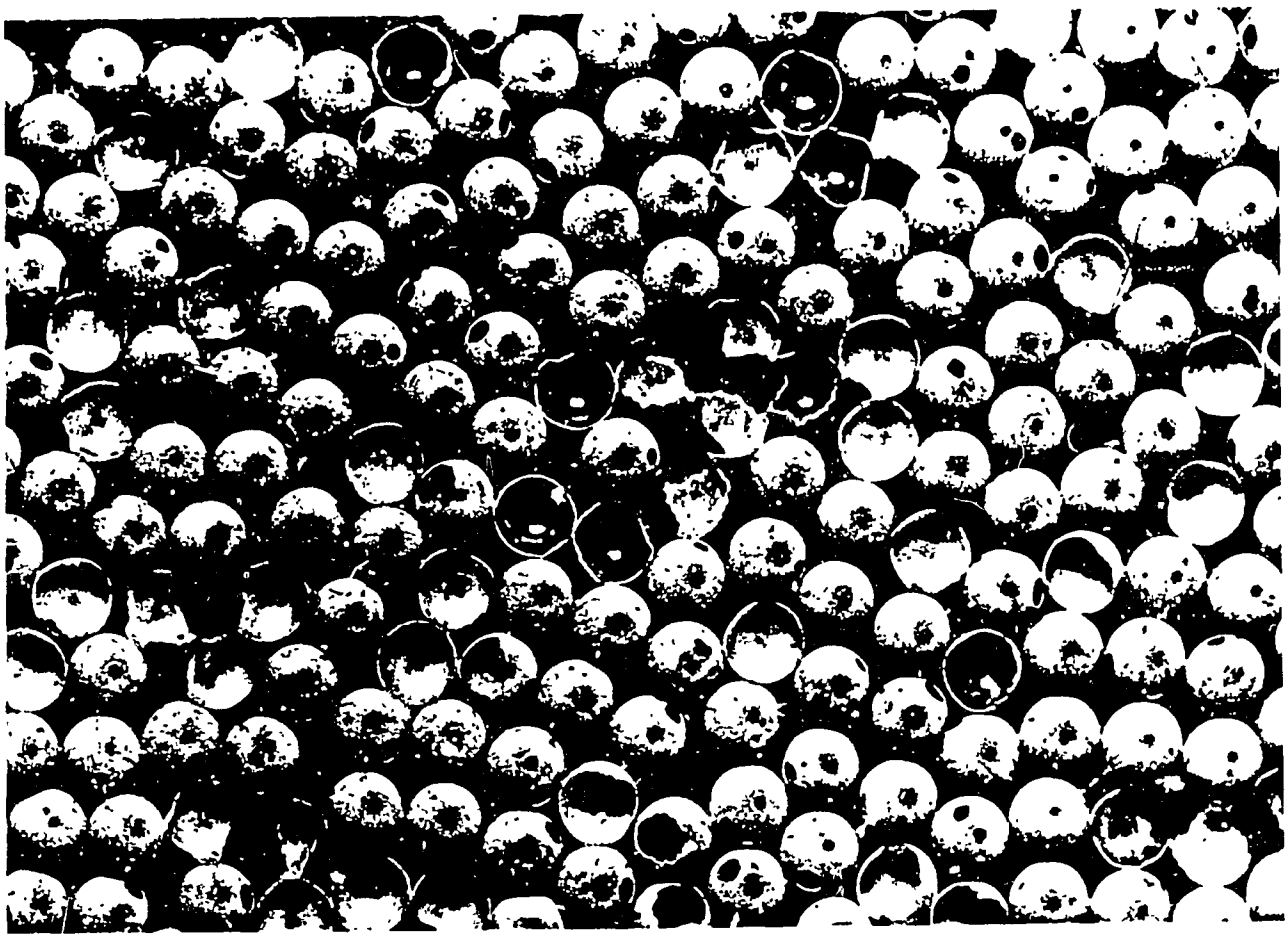

FIGURE 1

FIGURE 1

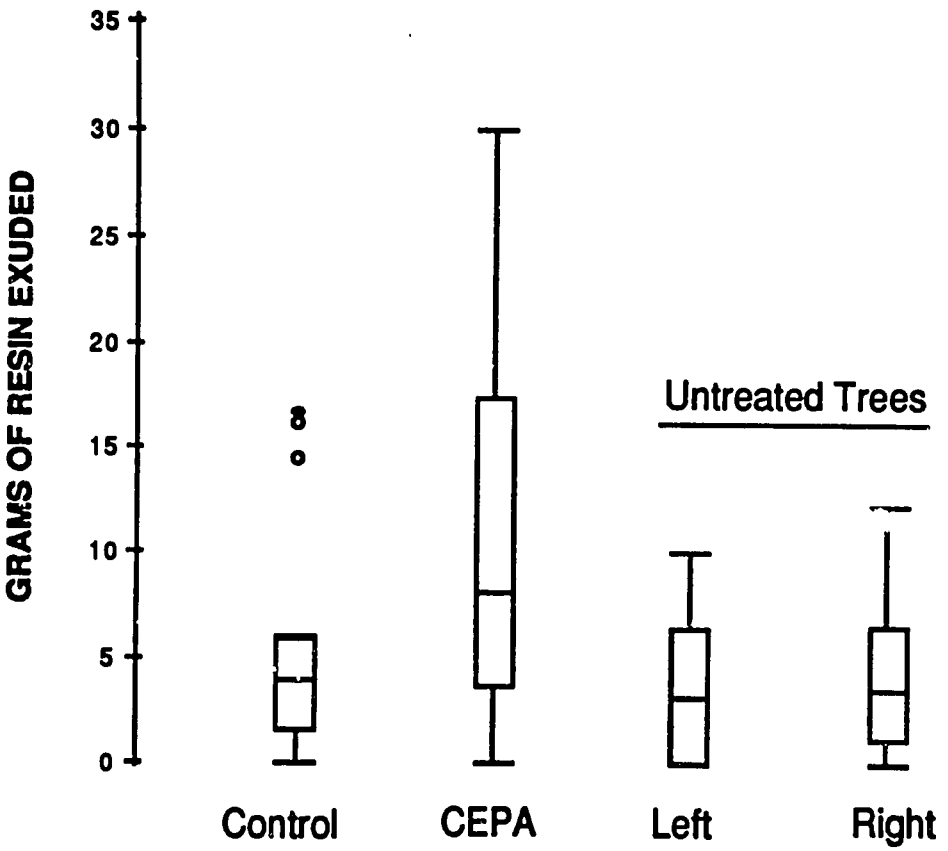
GRAMS OF RESIN EXUDED
35
30
25
20
15
10
5
0
Untreated Trees
Control
CEPA
Left
Right

FIGURE 2

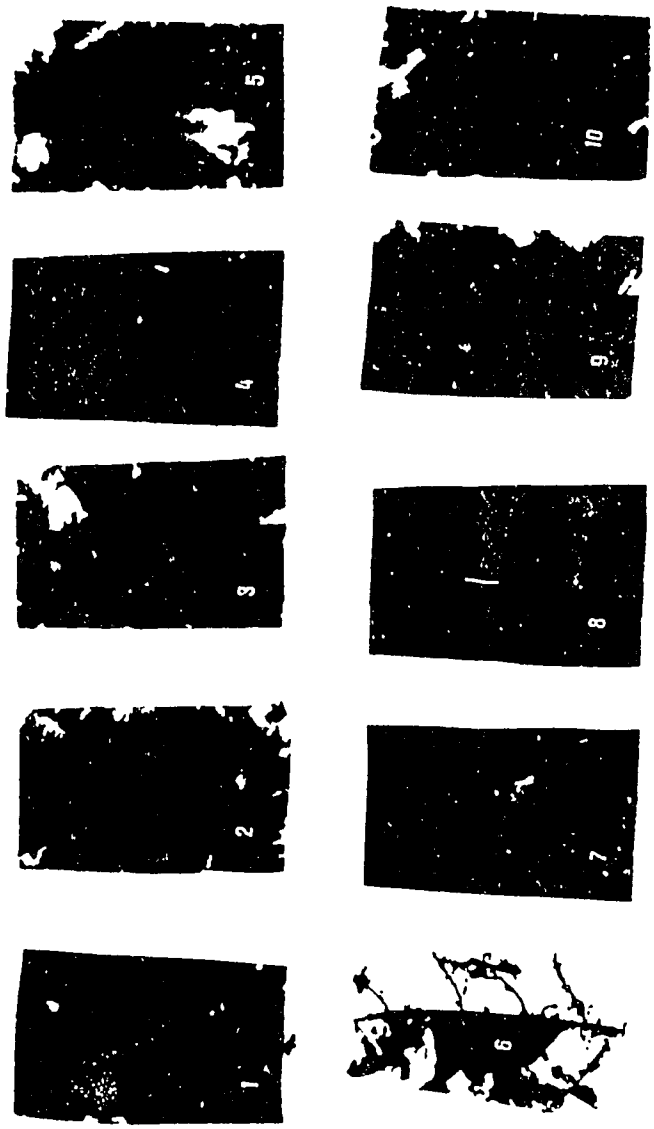

FIGURE 2

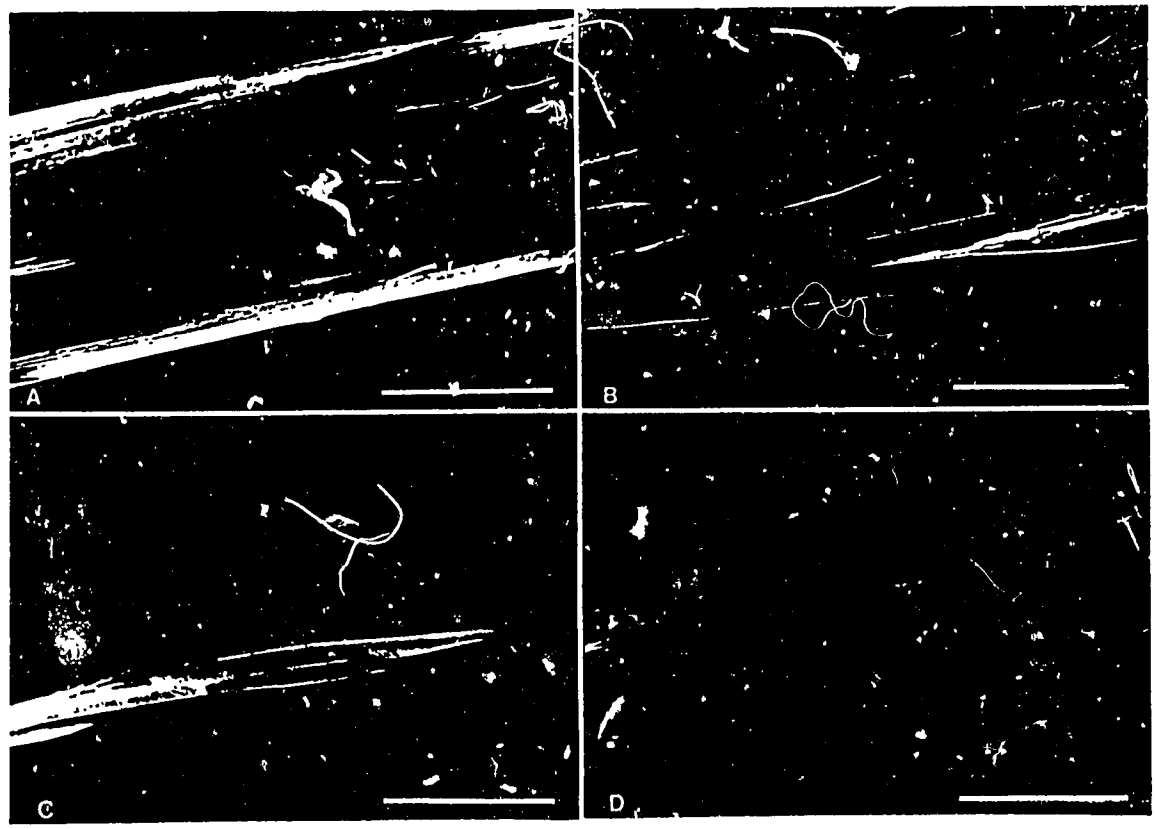

FIGURE #3

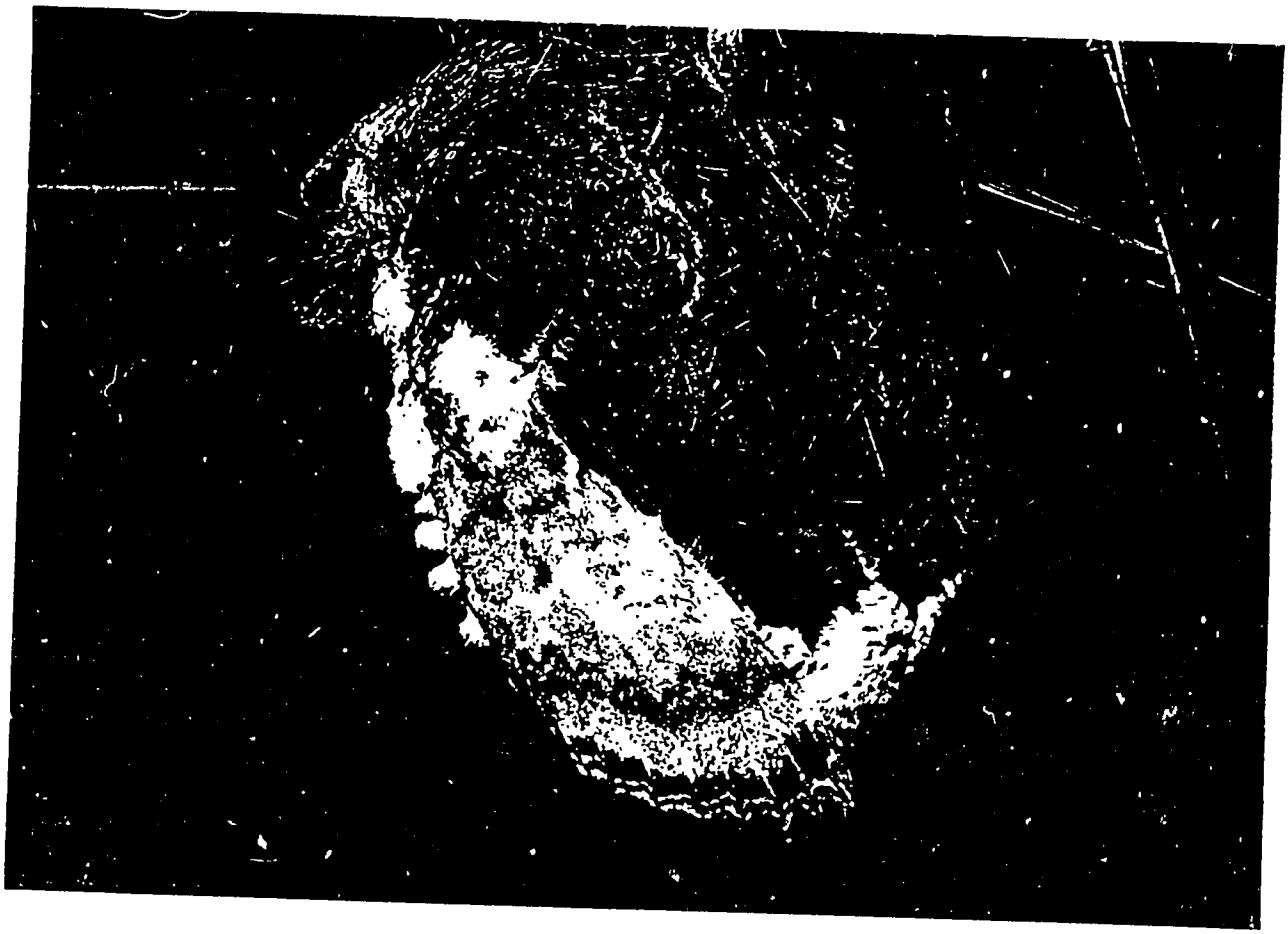

FIGURE 4

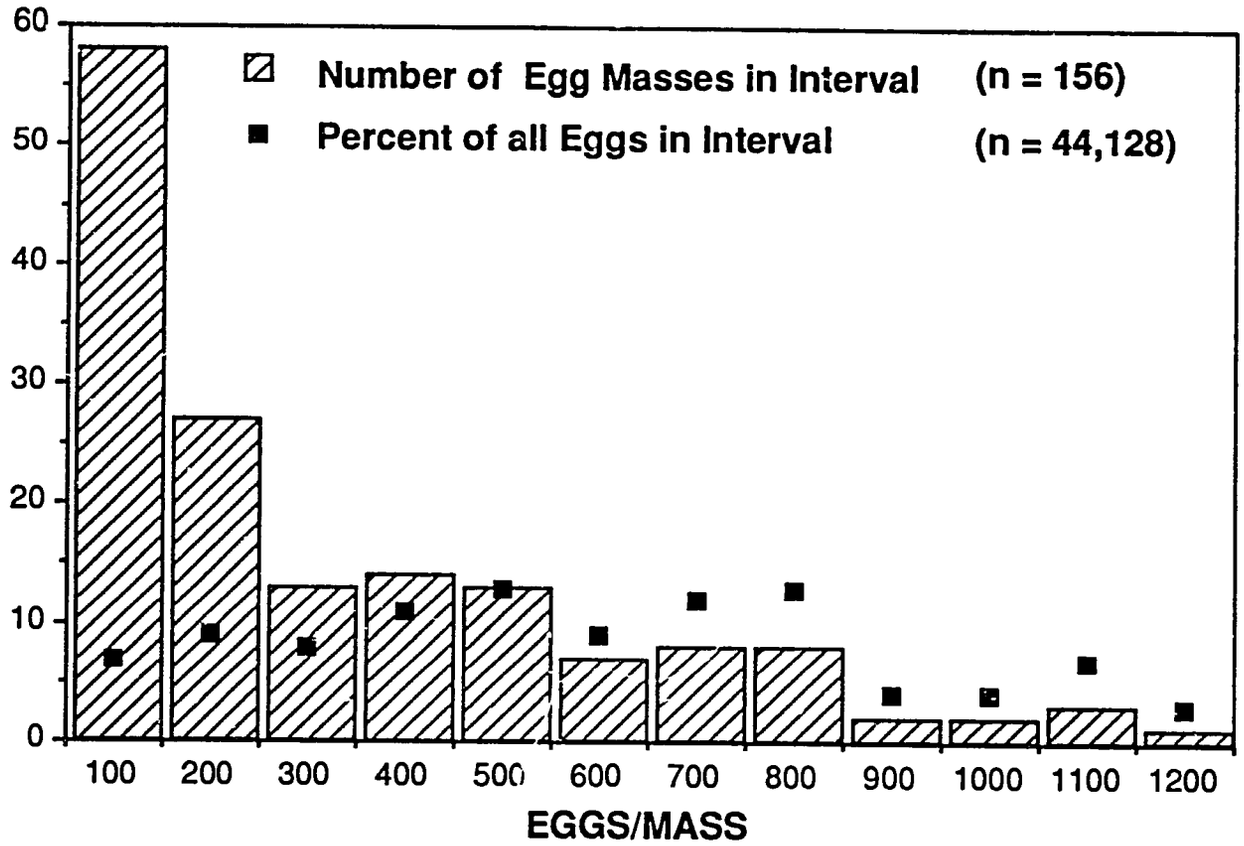
60
50
40
30
20
10
0
Number of Egg Masses in Interval (n = 156)
Percent of all Eggs in Interval (n = 44,128)
100
200
300
400
500
600
700
800
900
1000
1100
1200
EGGS/MASS

FIGURE 5

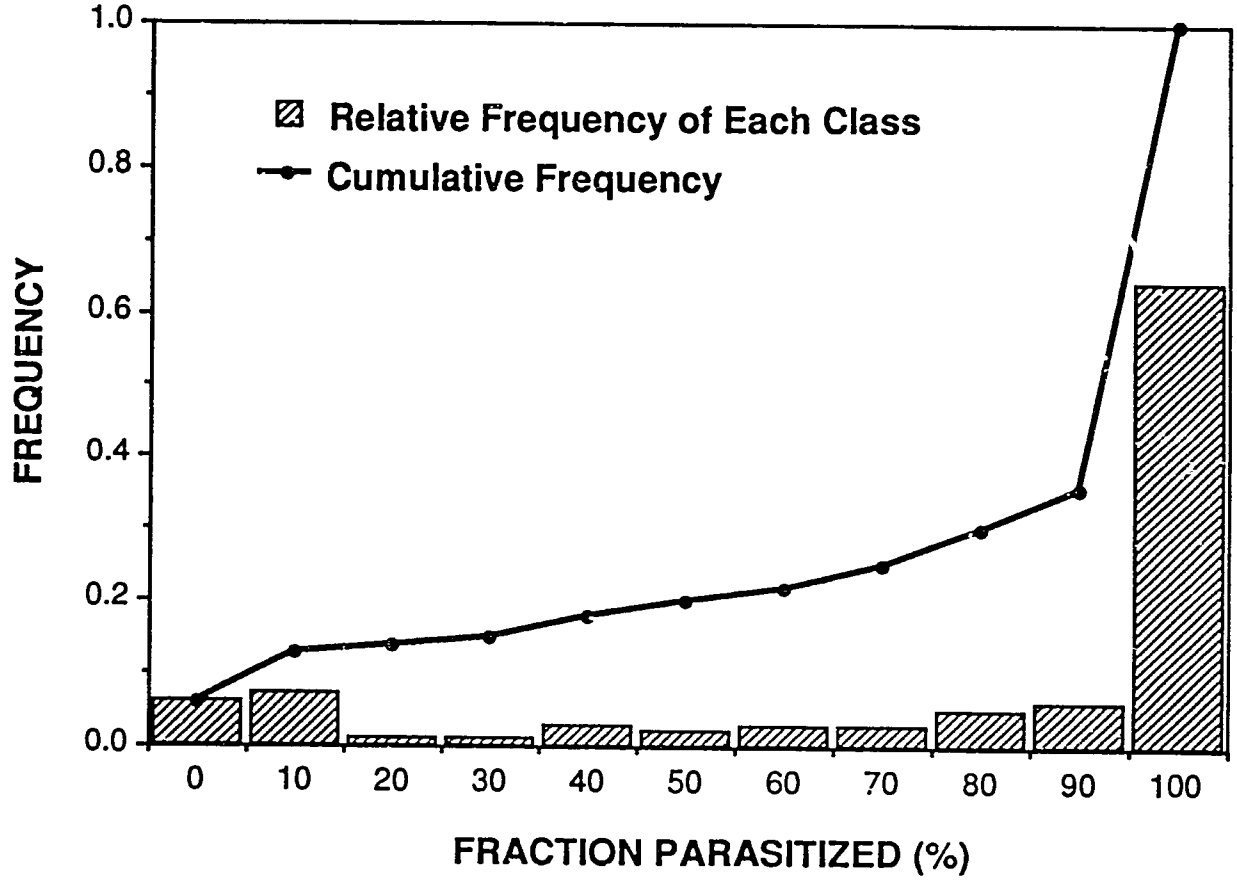
1.0
0.8
0.6
0.4
0.2
0.0
FREQUENCY
Relative Frequency of Each Class
Cumulative Frequency
0
10
20
30
40
50
60
70
80
90
100
FRACTION PARASITIZED (%)

FIGURE 6

Traditional and chemical techniques for stimulation of Shorea javanica (Dipterocarpaceae) resin exudation in Sumatra[1]

Adam Catton Messer[2]

Resin tappers in Sumatra induce resin flow in Shorea javanica by first opening vertical rows of small (3 cm) holes in the tree trunk. After 6-12 mo, larger (10-15 cm) holes are opened between the rows of small holes for resin harvest. Informants reported that opium applied to trees increased resin yields. Application of 10% 2-chloroethylphosphonic acid to artificial trunk wounds of S. javanica production trees increased resin yields by 110% relative to controls in 72-hour trials. Wounds of previously untapped trees exuded no resin in response to the same treatment.

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Les techniques traditionelles et chimiques por la stimulation de la exsudation de résin de l' arbre Shorea javanica (Dipterocarpaceae) en Sumatra. Les préleveurs de résine de Sumatra provoquent l'écoulement de la résine du Shorea javanica en pratiquant, d'abord, des rangées verticales de petits trous (3 cm) dans le tronc de l'arbre. Aprés 6 à 12 mois, des trous plus grands (10 à 15 cm) sont pratiqués, entre les rangées de petits trous, pour la récolte de la résine. Les informateurs ont noté que l'application d'opium aux arbres augmentait les rendements en résine. L'application d'acide 2-chloroéthylphosophonique à 10% aux plaies artificielles aux tronc d'arbres S. javanica de production augmentait les rendements en résine de 110% par rapport aux témoins dans les essais de 72 heures. Les plaies d'arbres non antérieurement saignés n'ont pas exsudé de résine en réponse au même traitement.

Dipterocarp tree resins, known generically as damar, are an important trade commodity in several Southeast Asian countries. Gathered by small landholders from man-made trunk wounds in a number of dipterocarp species, the resins are sold for export (Mantell et al. 1942).

Dipterocarp resins are collected from artificially-induced trunk wounds. The agroforesty ecosystem based on Shorea javanica K. & V. (Dipterocarpaceae), in which villagers harvest resins from cultivated trees, has been documented by Torquebiau (1984), and Mary and Michon (1987). The tapping methods used for S. javanica, in which triangular or semicircular holes are opened in the trunk, have been classified as the "'boxsystem" (Tschirch and Stock 1933).

As with other plant exudates, such as Hevea latex, techniques for increasing resin production by S. javanica might be of significant economic impact. Applications of liquid 2-chloroethylphosphonic acid (CEPA) to trunk wounds, or via solutions sprayed on foliage, have been used to stimulate yields of plant exudates. Under basic conditions, CEPA hydrolyzes to liberate ethylene, which produces a variety of physiological responses

(Abeles 1973). Chemical treatments with CEPA increase yields of latex of Hevea (Abraham et al. 1971), opium resins from Papaver somniferum L. (Ramanathan 1981), and resin of Pinus resinosa Ait. (Wolter and Zinkel 1984). Use of CEPA promoted resin exudation by a single Dipterocarpus kerrii King tree (Kadir et al. 1986).

Known traditional techniques of stimulating dipterocarp resin exudation include those involving the use of fire. To stimulate flow of Dipterocarpus resins in the Phillipines, resin harvesters ignited production wounds to increase the rapidity of the resin flow (Clover 1906). Semelai villagers in peninsular Malaysia briefly burn trunk wounds of D. kerrii to increase exudation of the resin locally known as minyak keruing (Gianno 1986).

This paper describes physical and chemical methods used by Indonesian villagers to stimulate resin exudation from Shorea javanica, and reports experimental results demonstrating a doubling of resin exudation from regularly tapped trees in response to CEPA treatments.

MATERIALS AND METHODS

Experiments and interviews were conducted in S. javanica agroforests and in villages near Krui, Lampung Utara, Sumatra, Indonesia, in July, 1987. Though a regional language predominates in this area, interviews were conducted in Standard Indonesian. Interviews took the form of discussions, and specific questions which might lead or prompt informants were not used.

Throughout this paper a distinction is made between two types of S. javanica trees. "Naive" trees were said by villagers to be 15-30 yr old, and had not been tapped for production prior to the application of the chemical stimulant. "Production" trees had been subjected to the traditional tapping and resin-collecting regime for at least 5 yr.

On naive trees, the southern exposure was determined with a compass. On each side of a line marking due South, a 2 × 15 cm diagonal slash was cut from the bark, exposing the wood beneath. The lowermost portion of the slashes was separated by a distance of approximately 10 cm. The slashes resembled an oblique V (Fig. 1).

Because of pre-existing trunk wounds used for resin harvest, slashes often could not be placed directly on the southern face of production trees. Otherwise these trees were prepared in a similar manner.

Immediately after cutting the slashes, 10 ml of 10% CEPA in carrier (Ethrel 10LS, Union Carbide) were applied with a plastic syringe to a single, randomly chosen slash from each pair. The chemical was distributed evenly over the wound with a paintbrush.

To test for uniform resin exudation between slashes of the same tree, several trees in each category were slashed, but not treated with the chemical.

Accumulated resin was scraped from the wounds into plastic bags 72 hr after CEPA application, and weighed within 12 hr.

Statistical testing was performed with Data Desk (Velleman 1989). All experiments were self-paired, with resin exudation compared between two slashes on the same tree. A normal distribution was confirmed for all data prior to statistical testing. Results are reported as $\bar{x} \pm$ S.D.

RESULTS

Traditional physical methods for increasing resin exudation

Informants claim that tapping unprepared trees will result in little, if any, resin exudation. When a tree has reached an age of 20-30 yr, it is prepared for tapping. Diameter at breast height (DBH) for such trees ranged from 30-49 cm ($\bar{x}$ = 37 cm, n = 7).

Three or four vertical rows of small (3 cm diameter × 2 cm depth) irregularly-shaped holes are cut in the trunk using a hatchet fitted with a 2 × 4 cm steel blade. The rows, containing 30-40 holes each, are cut into lowermost 1-2 m of the bole. Resin tappers cover the holes with a piece of bark. After a month, the hole is uncovered. Any exuded resin, or newly formed periderm, is stripped away and the bark cover is replaced. Though in most cases the holes fill with resin by the third month of the treatment, the procedure continues. After 6-12 mo, when there is substantial resin exudation from the small holes, larger triangular holes are cut in the bole for resin production. Measuring 10-15 cm on a side, and 5-10 cm deep, holes are shaped so that the triangle is upward pointing. Informants explained

that this configuration allows the hole to function as a foothold for tree climbing during resin harvesting.

Should the small holes fail to stimulate resin exudation, resin tappers may remove half of the bark from the lowermost 1-2 m of the tree. After resin flow begins, the stripped area is allowed to heal, and holes are opened in the manner described above.

None of the informants had used fire to stimulate resin exudation, or were aware of any resin tappers in the Krui area who had. Informants said they would be reluctant to ignite trees, for fear of damaging the tree or destroying it.

Traditional chemical methods for increasing resin exudation

Informants in two villages, Pahmungan and Gunung Kemala, said that opium (Indonesian, candu; Echols and Shadily 1989) had been used in the past to stimulate resin exudation. These informants did not know each other, and provided this information of their own accord during discussions about traditional techniques for stimulating resin flow. While exact details of dosage and treatment regime could not be obtained, informants were able

to outline the methods used. Small amounts of opium were applied to trunk wounds; within a week resin flow would begin. Use of opium required great care. An overdose would cause copious resin exudation, with subsequent defoliation resulting in death of the tree.

Treatments with 2-chloroethylphosphonic acid

Results are summarized in Fig. 2. Naive trees (n = 13) exuded no resin whatsoever. Both control and treatment slashes were dry on each naive tree.

Production trees (n = 14) showed a statistically significant 110% increase ($p < 0.031$) in resin exudation in response to chemical treatment (Fig. 1). Untreated slashes yielded 5.3 ± 5.9 grams of resin; CEPA-treated slashes exuded 11.2 ± 10.2 grams.

Comparison of the two slashes on untreated production trees (n = 9) showed no statistically significant difference in resin exudation between right and left sides ($p < 0.53$). Slashes on the left and right sides of the tree yielded 3.6 ± 3.8 grams and 4.9 ± 4.7 grams of resin respectively.

With respect to production trees, no statistically significant difference could be detected ($p < 0.56$) in a pooled t-test ($t = 0.59$ with 22df) of resin exudation between group means of control slashes from chemically-treated S. javanica ($\bar{x} = 5.3 \pm 5.9$ g, $n = 14$) and all slashes from untreated trees ($\bar{x} = 4.2 \pm 4.2$ g, $n = 18$).

DISCUSSION

Treatment of S. javanica trunk wounds with 10% CEPA resulted in a statistically significant 110% increase in resin exudation from production trees during the 72-hr duration of the experiments reported here. Naive trees exuded no resin during the same time course. The striking difference between the two groups of trees suggests that CEPA treatment heightens the existing wound response in S. javanica. The experimental results are also consistent with earlier descriptions, which noted that dipterocarp trees do not usually produce resin until they haved been tapped two or three times (Manteli et al. 1942; Watson 1927).

No statistically significant difference is apparent in resin exudation between groups of slashes on untreated trees. This result gives confidence

to the conclusion that the CEPA treatment caused the observed physiological differences. Further, the similar degree of resin exudation between control slashes on CEPA-treated production trees and all slashes on untreated production trees suggests that effects of CEPA are localized to the area of application. Systemic effects, which might require more than the 72-hr duration of these experiments to develop, would result in control slashes of CEPA-treated trees exuding more resin than slashes in untreated trees. Alternatively it is possible that ethylene gas liberated by the hydrolysis of CEPA is effective only over a short radius.

The experimental results are in line with aspects of traditional resin harvesting methodology. Informants noted that naive trees rarely produce resin if they are tapped for production prior to a conditioning period. This observation was substantiated by experiments with naive trees, none of which produced any resin, even under chemical treatments. Only after the wound response has been 'cranked up' will resin harvesters make larger holes to faciliate resin harvest. Failure of CEPA to stimulate resin exudation in naive trees may have resulted because these trees had not yet received

a challenge sufficient to induce resin exudation.

Precisely what induces the wound response is not clear, though fungal infection following cortical injury seems likely. Traditional methods of stimulating resin flow, with a series of small holes, may expose the tree to such a fungal infection. Induction of wound response in the lodgepole pine, Pinus contorta Dougl. var. latifolia Engelm., and the maritime pine P. pinaster Ait., occurs after these trees have been challenged with bark beetles, fungi, or chemicals of fungal origin (Cheniclet 1987; Miller et al. 1986). In general, the wound response of S. javanica parallels conifer resistance to biological attack (Berryman 1972).

The experiments reported here indicate that resin production by S. javanica is divisible into two phases. In the first phase, a tree is primed for resin production with the opening of numerous small holes. These presumably render the tree susceptible to fungal attack, which induces resin flow as part of the defensive response. Uninfected wounds, such as those on the naive trees in this study, produce only a weak, baseline response. Ultrastructural and anatomical changes, including sythesis of additional

rough endoplasmic reticulum and polysomes, filling of resin duct lumina with resin, and cyst and cavity formation in secondary phloem have been documented in other cases of enhanced resin flow (Bhatt and Shah 1985; Cheniclet 1987).

In the second phase, larger holes are opened for resin production. It is during this production phase that chemical yield stimulants such as CEPA amplify an existing wound response, by simulating the effects of additional wound or fungal infection through the release of ethylene. Igniting resins also amplifies any existing wound response, by causing actual tissue damage. In regions where fire has been used to increase resin flow, it would be interesting to discover whether trees are treated in any manner to initially prime the wound response.

A literature search failed to locate other reports of utilization of opium as a stimulant of plant exudates.

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FIGURE LEGENDS

Fig 1. A Shorea javanica production tree showing placement of slashes, and stimulation of resin exudation 72 hours after application of 10 mls of 10% 2-chloroethylphosphonic acid to the left slash. Resin is evident in the slash on the left, while the untreated slash on the right does not exhibit any resin exudation.

Fig. 2. Boxplots of experimental results obtained following treatment of production trees with CEPA. The CEPA treatment more than doubled the resin exudation at the 72-hour time point compared to untreated slashes on the same production tree. In untreated trees, resin exudation from the left and right slashes is not statistically significantly different. See text for details.