DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This description is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

Our objectives are to build a sustainable bioprospecting program in Panama, to enhance conservation, and to discover new products for medicine and agriculture. Panama has more forest cover than any other Central American country, and is considered a threatened diversity hotspot. Its unique position as a biological corridor joining the northern and southern hemispheres has implications for both regional and international conservation strategies. To promote conservation and sustainable bioprospecting in Panama, we are stressing technology transfer and effective drug discovery. First, we will use ecological research to target collections towards species and tissues that have a high biological activity. We will also extract fresh samples, minimizing the loss of active compounds. Second we are placing a large emphasis on training and technology transfer to Panama. We are establishing bioassays with HIV and cancer at the University of Panama and with tropical diseases at Gorgas Memorial Institute in Panama City.. Active compounds will purified at the University of Panama. In addition, protein extracts will be tested for activity against whitefly, a crop pest that could become one of the major pests worldwide. Both organic and protein extracts will be sent to Monsanto, our industrial collaborator. Monsanto has committed to providing strong technical support for the pharmaceutical and agricultural investigations in Panama, especially with the identification of active compounds, thus providing a substantial component of capacity building. This emphasis on training and technology transfer creates immediate benefits to Panama and helps establish the scientific infrastructure necessary to continue bioprospecting after the termination of the grant. In addition we will assist the Kuna and Naso Indians of Panama to make a permanent record of their ethnobotanical knowledge and to increase their involvement in conservation and land management planning.

PERFORMANCE SITE(S) (*organization, city, state*)

Smithsonian Tropical Research Institute, Panama City, Republic of Panama
University of Panama, Panama City, Republic of Panama
Gorgas Memorial Institute, Panama City, Republic of Panama
Conservation International, San Jose, Costa Rica and Washington, D.C., USA

KEY PERSONNEL. See instructions on Page 11. *Use continuation pages as needed* to provide the required information in the format shown below.

Name	Organization	Role on Project
Phyllis D. Coley	Smithsonian Tropical Research Institute	Group Leader
Todd L. Capson	Smithsonian Tropical Research Institute	Co-Leader
Thomas A. Kursar	Smithsonian Tropical Research Institute	Co-Leader
Mahabir P. Gupta	University of Panama, School of Pharmacy	Leader, Associate Program 2
Eduardo Ortega-Barria	Gorgas Memorial Institute of Health Research	Leader, Associate Program 3
Don Windsor	Smithsonian Tropical Research Institute	Leader, Associate Program 4
David G. Corley	Monsanto Company/Pharmaceutical	Associate Program 5
Leslie Harrison	Monsanto Company/Agriculture	Co-leader, Associate Program 5
Manuel Ramirez	Conservation International Rep. Costa Rica & Panama	Leader, Associate Program 6

1998

SPECIFIC AIMS

1. To collect plants, beetles and fungi from a wide variety of habitats within the Republic of Panama for bioassays.
   - Plants will be collected using ecological insight to target collections with greater biological activity.
   - Endophytic fungi will be cultured from identified leaves and will be sent to Monsanto.
   - Herbivorous beetles will be collected when our ecological and inventory data suggest they are both toxic and common.
2. To use fresh plant samples to make high-quality organic and protein extracts for bioassays and to culture endophytic fungi.
3. To test organic extracts for activity against:
   - cancer (University of Panama and the National Cancer Institute)
   - HIV (University of Panama and the National Cancer Institute)
   - tuberculosis (GWL Hansen's Disease Center at Louisiana State University).
   - tropical diseases including dengue, Chagas' disease, leishmaniasis and malaria (Gorgas Memorial Institute, Panama).
4. To test organic extracts in proprietary screens at Monsanto/Pharmaceutical.
5. To test protein extracts for their potential in crop plant transformation at Monsanto/Agricultural.
6. To establish bioassays in Panama testing protein extracts for activity against whitefly.
7. To inventory the diversity of herbivorous beetles and to establish permanent collections in Panama.
8. To work with indigenous communities in the Provinces of Bocas del Toro and San Blas to preserve their ethnobotanical traditions and to increase their role in conservation.
9. To formalize contracts between all of the participants in this program that promote: equitable sharing of benefits, collaborations among Panamanian scientists, collaborations between US and Panamanian institutions, and conservation of Panamanian biodiversity.
10. To channel a portion of revenues that may result from this work to a Panamanian foundation which will administer a competitive grants program to promote bioprospecting, conservation and rural community development.
11. To help build the scientific infrastructure and expertise necessary to support a sustainable bioprospecting program based in Panama.

BACKGROUND AND SIGNIFICANCE

A. COMPOSITION OF THE ICBG

The multiple goals of the International Cooperative Biodiversity Group (ICBG) program require the assembly of an interdisciplinary group of participants. The composition of our group is based on the participants' area of expertise and on a demonstrated ability to collaborate in the conception of and, in most cases, the execution, of a shared and complex project. The Associate Programs are listed on the next page, followed by a brief explanation of the role of each program.

Ecologically Guided Bioprospecting in Panama
Group leader: Dr. Phyllis Coley
Smithsonian Tropical Research Institute and the University of Utah

Associate Program 1: Collections, coordination & database management
Associate Program Leaders: Drs. Phyllis Coley, Todd Capson & Thomas Kursar
Smithsonian Tropical Research Institute (STRI)
Management and coordination of the Panama-based program
Dr. Todd Capson (STRI)
Collection of plants, preparation of extracts and culturing of endophytic fungi
Drs. Phyllis Coley, Thomas Kursar & Mireya Correa (STRI)

Associate Program 2: Panama-based screening, isolation, and characterization of biologically active natural products
Associate Program Leader: Dr. Mahabir P. Gupta
Center for Pharmacognostic Research on Panamanian Flora (CIFLORPAN), University of Panama
Anti-cancer screens and purification and identification of active compounds
Drs. Mahabir Gupta & Pablo Solis (CIFLORPAN, U. of Panama)
Screening of biological materials for activity against HIV
Professor Basilio Gómez (Faculty of Medicine, Department of Microbiology, U. of Panama)
Screening of biological materials for activity against agricultural pests
Professor Daniel Emmen, Dr. Cheslavo Korytkowski & Dr. Dora Quiros (Department of Zoology and Entomology, U. of Panama)

Associate Program 3. Panama-based screening for tropical diseases
Associate Program Leader: Eduardo Ortega-Barria, M.D.
Gorgas Memorial Institute for Health Research, Ministry of Health
Screening of biological materials for activity against dengue, Chagas' disease, leishmaniasis and malaria
Drs. Eduardo Ortega-Barria[1], Evelia Quiroz[1], Luz Romero[1] & Phillip Rosenthal[2] ([1]Gorgas Memorial Institute and [2]University of California at San Francisco)

Associate Program 4: Biodiversity inventories
Associate Program Leader: Dr. Donald Windsor
Smithsonian Tropical Research Institute
Inventory of herbivorous beetles and their host plants
Drs. Donald Windsor[1] & Hector Barrios[2] ([1]Smithsonian Tropical Research Institute and the [2]University of Panama)

Associate Program 5: Monsanto Company. Development of novel pharmaceutical agents and products for agricultural biotechnology
Associate Program Leaders: Dr. David Corley & Leslie Harrison

Associate Program 6: Ethnobotany and Conservation
Associate Program Leader: Manuel Ramirez
Conservation International

Group Leader and Central Operations
The Group Leader of our program will be Professor Phyllis D. Coley. Dr. Coley is an internationally recognized leader whose research focuses on the evolution and mechanisms of defense by plants against herbivores and pathogens. Her published theory on plant defenses (Coley et al. 1985) revolutionized the field and is still the dominant paradigm for understanding plant investment in defenses. Science Watch recently identified the field of plant/herbivore interactions as one of the hottest and most prominent research areas in ecology and environmental biology. Of the 10 most cited papers published in this field from 1981-1993, Dr. Coley is sole or first author on two of them (ranks 1 and 3). She recently (1995) received a Career Advancement Award from NSF. She has worked on the defenses of tropical trees for 20 years, and is an expert at vegetative identification of plants and quantitative analysis of fungal pathogen and herbivore damage to leaves. As a faculty member at the University of Utah for the past 15 years, Dr. Coley has trained 15 graduate students, including 7 Ph.D. students, all of whom have taken university positions.

The Smithsonian Tropical Research Institute (STRI)
STRI has a 75 year tradition of conducting ecological research in Panama, employs a staff of 34 world-renowned scientists, and hosts hundreds of international researchers annually. This ICBG project, based almost entirely in Panama, would be impossible without the comprehensive and sophisticated logistical support provided by STRI. In addition, our scientific research has benefitted immeasurably from the opportunity to interact with this large group of scientists working on such a diversity of ecological questions. The proposed project also has the strong support of Dr. Ira Rubinoff (see Letter of Support) who has been the Director of STRI for the last 25 years.

STRI has a history of successfully implementing complex projects, for example, mapping and censusing every 5 years over 500,000 stems in a 50 hectare plot on Barro Colorado Island, as well as establishing or facilitating 8 others world wide. In addition STRI managed a multi-million dollar, 5 year project to investigate the consequences of an oil spill near Colon, Panama. Managing projects of this dimension is feasible due to STRI's skilled staff, state-of-the-art computer and communications facilities, ease of shipping supplies from the US, a fully equipped library, lawyers, personnel managers, and accountants. Equally important to the success of STRI is their educational department which is engaged in publishing in the popular media, staging workshops, organizing educational programs for the public and running internships for students. The results presented in Associate Program 1 were obtainable only because STRI maintains modern laboratory facilities that are in the heart of a protected rainforest reserve. Indeed, the use of ecological criteria to drive a collection strategy is realistic only when one can carry out long term research in the forest. The advantages inherent in the research environment provided by STRI make clear why our Panama-based research program is more likely than most to yield results in the near future.

Associate Program 1: Collections, coordination and database management
This program will focus on the use of ecological insight for sample collection and the collection of fresh samples, two factors that should greatly improve the probability of finding active compounds. Dr. Coley will be the Program Leader and Drs. Capson and Kursar will serve as co-leaders. All three have interacted productively for three years in the planning and implementation of this program. Dr. Coley's perspective as an ecologist, Dr. Kursar's as a plant physiologist and ecologist and Dr. Capson's experience in organic chemistry, biochemistry and natural products chemistry have allowed them to create an innovative and productive program in Panama with minimal resources.

Dr. Capson is a Research Assistant Professor at the University of Utah and a Research Associate at STRI. His full-time presence in Panama will be an important component to the continued success of this project. Dr. Capson has had input on virtually every component of this application, in particular,

Associate Programs 1, 2, 3 and 6. In collaboration with Dr. Kursar, he has been involved in all of the contractual agreements that are being developed for this project. He was also the principle architect of the trust fund developed by Fundación Natura for the program's competitive grants program. He is solely responsible for establishing a productive dialogue with the Kuna indigenous group (Associate Program 6). He was instrumental in arranging the establishment of cancer and HIV assays at the University of Panama (Associate Program 2). He has organized and will play an active role in Marianne Guerin-McManus' contribution to developing a legal framework for bioprospecting in Panama (Associate Program 6). Dr. Capson will continue his role of facilitating and overseeing the programs in Panama, of coordinating sample distribution and compilation of results, and of promoting public relations. In addition, he will be involved in the purification and structure elucidation of active compounds in Dr. Gupta's laboratory at the University of Panama.

Dr. Kursar is an Associate Professor at the University of Utah and a Research Associate at STRI. He and Dr. Coley have worked together for over 15 years on the mechanisms by which rainforest plants are defended against attack by herbivores and pathogens. The insights obtained from these investigations have been incorporated into the sampling and extraction protocols of this ICBG. Dr. Kursar's research on proteins found in developing leaves led to the collaboration with Monsanto on plant transformation. In addition, his work with tropical endophytic fungi has enhanced the collaboration with Monsanto, as they will batch culture our endophytic collections and make extracts for testing in their own bioassays as well as those in Panama.

Drs. Coley and Kursar will be responsible for the collection and extraction of plants and for the isolation of of endophytic fungi. Dr. Kursar has considerable experience working with plant proteins and will be responsible for assuring the quality of the protein preparations. As in the past, Drs. Coley, Capson and Kursar will train Panamanian students in vegetative identification of rainforest plants, the making of organic and protein extracts and in the isolation of endophytic fungi from fresh leaves. Plant identifications will be confirmed by Professor Mireya Correa who has over 30 years of experience in identification of rainforest plants, resulting in 70 publications. Professor Correa is the Director of the herbaria at both STRI and the University of Panama. In summary, Drs. Coley, Capson and Kursar, by working as a team, provide the capacity, commitment and attention to detail that will be required to make this a productive ICBG.

Associate Program 2: Panama-based screening, isolation, and characterization of biologically active natural products

The Associate Program Leader, Dr. Gupta, will be responsible for supervising all of the biological assays to be run at the University of Panama and the use of those assays to guide the purification of selected compounds. The program has the strong support of the Rector (President) and Dean of Pharmacy at the University of Panama (see Letters of Support from Rector Garcia de Paredes and Dr. Angela Aguilar). Dr. Gupta, is a key colleague in Panama and is the founder of the Center for Pharmacognostic Research on Panamanian Flora (CIFLORPAN) at the University of Panama. He has published 90 papers and his contributions to natural products chemistry are recognized world-wide, in particular his efforts to promote technology transfer to developing countries. Associate Program 2 epitomizes our efforts to facilitate the technology-transfer and development of human resources necessary for a long-lasting and productive bioprospecting effort in Panama. Dr. Solis, also internationally recognized for his work in natural products chemistry, will play a major role in purification, dereplication and structure elucidation. Ms. Angela Calderon will manage the bioassays. In addition, Dr. Gupta will hire a postdoctoral associate with training in natural products chemistry. This team, along with two student trainees, assistance from Dr. Capson, and technical support (NMR and MS) from Monsanto, will have the capacity to effectively and rapidly carry out purification, dereplication and structural elucidation of active compounds.

Associate Program 2 also demonstrates our efforts to tap the most relevant expertise among Panama's academic community, and to spread, to the greatest practical degree, technology transfer to various research programs. Thus, the bioassays to detect activity against HIV will be run by Professor Basilio Gómez, a microbiologist from the Department of Microbiology in the Faculty of Medicine at the University of Panama.

As described below under Associate Program 5, an important component of our work in Panama is to perform bioassays to detect proteins with activity against a major agricultural pest, the whitefly. These assays will be accomplished by a team of qualified entomologists from the Entomology Program at the University of Panama. Purification of the active components in this assays will be accomplished in collaboration with Monsanto.

Associate Program 3: Panama-based screening for tropical diseases
The Associate Program Leader, Eduardo Ortega-Barria, M.D., is affiliated with the Panamanian Ministry of Public Health, through the Gorgas Memorial Institute for Health Research. Dr. Ortega-Barria has over 15 years experience as a parasitologist and specialist in pediatric infectious diseases, and trained at both Stanford University School of Medicine and Tufts University School of Medicine. This Associate Program represents another major emphasis of our ICBG program to develop a long-term, productive bioprospecting program, in which we provide support to qualified Panamanian scientists and create training opportunities for students. Dr. Ortega-Barria is committed to re-establishing Gorgas as a center for research and education in tropical diseases. The Director of Gorgas has endorsed this collaboration (see Memorandum of Understanding, Guillermo Casto). At Gorgas, Dr. Ortega-Barria will coordinate a program responsible for screening of biological materials for activity against dengue, Chagas' disease, and leishmaniasis. Extracts will also be screened for anti-malarial activity at the University of California at San Francisco by a collaborator of Dr. Ortega-Barria's, Dr. Phillip Rosenthal. Dr. Ortega-Barria's plans include running anti-malarial assays at Gorgas in years 3-5. In addition, Dr. Ortega-Barria will assist with rural health care in Associate Program 6.

Associate Program 4: Biodiversity inventories
The biodiversity inventory of this ICBG will focus on herbivorous beetles, collecting taxonomic, ecological and chemical information about one of the most diverse groups of animals in the tropics. Collections of insects will be the responsibility of two Panama-based entomologists, Dr. Donald Windsor and Hector Barrios, representing STRI and the University of Panama, respectively. Dr. Windsor has over 54 publications on the ecology and biology of tropical insects, and is a specialist in beetle-host plant interactions. He has worked closely for 10 years with Dr. Hector Barrios, an entomologist who has specialized in weevils (Curculionidae). Drs. Windsor and Barrios have collected insects throughout Panama and are ideal candidates for carrying out the biodiversity inventory component of this proposal. Their choice of collection locales will allow them to document beetle diversity in different biomes, greatly increasing our knowledge of the regional distribution of beetles in protected and threatened habitats. While the focus of our inventory is beetles, our work also addresses plant biodiversity in Panama. Professor Correa's work in Associate Program 1 will contribute substantially to the understanding of Panama's flora. In addition, funds from this ICBG will support botanical collecting trips by the University of Panama Herbarium to poorly collected regions, enhancing our knowledge of plant distributions, and creating training experiences for students.

Associate Program 5: Monsanto Company. Development of novel pharmaceutical agents and products for agricultural biotechnology
The Monsanto Company, with world headquarters at St. Louis, MO, adds enormous strength to our bioprospecting work in Panama, and is a critical component of this ICBG application. In addition to

bringing the resources of a company of global importance in the areas of agriculture and pharmaceutical agents, Monsanto provides expertise that will contribute to the success of our program in many ways. Monsanto's participation stems from their interest in our ecologically-driven rationale for the selection of plants, which they consider more likely to yield novel biologically active compounds than plants selected from random, ethnobotanically or taxonomically driven search strategies. Both the pharmaceutical and agricultural components of Monsanto benefit from our use of fresh materials: endophytic fungi isolated from leaves will be provided to Monsanto/Pharmaceutical and defensive proteins isolated from the same leaves will be provided to Monsanto/Agriculture. Another crucial role of Monsanto will be to obtain patents on important discoveries and develop and commercialize products. The association with the pharmaceutical and agricultural components results in two fundamentally different emphases, hence the leadership of this Associate Program is shared by Dr. David Corley and Leslie Harrison, from Monsanto/Pharmaceutical and Monsanto/Agriculture, respectively.

Monsanto/Pharmaceutical will address the discovery of medically useful metabolites from plants and endophytic fungi. Using organic extracts of biological materials provided by Associate Program 1, they will use state-of-the-art facilities to test for active metabolites and isolate and characterized any novel compounds, particularly alkaloids, in high-throughput screens. By offering the modern spectroscopy facilities to our Panamanian colleagues, they are also assisting in the development of scientific infrastructure related to bioprospecting.

Dr. Corley, an Associate Fellow in Natural Products Chemistry at Monsanto, is a nationally recognized leader in the field of natural products chemistry. His extensive training in the isolation and structural elucidation of biologically active natural products from microbial, marine and terrestrial sources has lead to numerous publications in leading journals. With ten years of industrial experience in high-throughput screening of natural products, he is considered an expert in bioassay-guided isolations. Through Dr. Corley's role as an Associate Program Leader on the Peruvian ICBG, he has gained experience in international negotiations with respect to bioprospecting issues as well as valuable team-building skills.

M. A. Miller-Wideman, a Research Group Leader, is responsible for high throughput screening at Monsanto. In addition, she will be responsible for the culturing of endophytic fungi. She has experience both in field and laboratory testing, and experience in multidisciplinary scientific research efforts including natural products, protein discovery, microbiology, and mycorrhizal projects. Organic extracts from endophytic fungi cultured by her program will be returned to Panama to be run in the bioassays of Associate Programs 2 and 3.

Monsanto/Agriculture. The association with Monsanto/Agriculture provides numerous opportunities not often emphasized in bioprospecting programs. First, we are using bioassays to discover proteins with activity against insect pests and plant pathogens. The ultimate goal is to transform crops so that plants protect themselves, eliminating the need for pesticides. Secondly, we will be in a position to collaborate with Panamanian scientists who deal with problems of great importance to local agriculture. This component is accompanied by explicit and practical provisions for technology transfer from Monsanto to Panamanian scientists.

Leslie Harrison, a Senior Research Biochemist at Monsanto, is a co-leader of this Associate Program. She has 9 years of experience with Monsanto, during which she has worked primarily on identifying gene products that can be used to develop crop plants that can defend themselves against pathogens and herbivores.

Associate Program 6: Conservation and ethnobotany
Manuel Ramirez serves as the Associate Program Leader, and brings 15 years of experience in conservation and sustainable development along with the expertise and resources of Conservation International. The program will hire an ethnobotanist and focus on assisting Panama's indigenous communities to preserve their ethnobotanical traditions. Another object of this program is to enhance the role of indigenous communities in the conservation of cultural and biological diversity in Panama. This program complements worldwide interest in establishing a protected, forested corridor throughout Central America.

B. GOALS AND SIGNIFICANCE OF THE ICBG

Tropical rainforests are the most diverse habitats in the world, yet are increasingly threatened with destruction. However, this trend might be slowed if the economic value of keeping a forest intact were greater than other uses, such as logging or agriculture. One promising mechanism for generating sustainable revenue from rainforests is the successful commercialization of drugs or other products from rainforest sources (Reid et al. 1993). The value of bioprospecting for conservation is still unproven, but it has received considerable attention. Unfortunately, we do not have the luxury of time to demonstrate that bioprospecting is an economically productive use of biodiversity. Several pharmaceutical companies have abandoned natural products and many governments and environmental groups are becoming disillusioned with bioprospecting as a tool for economic development and conservation. Thus, if bioprospecting is to be embraced by industry and biodiversity-rich nations, it is imperative to produce successful examples quickly. These will confirm the value of bioprospecting, and also provide a variety of demonstrably successful models that can guide future efforts around the world.

Highlights of the Proposed ICBG
The proposed ICBG program has several innovative attributes that should help us meet the goals of linking effective drug discovery with conservation and sustainable development in the Republic of Panama.

Effective Drug Discovery Several components of our program suggest that we will be successful at discovering novel compounds of importance to agriculture and medicine. First, Panama is extraordinarily diverse and is considered a "biodiversity hotspot" (Conservation International, 1997). Panama is at the center of the region with the greatest concentration of plant species in the world (Barthlott et al. 1996), and the isthmus provides a unique link between the Northern and Southern Hemispheres. Second, we plan to use our extensive ecological research on plant defenses to target collections towards species and tissues that have a higher biological activity. For example, our results show that organic extracts from young leaves, and particularly from slow-expanding young leaves, have significantly higher amounts and a greater diversity of active compounds than the more commonly collected mature leaves. Third, we will use ecological and taxonomic information on beetles collected as part of our inventory program, to identify beetles that are both toxic and common. Our approach to making ecologically guided collections of plants and beetles is unique, yet our research suggests they are extremely promising.
Another important component of our collection program is the extraction of fresh tissue immediately upon collection, thus minimizing the loss of active compounds. By using fresh samples and ecological insight, our organic extracts have 3-10 times the levels of biological activity found in conventional samples. Another advantage of fresh samples is that we will extract proteins. Proteins with fungicidal or insecticidal activity can be used to transform crop plants, a major focus of agricultural biotechnology. And finally, collection of fresh leaves allows the isolation of endophytic fungi, a diverse and chemically interesting group of organisms. Thus, because of the extraordinary biodiversity in Panama, and our innovative collection and extraction program, we suggest we will be

successful in finding promising lead compounds.

Sustainable Development and Conservation A key component of our proposal explicitly links drug discovery with sustainable development and conservation. To accomplish this goal, we have placed enormous emphasis on training and technology transfer to Panama. We are establishing numerous biomedical and agricultural bioassays, and enhancing the capabilities for purification of active compounds at a number of research laboratories in Panama. Our bioassay program includes diseases of importance to both developing and developed nations, as well as an agricultural component for protection of temperate and tropical crop plants. In every associate program, we provide numerous training opportunities for students, technicians and professors. Technology transfer is a cornerstone of our ICBG and creates immediate benefits to Panama as well as opportunities for substantially adding value to collections. It also establishes a scientific community that depends on intact forests to fuel both research and financial rewards. In addition, we are developing a mechanism whereby a substantial portion of the financial benefits returning to Panama will be used to promote conservation initiatives and sustainable bioprospecting research. And finally, because much of the remaining forests in Panama are inhabited by indigenous peoples, we are including plans to help them preserve their ethnobotanical knowledge and share information among themselves on conservation and land management strategies.

In summary, we have an excellent group of Panamanian colleagues who, over the last few years, have demonstrated their ability to collaborate and contribute productively to the ICBG project. Together we can build a sustainable bioprospecting program in Panama, one we hope will promote conservation and serve as a general model for other biodiversity-rich nations.

International Applications of the ICBG Program

We expect that our proposed bioprospecting program can serve as a useful model for other countries that are anticipating such research. Here we outline key elements, which we think will contribute the most to the success of our program, and which can easily be applied to other countries. (i) The specifics of our ecologically guided collection scheme can be followed in all tropical rainforests. (ii) We have emphasized the preparation of fresh, high-quality extracts, something that is easily accomplished if collection sites are near simple laboratory facilities. (iii) We propose to develop and test a field lab for preparing extracts from fresh samples at remote sites. (iv) We will demonstrate that substantial host-country benefits can be obtained through collaborations and technology transfer. (v) We have expended considerable effort to develop a Material Transfer Agreement that will be adequately detailed and sufficiently flexible for adaptation in other countries. (vi) We will provide internships to visitors from other biodiversity-rich countries to facilitate the transfer of all aspects of our bioprospecting model.

C. WHY PANAMA?

One of the principal goals of the ICBG program is to aid in the development of effective approaches to drug discovery, biodiversity conservation and sustained economic growth. Perhaps the most critical consideration is that highly visible bioprospecting programs such as the ICBGs are closely scrutinized to determine their viability as strategies for conservation and sustainable development (Simpson et al. 1996).

Bioprospecting in biodiversity-rich nations involves serious technical, legal and logistical difficulties. Hence, we believe that the best route to long-term success is to start with bioprospecting programs that emphasize practicality and rapid results. We therefore propose to work in a "biodiversity hotspot" that also has the infrastructure and political stability that will encourage successful drug discovery. In Panama, as in most other biodiversity-rich nations, bioprospecting is a nascent activity, and has

lacked the necessary financial and technical inputs to establish a self-sustaining program. However, we suggest that Panama is a country where the probability of success is great and where one might demonstrate a viable and productive bioprospecting program. Below we outline more specifically the characteristics and advantages of working in Panama.

High Biological Diversity

In the most comprehensive analysis to date of the global distribution of vascular plant diversity, Barthlott et al. (1996) calculated regional diversity on the basis of the number of species per 10,000 square kilometers. In their analysis, the Panama region (including the western Colombia and eastern Costa Rica) was ranked as having more species than anywhere else on the globe. This regional measure of diversity is the most useful spatial scale for evaluating diversity with respect to bioprospecting. Diversity measured at smaller scales, such as the number of species per hectare (local or alpha diversity) cannot be used to calculate diversity in a larger area. For example, Malaysian forests have extremely high local diversity (per hectare), but the same species occur throughout the country, resulting in a relatively low regional level of diversity (Condit et al. 1996). At the other spatial extreme, it can be misleading to compare species lists for entire countries if the countries differ in size. Thus, when biodiversity is measured at a scale relevant to bioprospecting, Panama compares favorably to any region in the world.

The extraordinary regional diversity in Panama results from an unusual mosaic of habitat types and contains plant species from both Central and South America (D'Arcy 1987, Condit et al. 1996). The thin 76,000 km^2 isthmus is oriented east west, and is divided down the middle by a mountain range that reaches 3500m. The montane habitats are straddled by wet aseasonal slopes on the Atlantic, and dry seasonal slopes on the Pacific. This diversity of physical and climatic characteristics has resulted in 13 life zones and over 8,500 species of flowering plants in a country about the size of South Carolina (Tosí 1971, Heckadon 1993, D'Arcy 1987, Professor M. Correa pers. comm.).

From the perspective of bioprospecting, the extremely high concentration of biodiversity in Panama is ideal, as new species are encountered across short distances. For example, annual rainfall on the Atlantic slopes is more than twice that on the Pacific slope 65 km away (S. Paton et al. unpublished data, STRI). Despite this short distance, 77% of the trees and shrubs are different between lowland forests on the Atlantic and Pacific sides (Condit et al. unpublished data). Thus the combination of extraordinarily high biodiversity in a small land area makes Panama an outstanding site for bioprospecting.

Biological Corridor

Panama's unique geographic position and high biodiversity make it a critical area for both regional and global conservation planning. These forests have served as important migratory corridors between Central and South America for the last 3.5 million years (Coates and Obando 1996). Today, 104 species of birds and 38 species of mammals are found only in the Panama region between Colombia and Costa Rica, and are thus completely dependent on protected habitats in Panama. Of the 900 species of birds that have been recorded in Panama, over 200 are North American migrants that use Panama as a wintertime destination. Destruction of migration destinations may be responsible for the observed decline in North American songbird populations (Robbins et al. 1989).

The role of Panama as a biological corridor is particularly vulnerable, because the isthmus is only 100km wide. Thus habitat destruction can easily disrupt this important link between the hemispheres. As extreme climate fluctuations become more severe, it becomes increasingly important for species to migrate in response to adverse conditions. Hence maintaining the Panamanian corridor will be vital for the long-term persistence of many tropical and temperate species. In recognition of the key role of

this biological corridor, 7 Central American Countries recently pledged to help preserve this forested connection (The Pantera Paseo Agenda, Illueca 1998).

Threats to Conservation of Forests

Panama is unique in Central America in having so much land still under forest (Figure 1). A remarkable 42% of its land is still forested (INRENARE 1992), and although 29% is under some form of protection, much of this is threatened (Guttierez 1992). Panama's population is growing and, as with most countries, there are increasing dangers to both protected and non-protected wildlands. If deforestation continues at the current rate of 70,000 hectares per year (2.7%), Panama's unique forests will disappear within 37 years (INRENARE, 1990). For example, the remarkably diverse Darien region of eastern Panama has been designated a World Heritage Site and a World Biosphere Reserve, yet it lacks sufficient funds for protection against encroachment by colonists and illegal loggers. Darien National Park is meant to protect 570,000 hectares but there are only 11 people assigned to this formidable task. This is equivalent to 520 km^2 of rugged terrain per park guard. The Darien province holds vast expanses of primary forests, yet also has the highest deforestation rate in the country, accounting for 93% of the area in Panama that has been given to logging concessions. Between 1985 and 1990, 360,000 hectares were converted to either agriculture or pasture. Similar habitat loss is occurring throughout Panama. Central Panama still contains large tracts of forest in the watershed of the Panama Canal that have been protected in the past. However, the future of these forests is uncertain, particularly since they lie adjacent to the major population center, Panama City. At present, very little of the biologically rich province of Bocas del Toro has been given any protection, yet mining exploration and newly planned roads pose serious threats to this pristine area (see Associate Program 6). Several of Panama's ecosystems were recently acknowledged by the World Bank (1997) as being of global significance for conservation. The crucial role Panama plays in the protection of global diversity is also manifest by Conservation International's classification of Panama as a "threatened biodiversity hotspot" (1997).

In summary, while extensive areas of Panama are still forested, these forests are under immediate and substantial threat. This is therefore a particularly critical time when effective planning can still make a difference to conservation.

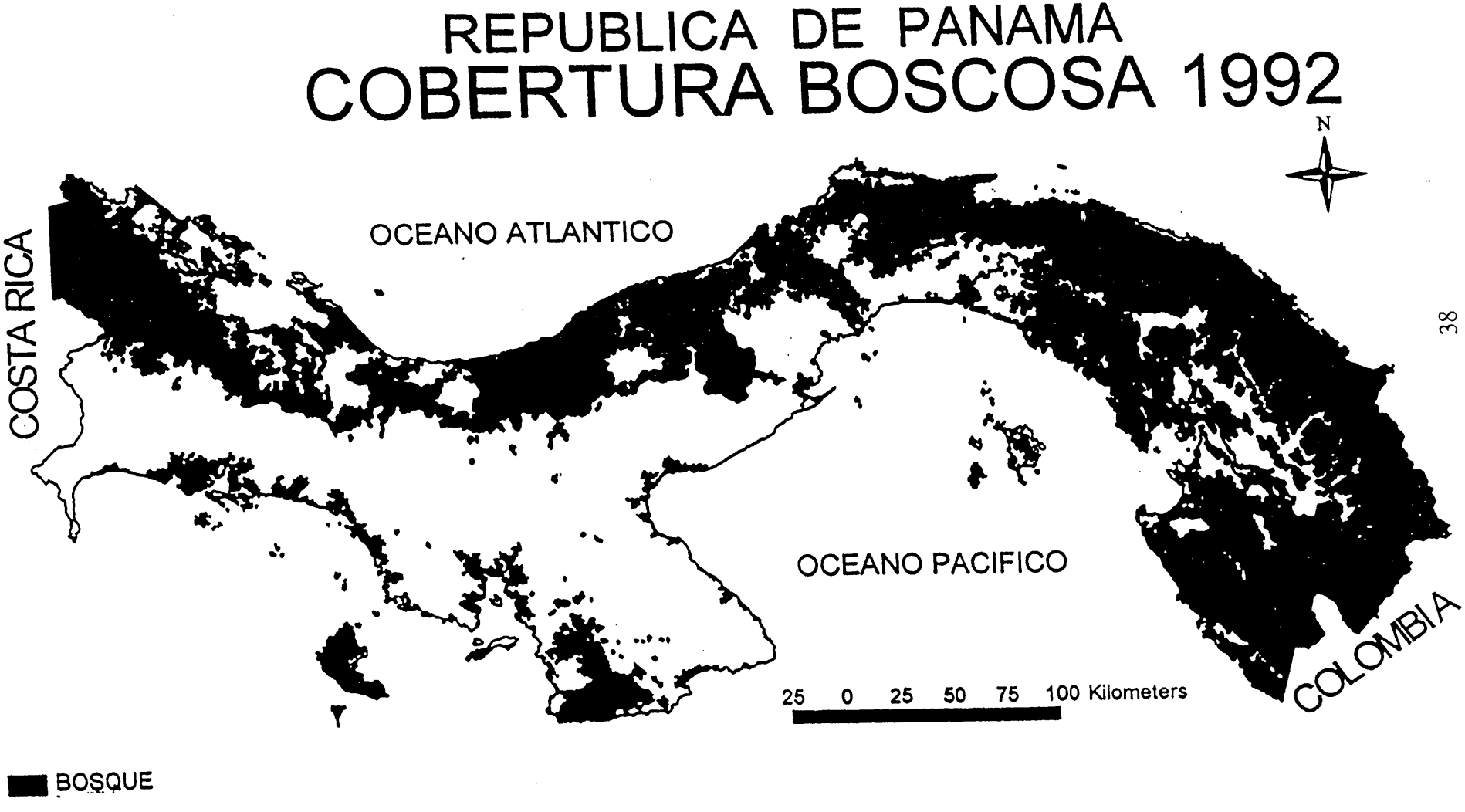
A Tradition of Research on Biodiversity

Because Panama is so diverse, there are many taxa and regions which are poorly known. Nonetheless, Panama has a long tradition of obtaining superb information on biodiversity and of providing the logistical support to make this happen. The existing knowledge of the flora is particularly impressive and will be invaluable for facilitating the identification of our samples. For example, excellent herbaria exist at both the University of Panama and STRI, and the Missouri Botanical Garden has published dozens of volumes of the Flora of Panama.

Accessibility of Wildlands

Furthermore, much of this biodiversity is accessible from Panama City, a situation almost unique to tropical forests. For example, within a 2 hour drive of Panama City, one can sample sites from sea level to 1100m, and lowland forests from "dry-deciduous" through "very wet-evergreen". More remote sites in Bocas province near Costa Rica and in the Darien province near Colombia can be reached by daily flights of approximately one hour. Thus, the extraordinary accessibility of a diversity of intact forests means we can readily collect across large gradients in rainfall and altitude and across biomes with primarily Central or South American influences.

Figure 1: Forest cover in Panama in 1992 (INRENARE, 1992).



Diversity of Indigenous Groups

According to the census of 1990, Panama has an indigenous population of 195,000 habitants comprised of 7 distinct groups (Ventocilla et al. 1995). Human presence in Panama dates to at least 10,000 B.C. Panama's indigenous cultures have a profound understanding of their physical world, reflected in their culture, knowledge and management of resources. Presently there are zones of extreme poverty that are found in ecosystems that are still largely intact: the fragile ecosystems along the Atlantic coast where the Naso, Ngöbe, and Kuna are found serve are examples (World Bank 1997). All three groups inhabit ecosystems of "high priority" for biodiversity conservation (World Bank 1997), the fate of which is intimately linked to the well-being of the indigenous inhabitants [Conservation International (CI) & General Secretary of the Organization of American States (OAS) 1994]. Some of Panama's indigenous groups are extraordinarily well organized, in particular, the Kuna whose unprecedented degree of cultural and political autonomy has earned them international recognition (Ventocilla et al. 1995, World Bank 1997). Among the Kuna are Western-educated professionals that are receptive to innovative strategies for biodiversity preservation. These circumstances provide new opportunities for incorporating indigenous Panamanians into effective conservation strategies that can serve as models for other indigenous communities worldwide.

Host Country Involvement

Essential to the success of any project combining bioprospecting, conservation and technology transfer is the enthusiastic participation of scientists, NGO's and government officials from the host country. All of these components have been incorporated into the ICBG program in Panama. There is a considerable scientific community whose research efforts could include bioprospecting. Furthermore, by collaborating with local academic institutions, we can combine research with education, an extremely effective way of enhancing scientific training. We have received valuable support and guidance from Panamanian NGOs as well as the director of Panama's INRENARE, the department with jurisdiction over natural resources. An example of the strong support we have received from within Panama is the fact that we were awarded a highly competitive grant from Fa Panamanian NGO.

As foreigners, we view our role as catalysts and collaborators. The long-term future of bioprospecting clearly lies with our Panamanian colleagues, many of whom have been working with us for two years in order to bring this project to fruition.

Logistics and Existing Research Infrastructure

The ability to effectively collect high quality samples for pharmaceutical and agricultural screening and to transfer technology to the host country demands good logistics. Many aspects of working in Panama make these goals realistic. Panama has a stable government and economy, increasing the likelihood of achieving meaningful accomplishments in conservation, and in the development of the scientific infrastructure and trained personnel necessary for drug discovery. We are collaborating with the University of Panama and a government-run research laboratory, Gorgas Memorial Institute of Health Research (GMIHR), as well as supporting 2 students in a Master's program in biotechnology at the University of Santa Maria La Antigua. In addition, the Smithsonian Tropical Research Institute (STRI) is highly respected and has a long history of interacting with scientific and educational institutions in Panama.

Modern laboratory facilities are available at STRI, many laboratories at the University of Panama, and GMIHR. In addition, a research and industrial park ("Ciudad del Saber") is being established with biodiversity as one of its two themes. A structure elucidation facility is being requested and could be operating by the end of 2001. This facility has received strong support from the National

Secretariat for Science and Technology in Panama and would be managed as a Central American and Caribbean facility.

Access to NAPRALERT, Chemical Abstracts Service (CAS), e-mail and the internet (via a direct satellite link to the US) are all possible through either STRI or the University of Panama. STRI obtains research permits, provides library facilities and offices, and manages a large protected forest reserve. Import of equipment and reagents is handled by the US Armed Services (APO) and is reliable and prompt. After the year 2000 when APO privileges expire, STRI will arrange for similar services to continue. Thus Panama provides a remarkable, and perhaps unprecedented opportunity, where the combined infrastructure and logistics permit productive interactions for bioprospecting.

Summary

In summary, Panama has one of the highest concentrations of plant biodiversity in the world, as well as superb logistical and scientific advantages that should ensure the success of the proposed project. Our program should help create lasting substantive changes in the scientific infrastructure, facilitating a sustained and productive bioprospecting effort. Because Panama houses both outstanding scientific expertise and infrastructure as well as enormous biodiversity, we think we are in an excellent position to successfully demonstrate the value of combining drug discovery with conservation.

D. RAINFOREST PLANTS ARE RICH IN BIOLOGICALLY ACTIVE COMPOUNDS

Rainforest plants have historically been a rich source of novel, biologically active compounds (Balick et al. 1996, Kinghorn and Balandrin 1993). Given that only a small percentage of the tropical flora has been examined (Cox and Balick 1994), investigations of wild tropical plants should produce a wealth of new compounds.

Leaves of tropical forest species have both higher levels of chemical defense as well as a greater diversity of compounds than temperate species (Gentry 1993, Coley and Kursar 1996). For example, an extensive survey of the distribution and activity of alkaloids showed that they are more common and more toxic in the tropics (Levin 1976, Levin and York 1978). All other classes of compounds that have been surveyed show similar patterns (Coley and Aide 1991). Tropical rainforest plants are so rich in secondary metabolites because they use chemical defenses against attack by a diverse and abundant set of herbivores and pathogens. And no where in the world are these biotic interactions more intense than in tropical rainforests (Price et al. 1991). Thus, the high biotic pressure in the tropics has lead to the evolution of an unsurpassed diversity of effective chemical defenses (Coley and Barone 1996).

E. RATIONALE FOR ECOLOGICALLY GUIDED COLLECTIONS

We have invested considerable thought and experimentation in the design of an ecologically guided collection scheme for plants, endophytic fungi (Associate Program 1), and beetles (Associate Program 4). The collections are complemented by an extraction protocol that ensures repeatable samples of high quality. The rationale and preliminary results are discussed in detail in Associate Programs 1, so here we simply summarize some key points.

Plants Our collection strategy is based on 20 years of ecological research on plant defenses to target classes of species and leaf tissues that are more likely to contain biologically active compounds. For example, our work has identified some simple leaf characters that serve as good field indicators of chemically well-defended species. Well-defended species are unrelated taxonomically, so a diversity of active compounds should be responsible for any observed biological activity. Because we are collecting novel plant tissues, and because we are extracting fresh tissue, we should discover new compounds, even from species that have been previously collected.

Beetles and endophytic fungi We will also use ecological insight to guide smaller collections of two other groups of organisms, herbivorous beetles and endophytic fungi. Both groups are extraordinarily diverse and both have complex, chemically mediated interactions with their hosts and predators. Herbivorous beetles are frequently well protected by chemicals against their predators. Primitive species tend to synthesize their own, and more advanced species tend to sequester plant toxins. Knowledge of the anti-predator behavior and host-plant use of beetles can suggest both beetle and plant species that may contain useful compounds.

The other group we will collect for screening in bioassays is the endophytic fungi. Endophytes are orders of magnitude more abundant in the tropics than in the temperate zone, and tens of species can co-occur in a single leaf. Endophytes are known to synthesize biologically active compounds that protect the leaf from herbivores and pathogens and that may also regulate competition among endophytes within the leaf. For example, taxol is a medically important compound that may be produced by a number of species of fungal endophyte (Stierle et al 1993), including *Pestalotiopsis* (Strobel, pers. comm.), a common genus in the tropics.

Thus, using decades of ecological research on tropical organisms that rely on chemical signaling and warfare should guide us towards previously unexplored sources of biologically active chemicals.

F. DISEASE TARGETS

We propose to screen the biological extracts in as many activities as is practically feasible. We have selected a wide variety of assays, to test for a wide variety of activities. All of the bioassays that we are using target diseases of global importance, all in need of innovative treatments. We have included an Associate Program that is dedicated exclusively to tropical diseases: diseases that are frequently overlooked in bioprospecting programs. Among the bioassays that are included in the proposed work: cancer, HIV, tuberculosis, dengue, malaria, leishmaniasis and Chagas' disease.

G. AGRICULTURAL PEST CONTROL

Agricultural "pests" (insects, nematodes, pathogens and weeds) cause the loss of about one third of all crops in the US and even more worldwide (Pimentel et al. 1975; Oerke and Heinz 1997). These losses occur at a time when the demand for food is increasing both due to increases in world population as well as increases in the standard of living in developing countries (Tzotzos and Leopold 1995). Despite substantial pesticide application, invasive species and the evolution of pest resistance have resulted in crop losses that have remained constant over the last 50 years. Recent experience demonstrates that chemically based anti-pest technologies are not adequate to lower crop loss rates. Even more striking is the observation that pesticides result in 8.3 billion dollars in socio-economic losses in the US each year (Pimentel and Greiner 1997). Crop losses and the socio-economic costs of chemical application are as severe, if not more so, in developing countries such as Panama. To confront these problems, Monsanto and others are currently investing considerable resources to identify protein-based defenses that, following transformation into crop plants, will allow plants to protect themselves. To date Monsanto has three new products, Bollgard Cotton, YieldGard Corn and NewLeaf Potato, for which transformation with defense proteins has resulted in improved productivity.

The goals of the Agricultural Pest Control program are to discover new protein-based defenses in Panama's plants, including those that inhibit the growth and development of whitefly (*Bemisia tabaci*; Homoptera: Aleyrodidae). *B. tabaci* is a major pest and virus vector in Panama, and without more effective control, may become one of the preeminent pest problems in the world during the next century (Brown 1994).

Protein extracts will be prepared from both plants and will be tested at Monsanto in a variety of proprietary assays. In addition, Monsanto will transfer their whitefly assay for detecting defense proteins to Professor Emmen, and Drs. Dora Quiros and Cheslavo Korytkowski of the Entomology Program at the University of Panama. Active extracts will be sent to Monsanto for purification and further characterization.

Preparation of protein extracts will be coordinated by Dr. T. Kursar, who has considerable previous experience working with plant proteins. Our preliminary data on young leaves indicate that highly abundant, developmentally regulated proteins occur frequently in tropical plants (Kursar and Coley 1992). Such proteins may play a role in defense. Based on the above observation, the high species diversity and the fact that numerous species interactions are mediated at the molecular level, we anticipate that new defense proteins will be discovered. Hence, this program has the potential to produce a considerable number of interesting leads in agricultural biotechnology, providing an exciting and highly visible use of biodiversity.

H. INTELLECTUAL PROPERTY RIGHTS

The use of forest resources in ways that could become commercialized has raised complex questions in the international community regarding the role of bioprospecting in conservation efforts. The Convention on Biological Diversity provides a framework for the manner in which bioprospecting activities should take place. To ensure clarity, the prior and planned arrangements that address intellectual property issues are all discussed in "Research Design and Methods".

PRELIMINARY RESULTS - Existing Relationships Among the ICBG Participants

The work proposed in this application is an extension of a program that has run productively for over two years. The ICBG award constitutes an opportunity to enhance the scope and size of our program. Equally important, we will increase the number of trained personnel, and facilitate the transfer of the necessary technology to ensure that the proposed bioprospecting program will survive beyond the funding period of the ICBG. Examples of our collaborative efforts are outlined below, and described in greater detail in the appropriate Associate Program.

Testing Ecological Theories: Collaborations Between STRI, the University of Panama and Monsanto

The ecological studies of Drs. Coley and Kursar over the past twenty years provided a unique perspective for selecting rainforest plants (Associate Program 1). Over the past two years, we have systematically tested those theories using chemical and biological assays in Panama. The results presented in Associate Program 1 reveal that ecological insights, coupled with our logistical facilities, provide the basis for a productive bioprospecting program. Most of the work was carried out by three Panamanian assistants we have employed, two of whom are obtaining Master's degrees in Biotechnology using funds provided by Coley, Capson and Kursar's research program.

In Associate Program 5, we discuss TLC and HPLC data that showed that young leaves have a greater concentration and diversity of alkaloids than mature leaves from the same species. The TLC experiments were performed by us with the assistance of Panamanian students. The HPLC experiments were performed in the CIFLORPAN laboratories in collaboration with Drs. Mahabir Gupta and Pablo Solis of the University of Panama (Associate Program 2). The work was funded in part by Monsanto/Pharmaceutical, which recognized that our collection and extraction approach may provide compounds not usually available from bioprospecting programs based in tropical forests (Associate Program 5).

Establishing Anti-cancer and HIV Screens at the University of Panama
As described in Associate Program 2, we have collaborated with scientists from the National Cancer Institute (NCI) and scientists at the University of Panama to establish assays to detect anti-cancer and anti-HIV activity. The NCI was involved in the selection of the cell lines and sent the biological materials to Panama via STRI. We are now at the stage of establishing the bioassays in the tissue culture facilities that are available in Professor Gómez' laboratory in the Department of Microbiology at the University of Panama. Dr. Gupta, with the assistance of Dr. Capson, is sponsoring a workshop in which scientists from all over Latin America will come to Panama to learn the techniques necessary for working with the cancer and HIV cell lines, and using them in biological assays. Dr. Anne Monks, the head of the NCI's Primary Drug Evaluation Laboratory in the Anticancer Drug Screening Program will demonstrate the use of the cancer cell lines at the Panamanian workshop. Similarly, Dr. David J. Clanton, the head of the AIDS Drug screening and Development Laboratory at the NCI, will demonstrate the use of the non-infectious HIV assay.

The successful completion of these bioassay and chromatography experiments demonstrates that we can work with institutions in the US and Panama to bring together the necessary expertise and resources for collection, recollection, extraction, chemical analysis and bioassays. Equally important, these projects have demonstrated the ability of the participants to work together productively.

Linking Bioprospecting and Biodiversity Conservation in Panama
Working with the Government of Panama An explicit goal from the beginning of our work has been to link our bioprospecting program to initiatives that promote conservation of biodiversity in Panama. To that end, we have worked closely with the Panamanian government through the National Institute of Renewable Natural Resources (INRENARE). A Letter of Understanding was signed between INRENARE and Coley, Capson and Kursar, in which INRENARE agreed to allow the investigators to carry out the preliminary experiments described in Associate Program 1.

Working with Panamanian Non-Governmental Organizations (NGO) We have worked closely with the Panamanian foundation, Fundación NATURA (Natura) to design a bioprospecting project with explicit ties to conservation. Natura is a non-profit organization devoted to biodiversity conservation in Panama, through the promotion, financing and strengthening of conservation initiatives. Natura was legally established in 1991. The money for Natura's projects comes from an endowment currently valued at $33 million that was provided by a variety of private and governmental sources. The trust fund created by this endowment, FIDECO, generates interest which has been the sole source of money available to Natura for funding projects. Proposals submitted to Natura are funded through a competitive grants program. They are reviewed by professional volunteers, the Technical Committee. Natura has received international ecognition for both its innovative design and the projects it funds.

Using STRI for institutional support, Coley and Kursar received $69,800 from Natura, for bioprospecting research in Panama. The approval of our project by the Technical Committee, the Board of Directors and the Executive Director, is an expression of the widespread support for our bioprospecting work. In their award letter, the Board of Directors indicated that "bioprospecting can constitute an important tool in the conservation of Panamanian rain forests".

The donors to FIDECO have stipulated that the majority of the projects supported by the interest on their endowment must be executed within the Panama canal watershed. Thus Natura is unable to support many worthwhile projects that lie outside of the canal watershed.

Creation of a trust fund to promote biodiversity research in Panama: Fundación NATURA
The infrastructure of Natura provides an excellent opportunity to effectively channel revenue from

bioprospecting to projects that promote conservation, sustainable development and continued bioprospecting research by Panamanian scientists. We have worked with officals from Natura to create a trust fund that takes advantage of Natura's infrastructure for supporting projects, but that can fund projects throughout the Republic of Panama. Natura has established a "National Environmental Fund" that will manage a portion of the funds that may result from the work described in this proposal (see Letter from Executive Director McKay and attached proposal for the fund). The creation of this mechanism before the arrival of funds, and before the signing of a formal agreement between the STRI and INRENARE, has obvious strategic advantages. There now exists a discrete entity, the National Environmental Fund, that is ideally poised to receive a portion of the funds that may be generated through our bioprospecting work.

RESEARCH DESIGN AND METHODS

The following organizational chart outlines the exchange of information, extracts, and students between the various participants in this ICBG group.

Communication, Material Transfer, Reports, and Coordination

The majority of the participants in our ICBG program are based in Panama City, greatly facilitating communication, the transfer of materials, and the sharing of data. All of the meetings, including annual meetings among the Associate Program leaders, will take place in Panama. All of the participants in this ICBG have committed to attending at least one meeting per year. The Program leaders not living in Panama have pledged to attend annual meetings. As described below, our affiliation with the Smithsonian Tropical Research Institute (STRI) makes possible our management of a complex program in Panama.

The exchange of materials will only occur following the signing by all of the relevant parties of Material Transfer Agreements that provide for equitable sharing of benefits and that protect the sovereignty of Panama and intellectual property rights of all the parties to this ICBG. Materials will be exchanged between the participants promptly in order to meet the demands of the proposed research except as restricted by the terms of material transfer and collaboration agreements.

All participants, including Monsanto Company, will report the results of their assays, inventories or other work in a timely manner and these data will be stored in a central database (see below).

Drs. Coley, Capson and Kursar will coordinate the collecting and extracting of biological materials, and the distribution of extracts to the participants in the US and Panama. Dr. Capson will be based in Panama for the duration of the project and will be in frequent contact with Drs. Coley and Kursar. Drs. Coley and Kursar will spend four months annually in Panama.

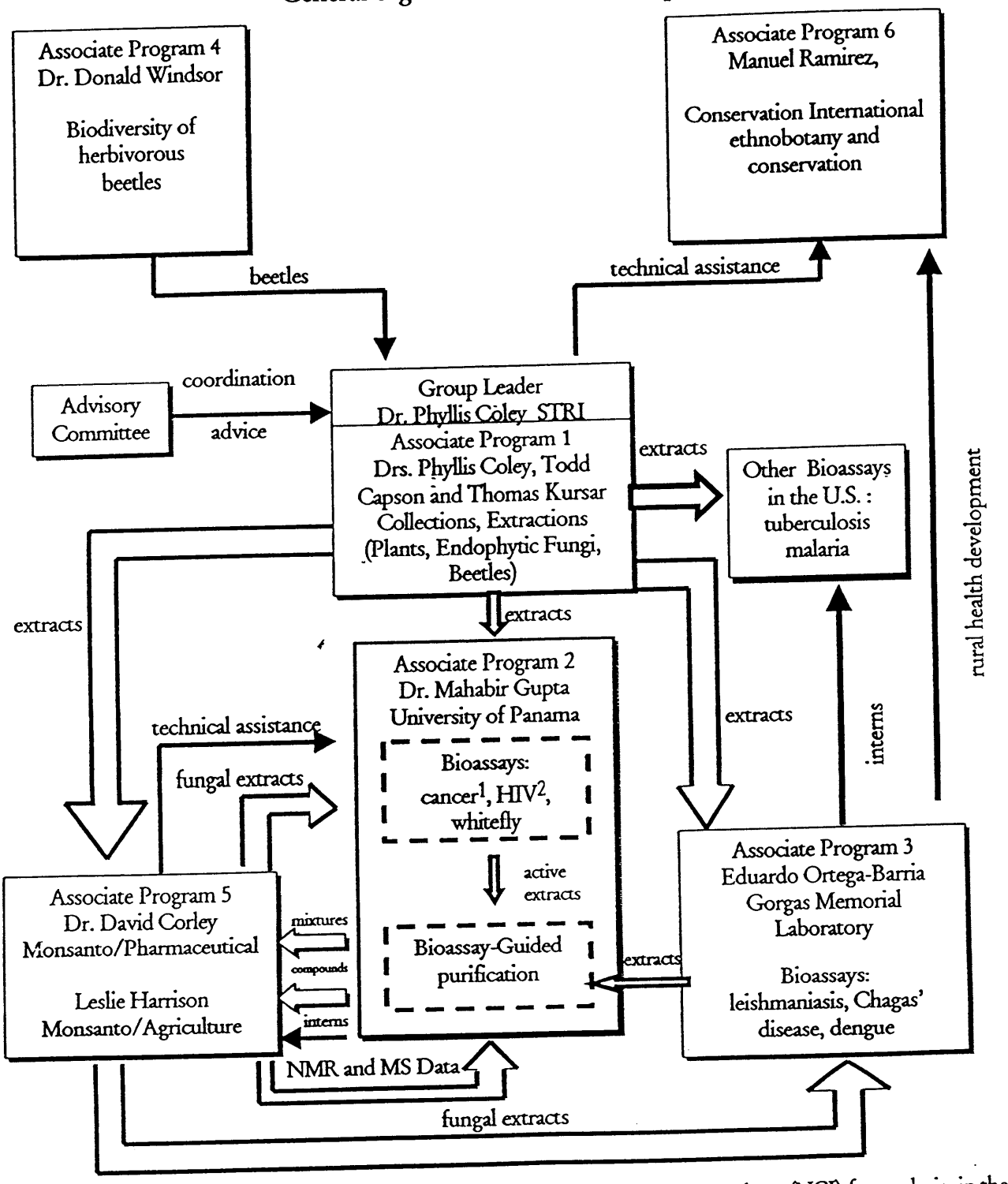
Data Management

Effective data management will be a crucial due to coordinate the extensive collecting, extracting programs we propose to carry out. A central database will be established at STRI to track barcoded samples and results (Associate Program 1). The database will include details of time and site of collection, scientific nomenclature, extraction procedures as well as bioassay results obtained from collaborators.

Collection

As described in "Background" above, our collection scheme focuses on making bioprospecting more effective. This program emphasizes obtaining organic extracts from plants (400 per year) of high

General organization of the ICBG program



1: Samples active in the anti-cancer assays will be sent to the National Cancer Institute (NCI) for analysis in their panel of 60 human tumor cell lines.

2: Samples active in the HIV assay will be sent to the AIDS drugs and screening laboratory at the NCI for further analysis.

quality by working with (i) fresh material, (ii) using vegetative plant identification in order to make recollections highly efficient, and (iii) the application of 20 years of ecological insight in order to select samples with greater biological activity. In Associate Program 1, we present preliminary results demonstrating the effectiveness of the proposed collection scheme. We also will prepare protein extracts from plants (400 per year), isolate endophytic fungi (approximately 1000 isolates per year), and make organic extracts from species of herbivorous beetles and other organisms that are likely to harbor active compounds (25 per year). Lastly, Monsanto will batch culture 100 samples/yr of endophytic fungi and provide us with organic extracts for our Panamanian bioassay program.

Field Laboratory

As many wildlands are not located near modern laboratories, we will develop a mobile field lab in years 4 and 5 with the capacity to make high quality extracts at remote sites. Our goal is to make the biodiversity of threatened areas more accessible to bioprospecting. Our experience with other small portable "laboratories" in Africa, Asia and Central America suggest that this should be feasible. A complete extraction laboratory can fit in a double cab jeep or small aircraft, and can be set up at selected field camps. These techniques will be applicable not only in Panama, but also in much of the developing world.

Bioassays

This component epitomizes our efforts to provide the basis for a long term, productive bioprospecting program in Panama. To maximize the possibilities for discovering useful compounds, we are using a broad range of bioassays. We will test organic extracts made through Associate Program 1 or obtained via Monsanto from cultured fungi (a total of 525 per year) in the bioassays described in Associate Programs 2, 3 and 5, and in laboratories in the US. The bioassays used in Associate Programs 2 and 3 can be run efficiently, safely, and at reasonable expense in Panama. Those assays will detect activity against cancer, HIV, dengue, Chagas' disease, malaria and leishmaniasis. Each of the laboratories carrying out bioassays in Panama will receive a bar-code reader to facilitate accurate collection of data and will feed results to the central database at STRI. Bioassays that are either proprietary, e.g., those at Monsanto (Associate Program 5), or that require special facilities, e.g., tuberculosis, are being run in the US. In addition, protein extracts obtained from Associate Program 1 (400 per year) will be assayed for activity against a major agricultural pest, *Bemisia tabaci* (whitefly) (Associate Program 2).

Our cancer screening efforts are typical of the coordination between Panamanian and US institutions. The three cancer cell lines we are using in Panama were selected by the head of the NCI's Anticancer Drug Screening Program, and serve as a pre-screen for the NCI's panel of 60 tumor cell lines. The tumor cell assays are complemented by mechanism-based anti-cancer screens in use by Monsanto. In addition, Dr. Frank Fitzpatrick, from the Huntsman Cancer Institute at the University of Utah, has had substantial experience in the pharmaceutical industry and has offered to act as a consultant in the evaluation of results from the bioassay program (see Letter of Support in Associate Program 2). Similarly, extracts and compounds that are active in the non-infectious HIV screen in Panama will be subjected to the wild-type HIV assay currently in use at the NCI's AIDS Drug Screening and Development Laboratory. The latter facility also conducts mechanism-based HIV screens.

Capacity Building

Training students in biological research

Providing educational opportunities for Panamanian scientists and students is a crucial component of our program. The ICBG program will train students in the collection of plants, fungi and beetles, as well as in extraction and bioassay techniques. We have requested funds for a total of 12 positions to be filled by Panamanian students, representing a substantial increase in the number of positions

available for graduate research in Panama. Each year one intern will spend 6 months at the GWL Hansen Disease Center in the US running assays for activity against tuberculosis (see Letter of Support, Associate Program 1). The ICBG program will also provide research opportunities for students participating the Master's program in Entomology at the University of Panama and in the newly created (1996) graduate program in Biotechnology at the University of Santa Maria La Antigua. Many of these students, as well as undergraduates majoring in botany, microbiology, pharmacy and chemistry, are looking for research opportunities in order to complete their thesis requirements. Thus the ICBG program could fill an important training need in Panama.

Bioassay-guided Purification and Structure Elucidation of Biologically Active Compounds

The Panama-based bioassays mentioned above will permit Drs. Gupta, Solis and collaborators to use bioassay-guided purification of the active components in the CIFLORPAN natural products laboratory, resulting in the isolation of numerous biologically active compounds. As there are no NMR or mass spectrometry facilities currently available in Panama, we have made arrangements with Monsanto to provide access to their state-of-the-art facilities (Associate Program 5). They will provide routine proton and carbon NMR data and mass spectral data, as well as consult closely with Drs. Gupta and Solis on interpretation, dereplication and structural elucidation. In addition, Monsanto will assist in the purification of natural products when the necessary equipment is unavailable in Panama. Finally, Monsanto has agreed to offer their facilities for use by our Panamanian colleagues for two months per year, affording valuable training and practice opportunities. Thus, materials and information will be exchanged between the scientists in Associate Programs 2 and 5. As there is the possibility of establishing a regional NMR facility in Panama, this training will facilitate the success of that endeavor.

Whitefly assay

This assay is designed to detect proteins that inhibit whitefly, *Bemisia tabaci*, a major crop pest in Panama and around the world for eventual use in crop plant transformation. Professor Daniel Emmen will learn the assay at Monsanto. This project will involve three members of the Entomology Program at the University of Panama, including Dr. C. Korytkowski, the Director (see Letter of Support in Associate Program 2). Because this approach to crop protection probably will become standard in the next century, such research experience represents valuable capacity building.

Gorgas Memorial Institute for Health Research

Gorgas Memorial Laboratory (now Gorgas Memorial Institute for Health Research) has a long tradition as world center for both investigation and research training in tropical diseases. Dr. Ortega-Barria's goal is to reestablish Gorgas as a center for education and research in tropical medicine. The work proposed by Dr. Ortega-Barria in Associate Program 3 provides a valuable first step towards this end. This program also will send several interns to laboratories working with dengue and malaria for training. This component has strong support from the Director, Guillermo Castro (see attached Letter of Support).

Intellectual Property Rights

Issues of intellectual property rights are central to agreements among our collaborating institutions and the government of Panama. As many of these issues have not previously been addressed in Panama, considerable education of all parties is involved. Thus we have attempted to encourage discussions and the dissemination of information to academic, industrial, and governmental representatives. For example, in February 1998, we have arranged for Ms. Marianne Guerin-McManus, a lawyer and expert in conservation finance from Conservation International, to visit Panama. She will give a presentation at an international workshop on bioprospecting as well as meet with officials from INRENARE and STRI.

Biodiversity Inventory
As described in Associate Program 4, we will study herbivorous beetles throughout Panama. Our work will shed light on important criteria such as their diversity, endemism and host-plant specificity. Our collection sites will include threatened forest ecosystems throughout Panama, possibly leading to the discovery of rare, geographically restricted or economically valuable insects and plants, thus providing incentives for conservation. This program represents another facet of our emphasis on ecologically guided bioprospecting. When ecological data suggest that a beetle may have chemicals of interest to our bioassay components, the beetles will be collected (assuming their numbers are sufficient) and tested in bioassays.

Intellectual Property and Benefit Sharing Mechanisms
The development of drugs, agricultural inputs, and other commercial products derived from genetic resources has raised complex questions in the international community regarding the role of bioprospecting in conservation efforts. The Convention on Biological Diversity, signed by more than 150 nations at the Earth Summit in Rio de Janeiro, in June, 1992, affirms States' sovereign rights over their own biological resources and encourages the equitable sharing of the benefits arising from the utilization of genetic resources and the knowledge of indigenous communities. Thus, the Convention provides a framework for the manner in which bioprospecting activities should take place.

This ICBG will comply in all regards with the Program Principles for the Treatment of Intellectual Property and assure adequate patent coverage of new inventions that may issue as a result of Government funding in accordance with Appendix 1 of RFA: TW-98-001 dated August 15, 1997.

This ICBG project will encompass the study and implementation of a variety of innovative benefit-sharing mechanisms that will be used in connection with bioprospecting to promote environmental conservation. The design of adequate benefit-sharing mechanisms can help to ensure that this economic activity encourages the preservation of Panama's biological diversity. The establishment of the National Environmental Fund (see "Preliminary Results") is an important first step in this direction. During the first year of the ICBG project, Ms. Guerin-McManus, a lawyer for Conservation International with expertise in conservation finance, will make 3 trips to Panama to consult with us as well as other academic and governmental representatives concerning these issues.

Another goal of the ICBG project is to develop a legal and contractual framework which can serve as a tool for developing a national policy and legislation to guide participants in bioprospecting projects. Our agreements address issues relevant to equitable bioprospecting, including biodiversity conservation, sustainable economic development, training of students and scientists, development of a scientific infrastructure in Panama, equitable sharing of benefits and many others. The final material transfer, intellectual property and contractual agreements are being developed through a participatory process involving Panamanian institutions and collaborating scientific researchers. The development of these contractual documents have entailed considerable dialogue among James Haisley (University of Utah lawyer), Mirei Endara (Director of INRENARE), INRENARE lawyers, Marianne Guerin-McManus (CI), Dr. David Corley (Monsanto), Monsanto's lawyers, Leonor Motta (Executive Officer and lawyer for STRI) and Drs. Capson and Kursar. A final draft of the appropriate contracts is currently being prepared by the parties to this ICBG project. Although the final form of the agreements must await completion of the on-going discussions among group members, we anticipate that it will generally implement the following principles, which have been agreed upon by the institutions participating in this application (see "Statements of Intellectual Property Rights" under Consultants/Collaborators):

"The participating institutions accept the principles for the treatment of intellectual property and

the sharing of benefits described in Section J-2 and Appendix 1 of RFA: TW-98-001 dated August 15, 1997. In particular, we understand and concur with the principles for the treatment of intellectual property and the sharing of benefits that have been outlined by Drs. Coley, Capson and Kursar for their ICBG. These include the following:

All collection of biological materials and research will be carried out in a manner consistent with the United Nations Convention on Biological Diversity, dated June 5, 1992 and adopted December 29, 1993 and ratified by the Republic of Panama on January 12, 1996.

The Republic of Panama will receive the majority of net revenue (such as royalties or other payments) which could be obtained from the commercialization of products that are derived from the ICBG project. The revenue will be distributed in the Republic of Panama for the benefit of projects within the country.

An agreement will be formulated that assures that a portion of the funds returned to Panama are explicitly committed to conservation and scientific research in Panama.

Appropriate agreements will be made that ensure adequate compensation for joint inventions made by collaborators in the proposed research.

We recognize that the ICBG guidelines specify that ICBG projects must have an industrial participant to develop and commercialize products. In cases where the participants in this ICBG group make discoveries that could be commercialized, Monsanto Company will have rights of first refusal."

Conservation

Of utmost importance is the explicit linkage of our bioprospecting program to concrete measures for promoting conservation of biodiversity. This will be accomplished by several means: (i) by establishing and supporting a conservation and bioprospecting trust fund, "National Environment Fund" in Panama, (ii) by enlisting the help of experts in conservation finance to draft contractual agreements for bioprospecting, (iii) by increasing the involvement of Panama's indigenous communities in conservation issues in Panama, (iv) by developing a scientific community of bioprospecting researchers that depends on intact forests, and (v) by promoting the public's appreciation of biodiversity.

Ethnobotany

We propose to help indigenous communities make a permanent record of their ethnobotanical traditions. This ICBG program will not use ethnobotanically guided collections unless invited to do so by indigenous groups. The recorded ethnobotanical information will remain the sole property of the respective indigenous community. Should any of the indigenous groups involved in this study choose to offer their knowledge or resources to the ICBG bioprospecting program, the appropriate legal agreements will be carefully conceived and drafted.

The ethnobotany program will be carried out with the Kuna and Naso indigenous groups. They inhabit two distinct Atlantic coastal ecosystems that are both still forested but threatened (Associate Program 6). In both regions, ethnobotanical traditions are still widely used, but are disappearing quickly. Our ethnobotany program represents an ideal means of building trust between ourselves and these communities who are crucial participants in any well-conceived scheme to promote biodiversity conservation in Panama. In support of this effort, Dr. Ortega-Barria (Associate Program 3), a pediatrician with training in infectious diseases, has agreed to visit the study sites of our

ethnobotanical studies to assess the state of health care and offer assistance when possible at no cost to the grant, either for time or travel.

Dissemination of Results

To work effectively in Panama, and to raise awareness of the importance of conservation issues, requires that we inform the general public as well as our professional colleagues. Results will be presented in a variety of fora, including internet access. Drs. Coley, Capson and Kursar have all presented results of this work in Panama and will continue to do so.

Scientific Community

We intend to submit manuscripts to the appropriate refereed journals whenever possible. We anticipate that the majority of our publications will be joint papers between colleagues in the US and Panama. We have given scientific talks in Panama, Colombia, Venezuela, Mexico, Costa Rica and the US. A recent visit to Panama by Monsanto's Leslie Harrison (Associate Program 5) was accompanied by a lecture at the Department of Zoology and Entomology at the University of Panama. Harrison's lecture led to the contacts that now constitute the whitefly screening project (Associate Program 2).

Policy

A recent invitation to a World Bank workshop on bioprospecting policy in Bogotá, Colombia, suggests that the model we have developed is of interest in other countries that are formulating their bioprospecting policy. Previously we also were invited to submit an editorial outlining our approach to Nature Biotechnology (Capson et al. 1996). We envision becoming increasingly involved in national and international policy issues.

Public Fora

Our project has been featured at least 8 times in the popular and international media, including the BBC, newspapers in the US and Panama, Panamanian television, etc. Public lectures and articles will continue to be an important medium for informing the scientific and lay public of our work in Panama.

Provisions for a Long-term, Self-sustaining Bioprospecting Program

Each component of our work in Panama is designed to contribute to a long-term, self-sustaining bioprospecting program: one that will endure far longer than the five years supported by the ICBG program. This is reflected in our emphasis on (i) internships for students, (ii) establishing bioassays in four different laboratories in Panama, (iii) returning a portion of the revenue to support conservation and bioprospecting research and (iv) transferring of ethnobotanical knowledge between generations in indigenous communities. The infrastructure and the human resources necessary for a viable bioprospecting program must be in place before Panamanian scientists will be in a position to solicit financial support, in the context of either a commercial or academic enterprise, for bioprospecting research. A component of our program that complements the development of the human and scientific capital is the trust fund established in collaboration with Fundación Natura: their competitive grants program provides a potential means of funding research for a well-conceived bioprospecting program. We will also pursue mechanisms for channeling revenue to Panama at as early a stage in the drug-discovery process as possible, for example, by negotiating for early milestone payments.

International Outreach

A central goal of our ICBG program is to establish a successful model of bioprospecting and to make this available to the international community. To facilitate the transfer of all aspects of our program to other biodiverse countries, we will host interns and visiting scientists who are interested in

starting bioprospecting programs. Because we are conducting all components of our ICBG project in Panama, it is possible for visitors to study the entire program. This includes (i) ecological criteria for making collections, (ii) procedures for extracting fresh samples, (iii) numerous biomedical and agricultural bioassays, (iv) purification of active compounds, and (v) financial and legal agreements for benefit sharing. We will host short-term visitors who would like to obtain an overview of the program, as well as interns who wish to learn about a particular component in more detail. We have budgeted for several visitors each year. By sharing our experience and results with other countries, we hope to promote sustainable bioprospecting as a successful tool for conservation.

Statement of Acceptance of "Terms and Conditions" of Award

This ICBG accepts the provisions of the "Terms and Conditions", as described in Section J, Part 3, of RFA: TW-98-001 dated August 15, 1997 including contributing to health benefits, the understanding and conservation of biological diversity and the provision of economic benefits for Panama.

HUMAN SUBJECTS: None

VERTEBRATE ANIMALS: None

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