Chapter 8
Iwokrama Fungal/Plant Bioprospecting Project 2000–2003 – A Model for the Future?

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Abstract In 2000 the Iwokrama International Centre for Rainforest Conservation and Development (IIC), Guyana embarked on a bioprospecting and bioinventory project of endophytic fungi and their plant hosts in partnership with other institutions. The project was called the Conservation and Sustainable Utilization of Biodiversity in the Iwokrama Forest (CSUBIF) programme and was funded by the European Commission (EC). The main objectives were (a) establishment of a bioprospecting-bioinventory laboratory in Guyana, (b) an inventory of Iwokrama's unknown endophytic fungi, (c) isolation of bioactive natural compounds from fungal endophytes and host plants, and (d) development/commercialisation of natural products. A local laboratory was established at the University of Guyana to collect, identify and analyse samples. The project was an overall success having identified (a) hitherto unknown endophytic fungi and (b) 110 fungal and 29 plant extract leads for further research and development. The major constraints of the project was its short duration, lack of Intellectual Property Rights (IPR) agreements and access to genetic resources and benefit sharing (ABS) protocols. There has never been a more important time to reassess efforts such as the Iwokrama Bioprospecting Project, given the current crisis caused by resistant bacterial and fungal diseases and the lack of effective antibiotics with which to treat them.

8.1 Background

Guyana dedicated the Iwokrama Forest (Fig. 8.1) to the international community to demonstrate how tropical forests could provide economic benefits at the 1989 Commonwealth of Nations Heads of Government meeting. This pristine forest comprises 371,000 hectares which should be utilized without compromising ecological integrity or biodiversity. The Iwokrama International Centre for Rainforest Conservation and Development (IIC) was legally established by Guyana and the

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Fig. 8.1 Map of Guyana showing the location of the Iwokrama forest

Commonwealth Secretariat in 1996 as an autonomous, not-for-profit institution governed by an International Board of Trustees with the mission to:

> "Promote the conservation and sustainable and equitable use of tropical rain forest in a manner that will lead to lasting ecological, economic and social benefits to the people of Guyana and to the world in general, by undertaking research, training and the development and dissemination of technologies."

From 1993 to 2000 funding to establish IIC came from the Government of Guyana and three main donor agencies, the: (a) Global Environmental Facility (GEF) through the United Nations Development Programme (UNDP), (b) Commonwealth of Nations Secretariat and (c) International Development Research Centre (IDRC), Canada. From 2000–2007, Iwokrama's work centered on the collection of baseline information, such as biodiversity inventory surveys, to assist with the management of the reserve which included zoning of the forest, forest management and surveillance. Other research projects conducted during that time included forest utilization, surveys of the wetlands and river systems bordering the Iwokrama forest, marketing, social research and biodiversity prospecting. The IIC was supported by several donor funded programmes, the major ones being: (a) Sustainable Human Development, (Department for International Development (DFID), UK), (b) Sustainable Forest Management (International Tropical Timber Organisation (ITTO), (c) Ecotourism Development and Programme Support (Canadian International Development Agency (CIDA), and (d) Conservation and Sustainable Utilization of Biodiversity in the Iwokrama Forest (CSUBIF) (European Commission (EC). The CSUBIF project included a component concerning bioprospecting.

8.2 Conservation and Sustainable Utilisation of Biodiversity in the Iwokrama Forest Project

8.2.1 Project Conception

The CSUBIF project proposal was conceived by IIC in accordance with its Operations Plan 1998–2002 and Business Plan 1998–2007 (Baines and Warner 2000), both of which featured bioprospecting as one of the prominent potential revenue generating activities for the Centre, together with eco-forestry, certified logging, sustainable production of non-timber forest products, eco-tourism, training services and the sale of expert forest management services (Gilmour 1999). The project proposal, comprising three components - Wilderness Preserve, Bioinventory, and Bioprospecting – was presented to the EC for consideration in 1998 and was accepted on 20th January 1999. A three-year Financing Agreement was signed between IIC and the EC with funding from the Tropical Forest Budget, line B7-6201.

The Wilderness Preserve component of the project focused on the development and participative implementation of management plans for the Wilderness Preserve area of the forest and the road corridor that runs diagonally through the forest from Kurupukari (Fairview Village) in the north east to Corkwood on the south western edge of the forest (Fig. 8.2) established through extensive stakeholder consensus. Demarcation of the Iwokrama forest into two areas divided equally into the (i) Wilderness Preserve for the conservation of nature and natural processes and (ii) Sustainable Utilisation Area for the judicious use of the multiple resources of the

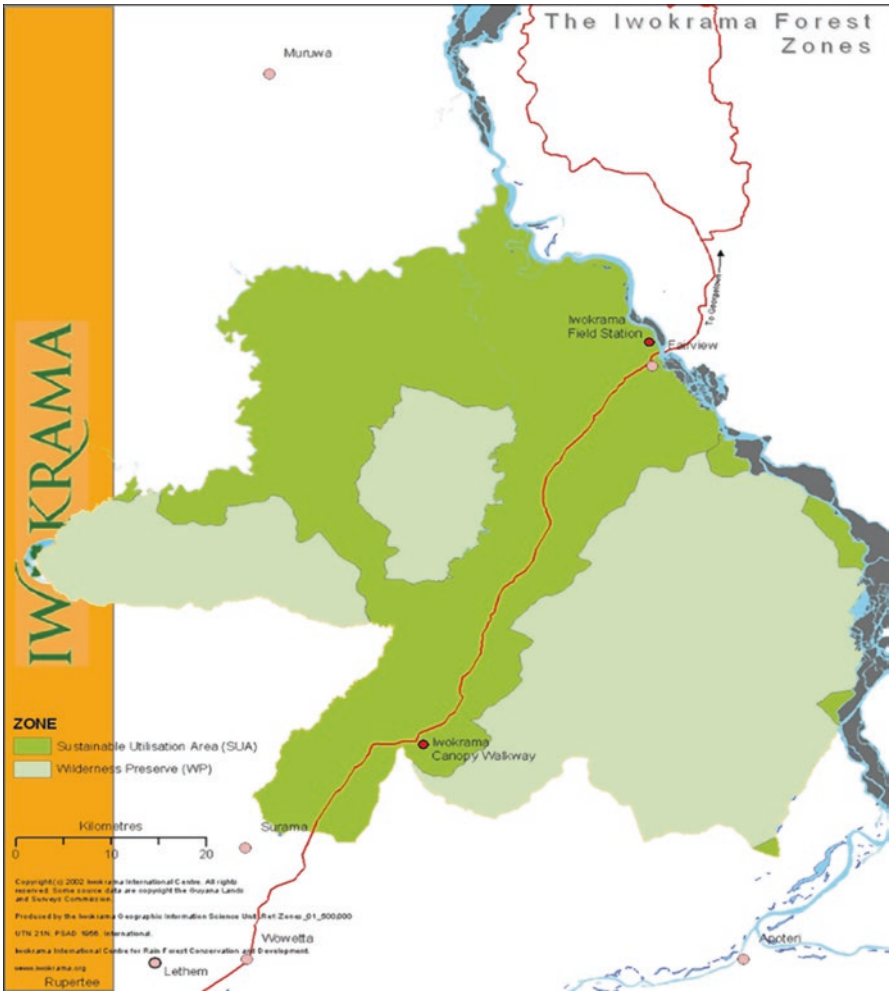

Fig. 8.2 Map of Iwokrama Forest showing the sustainable utilization area and wilderness preserve zones, road corridor, river systems bordering Iwokrama and surrounding villages

tropical rain forest to yield benefits to the peoples of Guyana and the world in general, without compromise of these resources for future generations. These were requirements of the IIC Act of 1996. Zoning of the forest was carried out with funding from DFID, EC and ITTO.

The Biodiversity Inventory component of the Iwokrama project under discussion herein was originally designed for a comprehensive inventory of the biodiversity of the Iwokrama Forest serving as a basis for sustainable forest management, further research and diversified forms of economic utilization. It was anticipated that this would create opportunities for training, capacity building, employment and the dissemination of knowledge to the wider scientific community. However, bioinventory

activities were modified to complement the bioprospecting project to avoid only supplementing the existing flora inventory of over 1,075 plant species and the vertebrate inventory (90 % completed) (Smith and Kerry 1996) previously carried out by the (a) Smithsonian Institution (Clarke et al. 2001), (b) Philadelphia Academy of Sciences, (c) Royal Ontario Museum (Engstrom et al. 1999, Lim and Engstrom 2001a, b, 2005), (d) University of Kansas, (e) Florida International University, and (f) American Museum of Natural History.

A major goal of the bioprospecting component was to identify, develop and commercialize bioactive compounds. Another was to initiate pilot ventures based on clear legal foundations, making best use of national and regional laboratory facilities, and operating through equitable business partnerships with local communities, the private sector and other stakeholders. Recognizing the lack of intellectual property rights (IPR) and access to genetic resources and benefit sharing (ABS) agreements at IIC and the absence of national legislation to enact such agreements from the outset, the EC recommended that these agreements be put in place prior to natural product commercialisation or publishing. IIC proceeded with its bioprospecting project contingent on the expectation that once protocols on IPR and ABS were established by IIC and high quality leads with good potential market value were generated, national legal legislation would follow to enable these agreements to work and for commercialisation to proceed thereafter.

8.2.2 Bioprospecting-Bioinventory Project Implementation

Work on the Bioinventory and Bioprospecting components commenced on June 2000, 16 months after the contractual agreement between IIC and EC was signed. The later than anticipated commencement was due in part to the time taken to establish a unified strategy and establishing a broad enough framework for institutional collaborators to agree the project objectives, as stated in Iwokrama's CSUBIF final project report to the EC in 2003. The initial three-year funded programme was therefore given an 11 month extension by the EC to complete proposed objectives.

In 1999, IIC decided that the best way to enhance the outputs of the bioinventory work and simultaneously maximize the likelihood of commercial development of bioactive products from projects involving the forest, was to focus on lesser known smaller-bodied organisms and microorganisms well-known to be rich sources of novel bioactive compounds in some cases (Chin et al. 2006; Giddings and Newman 2013; Sneader 1996). IIC realized that it was very unlikely for a single natural product chemist (NPC) to achieve a high measure of success without some level of external collaboration with institutions having modern laboratory facilities and marketing capacities. With this strategy in mind, IIC advertised for institutional partners with a track-record of product development and commercialisation from local and international sources to work with the Centre. In August 1999 two consortiums were chosen, (a) CABI Bioscience, UK and the Royal Botanic Gardens (RBG), Kew, UK and the (b) University of the West Indies (UWI), Trinidad and Jamaica,

University of Guyana (UG), Guyana, and Institute of Applied Science and Technology (IAST), Guyana, from a field of 12 institutional and individual applicants. CABI was selected on the basis of providing training and technical support on macro and endophytic fungi. In addition, their proposal was designed to be closely linked to the parallel bioprospecting programme, in which potentially valuable biochemicals from fungi during the bioinventory activities would be screened for biological activity in collaboration with RBG Kew. The UWI-UG-IAST group, were selected on the basis of their extensive experience in plant natural products research and ability to provide local and regional laboratory facilities to the project, together with technical support for local bioprospecting staff.

Consortium members met for the first time with IIC staff in Guyana in January 2000 to decide how to utilize capacities in natural product chemistry and product development by research collaborators. The need to establish clear objectives for each collaborator was discussed. IIC agreed that the (a) CABI-RBG Kew group comprising Drs. Joan Kelley, Paul Cannon, Russell Paterson, Paul Kirk, and Tetsuo Kokubun, and Prof. Monique Simmonds, and (b) Guyana-based NPC, should focus on endophytic fungi isolated during the biodiversity inventory programme. This involved the production of extracts for screening by bioassays developed by CABI and RBG Kew. IIC established a bioprospecting lab in Guyana to facilitate the work and provide a useful output *per se*. The UWI-UG-IAST group comprising (a) Profs. Bladwin Mootoo, and Wilfred Chan, (b) Drs. Anderson Maxwell, Helen Jacobs, Marlene Cox, and David Singh and (c) Mr. John Caesar would focus on structural elucidation and biotesting of compounds isolated from host plant species to examine the interaction between host plant and endophyte chemistry.

A target of producing 2000 extracts was considered suitable to establish linkages with companies for more in depth examination and screening. Subsequent interests in commercial development of extracts produced by the project would be contingent on clear and well-constructed IPR and ABS protocols within the framework of a national IPR and ABS policy. This is stipulated under the Convention on Biological Diversity (CBD) of which Guyana is party to since 27 November 1994.

8.2.3 Recruitment and Training of Bioprospecting and Bioinventory Staff

IIC advertised internationally for applicants to undertake the bioinventory and bioprospecting components in Guyana. In June 2000, Coralie Simmons, a Guyanese national and biology graduate with experience in fungal systematics from UG, was hired as the Biodiversity Inventory Scientist (BIS). Vijay Datadin, a Guyanese national, was hired as a Geographical Information System specialist to manage the georeferenced field sample collection data from the different collection sites in the forest and to integrate this data into maps. Dr Ramish Pingal (the author of the current chapter), a Trinidadian NPC and recent PhD chemistry graduate from the

UWI, was recruited to manage the bioprospecting component in Guyana who entered the programme with over 5 years of research experience in the extraction, isolation, characterization and bioassay analysis of natural products from terrestrial plants.

Bioinventory and bioprospecting activities commenced shortly after the BIS and NPC were hired with a 4.5 month training period of the BIS on endophytic fungal isolation, identification at CABI Bioscience. The NPC also underwent training on aseptic culture and bioassay techniques, isolation of fungal metabolites and laboratory safety training split between CABI Bioscience and RBG Kew from July–November 2000. Plants collected from the Iwokrama forest prior to the departure of the BIS and NPC to the UK were used for isolating fungal endophytes during the training period in order to yield immediate results for both components. Four Guyanese nationals (a) Henry James and Linsford Lagoudou (parataxonomists, field technicians and research assistants), and (b) Melanie McTurk (BSc, Chemistry, UG), were hired in early 2001 to fill the positions of (a) two full time field/laboratory technicians and (b) laboratory technician, respectively. The main duties of the technicians were to collect plant samples from the rainforest and to prepare fungal cultures, plant extract generation and other routine activities in the lab in Guyana. Technicians were trained in-house by the BIS and NPC on plant collecting, aseptic and mycological culturing techniques, preparation of fungal and plant extracts, handling and disposal of hazardous materials, and laboratory health and safety procedures.

8.2.4 Project Management

The BIS and NPC were responsible for the day-to-day management of the bioinventory and bioprospecting components of the EC project, respectively. The Centre's Principal Forest Ecologist, Dr. David Hammond, served as the component head of the Wilderness Preserve aspect of the project and the team leader for the entire EC project. The BIS and NPC reported to the team leader and each component head also reported directly to the Director General of Iwokrama Mr. David Cassells July 1997–June 2001 and Dr. Kathryn Monk July 2001–May 2003.

8.2.5 Rational for Selection of Endophytic Fungi

Fungi were selected as the microorganisms to study because they had not been studied in Iwokrama, and some of the most valuable and widely used pharmaceuticals from natural products originate from these organisms (Kelley et al. 2003; Butler 2004, 2008; Butler et al. 2014, Newman and Cragg 2007). These include penicillin (Sneader 1996), the antifungal cyclosporins used in transplant surgeries, and cholesterol-busting drugs based on zaragozic acids (Bergstrom et al. 1995; Dreyfuss and Chapela 1994). Other fungi-derived metabolites undergoing development

include the diketopiperazine, plinabulin from *Aspergillus*, which was under clinical trials for the treatment of lung cancer (Bhatnagar and Kim 2010).

Other compelling reasons that made fungi the organism of choice included (a) they are the second most diverse major organism group on Earth with species estimated at 1.5–5.1 million (Hawksworth 2012; Blackwell 2011; O'Brien et al. 2005; Hawksworth 1991; Hammond 1992; Cannon and Hawksworth 1995; Rossman 1994), (b) 10,000 to 15,000 fungi are estimated in the Iwokrama forest, of which 50–80 % were expected to be undescribed (Rossman et al. 1998; Kelley et al. 2003), (c) they have close associations with a wide range of other organisms; (d) host-fungi specificity, (e) the major functional roles fungi play in terrestrial and aquatic ecosystems; (f) their anticipated high diversity within tropical ecosystems in general and (g) the ease of maintaining ex-situ in some cases (Arx 1980; Cannon 1996; Domsch et al. 1993).

Although fungi can be challenging to isolate and culture, studying fungi in specific fungi-host associations makes the sampling process easier enabling initial collection to focus on the host rather than the fungus. This approach (a) facilitates isolation from many species where the fungal fruiting bodies are difficult to sample directly because of small size, (b) reduces the risk of sample contamination from other organisms, and (c) allows simple reproducible manipulations of culture conditions for effective isolation.

8.2.6 Field Sample Collection

Thirteen plant host species from the genera *Carapa, Catostemma, Cecropia, Chlorocardium, Eperua, Eschweilera, Euterpe, Goupia, Jacaranda, Manilkara*, *Mora*, *Sclerolobium* and *Swartzia* were selected to study their endophytic fungi. Seven bioinventory plots of one ha were established within the Sustainable Utilization Area of the Iwokrama Forest representing different (a) vegetation and edaphic types, (b) spatial distributions and (c) levels of disturbance within the forest. Selected host trees were tagged and georeferenced. The plots were located at the "3 Mile" and "8 Mile" satellite camps along the road corridor that runs through the Iwokrama Forest and Corkwood swamp, Kabokalli, White Water, Pakatau falls and Moco-Moco. A map showing the spatial distribution of the plots was generated by the Geographical Information Staff at IIC using the georeferenced data collected for each of the seven plots. A total of six field collections of healthy leaves and soft stems were carried out at six different times over the project duration: June 2000; April, October and December 2001; and February and June 2002. From the six field collections, leaf samples of 13 host plant species representing 10 families were collected. A secondary investigation into wood and bark endophytes was undertaken. Furthermore, two macro fungi collections were made from within the sample plots and other areas within the sustainable utilization area. A total of 125 macro fungi samples were collected that yielded 71 different species.

For the phytochemical work, aerial parts of all 13 host plant collections were made from two field collections in April and August 2001. A third collection was made in March 2002 of nine additional plant species from six genera (*Annona*, *Clusia*, *Dugetia*, *Piper*, *Tovomita*, and *Vismia*). This represented 22 different plant species. All plant collections were made from within the Sustainable Utilization Area and voucher specimens of each plant species collected have been lodged at the herbarium of the Centre for the Study of Biodiversity at UG.

8.2.7 Rational for Selection of Host Plants

Thirteen plant host species were selected for study from which endophytic fungi were collected based on (a) their commercial importance as timber species, (b) their abundance in the Iwokrama forest and (c) no, or very limited, phytochemical information on seven of these species. Selection of the nine additional plant species for phytochemical analysis was made on the basis of the known bioactivities of plants from the genera to which they belonged. Importantly, use was not made of ethnobotanical information or traditional knowledge in the selection of plants because of the lack of IPR and ABS protocols at Iwokrama and in Guyana more generally.

8.2.8 The Biodiversity Inventory Programme

8.2.8.1 Endophytic Fungal Isolation and Characterisation

The inventory programme focused on isolation of endophytic fungi from healthy, mature and symptomless plant leaf samples from saplings and young trees. Leaf samples of 13 host plants were collected from which a total of 912 pure fungal cultures were obtained representing over 10,000 isolations of endophytic fungi. Fungal cultures were identified using morphological techniques, including observation of cultures grown under defined conditions, and description and measurement of microscopic features. Initial fungal identification was carried out to the species aggregate level, followed by assignment to a morpho-species using a combination of cultural and micromorphological characters to make the overall process manageable.

Sixty-four fungal morpho-taxa were characterized from 2,492 cultures. Most of the endophytic fungi samples isolated belonged to four genera: *Colletotrichum, Phomopsis, Nodulisporium* and *Pestalotiopsis*. The complete strain set of *Colletotrichum* and *Pestalotiopsis*, ca. eighty strains each, was selected for further studies using inter-simple sequence repeat polymerase chain reaction (ISSR-PCR) and random amplified polymorphic deoxyribonucleic acid (RAPD) techniques. Analysis of the data from both techniques indicated that host specificity could not be detected in *Colletotrichum* nor *Pestalotiopsis* even at the strain level (Lu et al.

2004). *Colletotrichum* strains only were selected for further analysis with the aid of ribosomal deoxyribonucleic acid internal transcribed spacer (rDNA ITS) sequencing, owing to the large number of strains to be analysed within a short time, and the fact that *Colletotrichum* is an important and large genus of over 900 species comprising numerous endophytes and a few phytopathogens of tropical plants.

8.2.9 The Bioprospecting Programme

8.2.9.1 Extract Profiling and Biotesting

Over 360 endophytic fungal cultures and 17 macrofungi samples were received from the bioinventory component. These were subcultured and bulked up for extraction with a chloroform-methanol mixture followed by extraction with pure methanol to give a total of over 900 organic and aqueous extracts.

Plant Samples A total of 13 host plant species and nine additional species of plants were collected, air dried, milled and extracted with a chloroform-methanol mixture followed by extraction with methanol. A total of 60 organic and aqueous plant extracts were prepared.

Metabolic Profiling A comparison of the profiles of compounds in the fungus and plant extracts was determined by thin layer chromatography (TLC) using a range of different spray reagents and high-performance liquid chromatography (HPLC). Liquid Chromatography-Mass Spectrometry (LCMS) profile was also obtained for selected extracts. The chemical profiles of ca. 880 fungal and host plant extracts were determined at RBG Kew using HPLC equipped with a photodiode array detector.

Bioassay of Fungus and Plant Extracts Over 2800 fungal extracts obtained from 332 fungal isolates, and host plant extracts were tested by RBG. These were subjected to up to six bioassays using insects, bacteria and fungi as test organisms. Cultures of all fungal isolates extracted and assayed have been stored in Guyana and 256 of these cultures are also held in a reserve collection at CABI Bioscience Genetic Resource Collection.

A total of 60 plant extracts transferred each to UG, the UWI Trinidad and UWI Jamaica were subjected to 16 different assays which tested for antibacterial, antifungal, anti-insect, antioxidant, antiretroviral and cytotoxic activities. At the bioprospecting natural products lab in Guyana, 547 fungus extracts and 60 plant extracts were tested in cytotoxicity (using brine shrimp nauplii, antifungal and antioxidant assays).

8.2.9.2 Bioassay Results

Endophytic Fungal Extracts Of the 332 fungal isolates cultured and extracted, 185 derived from 152 fungus strains showed bioactivities in at least one of the six assays. The activities were: 13 antibacterial, 14 antifungal, 92 anti-insectal and 103 cytotoxic. Of the 185 fungus extracts that were active in at least one of the assays, 130 were of organic origin whereas 55 were aqueous. Furthermore, 33 fungal isolates had organic and aqueous extracts that were active in at least one assay. Aqueous extracts showed only cytotoxic (40 % of aqueous extracts) and anti-insect (60 % of aqueous extracts) activities whereas organic extracts showed cytotoxic, antibacterial, anti-insect and antifungal activities. From the 185 active fungus extracts identified, 110 extracts derived from 100 fungus isolates were selected as possible leads for further research to continue bioprospecting in the future. This was reported in the Iwokrama's final CSUBIF report to the EC in 2003, and the final evaluation report of the CSUBIF by Stephen Devenish, 2003 for the EC.

Plant Extracts Host plant extracts tested in the six assays at RBG Kew all showed activity in at least one assay. The bioassay results showed that a high proportion of the extracts exhibited anti-bacterial activity, whereas extracts from only two plants showed anti-insect activity. This contrasts with the high levels of anti-insect activity observed from screening the endophyte fungal extracts of organic and aqueous origin.

Of the 60 plant extracts obtained from 13 host plants and nine additional plants, tested at the lab in Guyana and by UWI-UG project collaborators, a total of 29 were selected as possible leads for further work as indicated in Iwokrama's CSUBIF final report, 2003. Only one extract showed activity in the anti-retroviral assay and in antibacterial and antifungal assays. Three extracts showed activity in cytotoxic, antioxidant and antibacterial assays, six extracts showed activity in only two assays, and two extracts each demonstrated activity against two bacterial and two fungal strains. Of the extracts tested in anti-insect assays, only four showed activity against only one insect, whereas one showed activity against two insects.

8.2.10 Analysis of Results and Summary of Achievements

8.2.10.1 Bioinventory

Although more work is required, no correlation was observed between any defined fungal communities and individual plant species, indicating that host specificity is low in the Iwokrama Forest and by extension tropical rain forests, in contrast to temperate forests. Further, molecular fingerprinting studies carried out on *Colletotrichum* and *Pestalotiopsis* did not reveal any host specificity even down to

the individual strain level. Analysis of the sequences of the ITS region of the rDNA of a subset of the *Colletotrichum* strains revealed significant variation within species. Based on the analyses, several taxa appeared to be new, including at least two species of *Colletotrichum* and an apparently undescribed genus of anamorphic fungi (Lu et al. 2004).

The main achievements of the bioinventory aspect of the project were:

- First inventory of endophytic fungi and macrofungi within the Iwokrama Reserve
- Establishment of a herbarium collection at the Iwokrama field station
- Establishment of a comprehensive database on endophytic and macro fungi
- Publication of two scientific papers on endophytic fungi from the Iwokrama forest (Cannon et al. 2002; Lu et al. 2004) and presentations on Iwokrama's bioinventory programme at four international, regional and local conferences and workshops (Simmons 2002a, b, c; Simmons and Cannon 2002).

8.2.10.2 Bioprospecting

The results of the initial bioassays on the fungal and plant extracts gave very good bioactive hit rates of 3.9 % (110 bioactive fungal extracts from 2800 extracts tested) and 48 % (29 bioactive plant extracts from 60 extracts tested) respectively compared to the average 'hit' rate of 0.03 % in carrying pre-screened samples to market (Lesser and Krattiger 2007). Bioactive hit rate refers to that percentage of samples which show biological activity in a given series of chemical assays (Guérin-McManus et al. 2011). Lead rate may be defined as the proportion of expected lead compounds identified from the total number of samples screened to yield a given lead compound (Lesser and Krattiger 2007). Further research on these 139 selected bioactive extracts would involve secondary screening: bioassay directed fractionation, isolation, purification and characterization of bioactive compounds using High Performance Liquid Chromatography (HPLC), Nuclear Magnetic Resonance spectrometry (NMR) and Mass Spectrometry (MS) to rapidly identify new compounds from those that are known. Further work would also involve the (a) testing of purified bioactive compounds in several specialized assays e.g. anti-inflammatory, antioxidant, and anticancer to assess their suitability for a specific use, (b) toxicological assessment of compounds for possible drug development, and (c) structure activity relationship studies to enhance activity.

The striking difference in bioactivities exhibited by fungal and host plant extracts in antiinsect and antimicrobial assays, where a higher proportion of plant extracts showed antimicrobial activity, compared to fungal extracts which showed mainly antiinsect activity, suggests that the metabolites of fungal endophytes may be responsible for providing protection to the plants against predatory insects. The poor antiinsect activity exhibited by host plant extracts compared to endophyte extracts was corroborated in independent assays conducted by UWI-UG project collaborators and further points to a possible role of fungal endophytes in the fitness and survival strategy of their plant hosts. Early work on plant endophytes centered

mainly on the endophytes of grasses and crops (Clay 1988, 1990; Márqueza et al. 2012; Saikkonen et al. 2013). Research on plant endophytes has mushroomed over the past two decades owing to the paucity of knowledge on fungal endophytes from other plant sources and the significance of endophyte-plant interactions and endophyte secondary metabolites. The emerging insecticidal (Omacini et al. 2001; Shrivastava et al. 2015; Simons et al. 2008; Zhao et al. 2011) and antimicrobial (Gutierrez et al. 2012; Guzman-Trampe et al. 2015; Lv et al. 2010; Mousa and Raizada 2013; Nisa et al. 2015) activities of endophyte metabolites and their potential for use as biopesticides and pharmaceuticals have played a major role in influencing and promoting research in this field. Despite the recent intensive work on plant endophytes, research on the endophytes of tropical rainforest plants remains limited (Arnold et al. 2001). However, data from this present study points to a low diversity of fungal endophytes and a high diversity of endophyte metabolites contrary to initial predictions. An investigation of the secondary metabolic profile of the leaves of the Iwokrama host plants with and without fungal endophytes and their resultant bioactivities is required to determine the role played by endophytes in plant-fungal associations. Wider research on endophytes of tropical forest plants is a priority to develop a more complete understanding of endophyte-plant interactions and the metabolites they produce. In summary, this project has built capacity within Guyana to carry out endophyte fungal isolations and identification, together with bioprospecting activities, and has established a collection of fungi, plants, and extracts thereof of interest to the agrochemical and pharmaceutical industries.

Major accomplishments during the implementation phase of the bioprospecting project component include:

- Establishment of a natural products bioprospecting laboratory in Guyana currently operated by UG through a MOU with Iwokrama.
- Preparation and screening of over 3400 fungal and 60 plant extracts for biological activities in 23 different assays
- Metabolic profiling of over 880 fungus and plant extracts
- Fractionation of eight bioactive extracts
- 110 fungus and 29 plant extract leads with anti-insect, anti-bacterial, anti-fungal, antioxidant and/or cytotoxic properties identified
- Establishment of a comprehensive database on fungi and plant extracts
- Antifungal, antioxidant and cytotoxicity assays established at lab in Guyana
- Laboratory technician trained in microbiological culturing, extraction, bioassay techniques and data management
- Two UG students trained in bioassay techniques and four UG students assisted with projects
- Bioprospecting project information disseminated at three international/regional conferences (Pingal 2002b; Pingal et al. 2001, 2002). Two public lectures (Pingal 2001; Pingal and McTurk 2002) were given and two national meetings with representatives of the 16 Amerindian communities that have legal rights over the Iwokrama forest were made. Two talks on biodiversity and natural product development were given at two international workshops (Pingal 2002a, c). In addition, the current chapter represents an output.

Table 8.1 Bioprospecting objectives and level of achievement (X = the level achieved)

Objectives of bioprospecting component	Level of achievement		
	Not done	Partially completed	Completed
Sample selection: Select plants for endophytic fungal and phytochemical research			**X**
Sample preparation: Prepare endophytic fungal cultures for chemical profiling and subsequent fractionation			**X**
Extract preparation: Prepare at least 2000 fungal and plant extracts for metabolite profiling and analysis			**X**
Bioassays: Establish a set of bioassays at the bioprospecting lab in Guyana			**X**
Analyse & Test 2000 Extracts: Test at least 2000 fungal and plant extracts in a series of bioassays in Guyana and through regional and UK collaborators,			**X**
Isolate & Identify Bioactive Compounds: Isolate and characterize bioactive compounds from fungi and plant extracts		**X**	
Database Tracker: Establish a database to track extracts prepared			**X**
Establish Local Lab Facilities: Establish a bioprospecting lab in Guyana			**X**
Commercial Products: Develop bioactive compounds for commercialization	**X**		
Pilot Ventures: Initiate pilot ventures based on clear legal equitable business partnerships with local communities, private sector and other stakeholders	**X**		

The extent to which the original objectives and activities of the bioprospecting aspect of the project was achieved during the project's life cycle is indicated in Table 8.1.

8.3 Project Analysis

8.3.1 Aspects of the Programme That Worked Well

The project ran smoothly throughout its entire cycle despite the complexity of the relationship among individual partners comprising the consortium. Initial training of the BIS at CABI Bioscience on endophytic fungal isolation, aseptic culturing techniques and identification before the project activities started in Guyana, was essential in the provision of pure fungal isolates of the highest integrity to the bioprospecting programme. Similarly, training of the NPC at CABI Bioscience and Kew Gardens UK on aseptic culturing techniques, basic fungal identification,

natural products research, lab/database management and health and safety procedures assisted tremendously in the establishment of the lab in Guyana. Preparing, analyzing and tracking extracts at all stages of the process ensured accountability. Ample support and technical assistance was provided by both groups of consortium partners over the entire course of the project facilitated by eight in country visits by eight persons and regular communication via email. CABI Bioscience assisted with ordering vital general equipment for the lab during the latter half of 2000 in order for these to arrive in Guyana in time for the establishment of the laboratory. A stock of general lab supplies and reagents sufficient for 1 year's work was ordered initially in early 2001 to avoid any delays in the progress of the project owing to the slow shipping (up to 2 months) of foreign purchased goods from Europe, the UK or EC based companies in the USA to Guyana. This core stock of materials was maintained by placing orders for spent stock at least 1 month in advance to give sufficient time for items to be delivered to Guyana on time to prevent shortages and project work delays.

There were no injuries and loss of time during the entire project cycle owing to the strict adherence to standard laboratory safety procedures as specified in IIC's bioinventory-bioprospecting laboratory safety manual, regarding the (a) safe storage, handling and disposal of biological specimens and hazardous chemical substances; (b) adequate training of all laboratory personnel on the collection of biological specimens from the forest, and the handling of plant and fungi specimens and hazardous chemicals; (c) the use of personal protective equipment e.g. respirators, gloves, safety goggles, lab coats; and (d) other safety engineering controls e.g. fume hoods, eye wash station, emergency showers and safety cabinets by all laboratory personnel. Standard laboratory health and safety rules were enforced by the BIS and NPC.

Staff at the Iwokrama Centre and Field Station provided excellent general project administration services and support in Georgetown, Guyana and in the field that enabled both components of the project to proceed without interruption. Collection of plant samples was carried out with the assistance of the field technicians, lab technician and BIS as well as the NPC upon occasion. Since collection of plant samples was restricted mainly to timber host trees ranging in height from a few metres to several metres high, collection of some leaf samples required a trained tree climber. Mr. Rodrigues Antone (now deceased) an Amerindian - a descendant of the indigenous peoples of the Americas, was hired on contract from one of the nearby communities to collect leaf samples from especially tall trees.

Linking the bioinventory activities with the bioprospecting project was a prudent decision at the time that enabled both components to work as one unit. The bioinventory component provided excellent support to the bioprospecting component by furnishing it with a continuous supply of large numbers of high quality pure strains of fungi (over 258) ready for bulking up, extraction and bioassay testing. The linking of both components eliminated the need for duplicate plant field collections for the endophyte fungal work and afforded the project significant reduction in time and resources as a major benefit.

Immediately fungal samples were received by the NPC, these were quickly subcultured by the lab technician and then bulked up for extraction, metabolic profiling and bioassay testing before being shipped to the UK collaborators. Similarly plant extracts were prepared quickly after collection following a period of air drying, milling, extraction, metabolic profiling and bioassay testing before delivery to UG and the UWI collaborators. So there was a steady progression at all stages of the process from plant collection, to (a) fungal isolation, (b) culturing, (c) extract preparation, (d) extract profiling, (e) extract biotesting, (f) extract tracking and (g) extract delivery to collaborators.

Semi purified extracts and isolates exported to collaborators in the UK, Trinidad and Jamaica for further analysis required the normal adherence to the regulatory procedures of the Environmental Protection Agency (EPA) of Guyana for access to genetic resources. The EPA, Guyana and the UG facilitated this process through the acquisition of relevant export permits on a timely basis. The UK and regional partners at the UWI and UG also provided valuable laboratory services in terms of testing of extracts in a series of insect and microbial assays as well as in the purification and analysis of extracts using modern analytical techniques: HPLC-Diode Array Detector, Liquid Chromatography-MS-MS and NMR spectroscopy. These analytical instruments were unavailable at the lab in Guyana. Reports on results of fungal identification and extract analysis and testing were provided by both UK and regional partners to IIC in very good time.

8.3.2 Aspects of the Programme That Did Not Work Well

The Iwokrama Forest lies 274 km south of Georgetown – a 7–11 h drive by road depending on road conditions. Since the majority of the road comprises unpaved laterite, road conditions especially during the May-June rainy season delayed accessibility to the forest and sample collection on a few occasions. In addition, intermittent bouts of malaria and dengue fever among field staff were a constant threat to sample collection, but did not affect the project significantly overall. There were some delays experienced in upgrading the laboratory at the UG and this coupled with power cuts/irregular power supply resulted in lost time and degradation of some fungal cultures. Contamination of sterile cultures through air borne fungi and bacteria severely hampered easy retrieval and re-culturing of fungus strains and the progress of antifungal assaying activities. The lack of basic analytical instruments e.g. Ultra Violet-Visible (UV-Vis) and Fourier Transform Infrared (FTIR) spectrometers and HPLC in the lab in Guyana prevented bioprospecting staff from preparing more extracts of higher quality.

The reference herbarium established at the Iwokrama Field Station by Bioinventory project component staff containing 140 herbarium sheets collected by

the Ecology Unit at Iwokrama and over 400 sheets donated by the Smithsonian Institution through a MOU with Iwokrama, have been infested with cockroaches from time to time leading to damage to the collection. This problem needs to be rectified to preserve the remaining current collection.

Despite the fact that 50 species of macrofungi were collected and photographed for the production of a field guide to the macrofungi of the Iwokrama Forest, images obtained were not of sufficient high quality for completion of the guide. Improved photography equipment is required.

The absence of legal IPR and ABS protocols in Guyana prevented access to Amerindian and other traditional knowledge, which would have provided excellent support to sampling of plant samples for endophytic fungi and general plant samples. The exclusion of Amerindian traditional knowledge in this project prevented the targeted selection of flora and fauna which may have resulted in higher isolation of potentially useful and marketable natural products upon which bioprospecting depends, as indicated by Stephen Devenish in the final evaluation report on the CSUBIF project in 2003. One must be mindful though of the intricacies involved in the use of traditional knowledge and medicine guided selection of samples for the development and commercialisation of natural products. Although an attractive, more selective and cost-effective approach than random screening, this type of selection is fraught with challenges, relating to the long periods of time and general difficulties involved in arriving at mutually accepted legal agreements between the knowledge holders and commercial companies relating to ownership of intellectual property and equitable Benefit Sharing (BS) (Kingston 2011).

The expected pilot level commercial outputs from the bioprospecting component did not materialize. This was due to the short time frame of the project, and the speculative nature of bioprospecting. Most importantly, the lack of (a) IPR and ABS protocols at IIC and (b) national legislation to enable these agreements to work and facilitate extract leasing, development and commercialization meant commercial outputs were impossible. As such, companies could not be approached for private sector funding to continue bioprospecting activities or entering into commercial ventures based on the extracts generated from the project as highlighted by Stephen Devenish, final evaluation report of the CSUBIF project. Despite this, exploratory meetings were held between the NPC and CABI-Kew collaborators and three private sector and semi-autonomous companies in Guyana in the latter half of 2002 on possible collaborations and financial support for the bioprospecting initiative. These meetings revealed that whereas private sector and semi-autonomous companies showed a high interest in, and support for, the programme and that they would have liked to provide financial assistance, they did not have the liquidity at the time to do so.

8.4 Suggestions for Improvement: Future of Bioprospecting at Iwokrama and Lessons Learned

Funding Iwokrama should aim to secure funding of at least 10 years for future bioprospecting projects owing to the well-known (a) speculative nature of bioprospecting, (b) significant investment in time required (ten to twenty years (Conniff 2012; Guérin-McManus et al. 2011; Lesser and Krattiger 2007) and (c) money needed (US$230 million to US$1 billion (Conniff 2012; Lesser and Krattiger 2007). This would give enough time and resources to transform biota into potentially marketable products, especially in light of the on-going crisis with resistant bacterial infections. Funding should be considered from a variety of multiple financial bodies such as local and international donors and private sector. It is important that governments of large countries become involved and provide incentives for pharmaceutical companies to work in these areas. One can consider using funds obtained from multiple donors to fund one large project or individual donor funds to finance individual small projects. Although each approach would have its inherent strengths and weaknesses, the former approach is more likely to lead to the successful development and marketing of a useful product through long term and larger financial investments. One major disadvantage of multiple donor funding of a large project is the resolving of mutually acceptable legal agreements between donors and their client and any third party beneficiaries (Cordell and Colvard 2005; Kingston 2011) such as the 16 Amerindian communities that have legal rights to the Iwokrama forest in this case, before the project starts, which could take a very long time. Whereas the latter approach, where a single donor funds a smaller project, is more likely to result in partial success owing to smaller financial support and an expected shorter time period of funding. Private sector funding should be used principally to advance natural product research, and the development and commercialisation of useful bioproducts. Funding from donor agencies should be reserved for short term aspects of the project such as capacity building, outfitting the national bioprospecting lab at UG with basic analytical instruments, and maintenance of laboratory infrastructure. Not to be overlooked though is funding from academic institutions and groups engaged in bioprospecting. Although individual institutions may not have the financial leverage to fund bioprospecting initiatives they certainly have the technical expertise and modern analytical tools and facilities to identify lead compounds suitable for further development into potential marketable products if efforts and resources from a few institutions are combined (Harvey and Gericke 2011). It is important to reiterate that governments of wealthy countries especially should provide financial incentives for pharmaceutical companies to become involved in bioprospecting projects because of the current problems with resistant bacterial infections.

Collaborators In selecting collaborators to continue its bioprospecting programme, IIC should consider to advantages of working with the previous consortium or new specialist service providers. The major advantage of working with the

same consortium partners is the ease with which previous work completed can be reinstated and further built upon. Furthermore, 256 of the original endophytic fungal cultures are held in reserve at CABI Bioscience Genetic Resource Collection for retrieval in the future.

Alternatively, since the bioprospecting lab is already established at UG and currently being maintained by UG, IIC can work with UG together with other organisations. These could be regional and/ or international academic institutions, industrial groups, and pharmaceutical companies, capable of conducting modern natural products research and development, coupled with business and marketing companies. For IIC to maximize its chances of finding and commercializing a bioactive compound, collaborators should be carefully selected to include those who are capable of:

(i) conducting dereplication of extracts using HPLC coupled with MS or MS/MS and IR-MS in combination with bioassays and reference libraries of natural compounds;
(ii) selectively identifying bioactive compounds early in crude extracts with the aid of modern techniques e.g. Liquid Chromatography-MS, 1D NMR and 2D NMR for faster purification and isolation of compounds;
(iii) characterizing pure isolates using automated structure elucidation software and routine techniques such as UV-Vis, FTIR, MS, 1D and 2D NMR, in addition to the latest NMR experiments e.g. Long Range Heteronuclear Sequence Quantum Multiple-Bond Correlation (LR-HSQMBC) experiment optimized to obtain very long-range ($\geq {}^{4}J_{\text{H-C}}$) heteronuclear correlations to assist with the elucidation of proton deficient compounds (Saurí et al. 2015). Microscale NMR techniques would be especially useful for the elucidation of metabolites of 1 mg or less (Kingston 2011), together with Comprehensive Multi-Phase NMR to characterize samples in their natural state (Monette et al. 2015);
(iv) screening extracts against a broad range of targets (e.g. antimicrobial, anticancer, insecticidal, immunological) and conducting new screening approaches even on crude extracts such as cell-based assays focusing on specific mechanisms of action and
(v) synthesizing a prospective lead natural compound to reduce the impact of collection from its natural source and to ensure that supply is unlimited (Kingston 2011).

Should a new specialist service provider be selected to take Iwokrama's bioprospecting programme to the next level, IIC should consider limiting collaboration to as few partners as is necessary per project to simplify working relationships and project implementation as recommended by Stephen Devenish in the CSUBIF final evaluation report, 2003.

National Laboratory One of the major disadvantages of the previous project was the lack of basic analytical instruments to help with the isolation and purification of natural products. The bioprospecting lab at UG should be outfitted with basic analytical instruments e.g. UV-Visible and FTIR spectrometers and HPLC to assist

with preliminary extraction, isolation, purification and analysis of natural products, if this project is to continue at some stage. Acquisition of an FTIR spectrometer could be justified from its two-fold use in the analysis of natural products, and in plant research to discriminate closely related plant species through analysis of leaf samples in the Near-IR region (Durgante et al. 2013). Having these instruments would assist the lab at UG to prepare purer extracts and compounds of higher quality faster for regional and international collaborators on which to work. Essential to the isolation of high quality, value added potential leads for further development is the establishment of a set of routine and easy to maintain bioassays, such as antimicrobial, insecticidal and cytotoxic, in the field laboratory. The results of these preliminary bioassays can give collaborators an insight into the type of activities to expect and so guide further testing. In addition, facilities for local long term storage of pure fungal strains and plant samples is recommended to avoid the loss of temporarily stored samples. The avoidance of, or provisions for, power cuts is essential.

Reference Herbarium To preserve and protect the over 500 herbarium sheets lodged in the reference herbarium at the Iwokrama Field Station, the room should be sealed and air conditioned, to prevent entry by cockroaches and other destructive insects. New and adequate storage cupboards should be installed.

Traditional Knowledge Use, IPR and BS Protocols Traditional knowledge should be employed from the beginning of any new bioprospecting initiatives at the IIC to reduce the time spent on selecting flora and fauna and to improve the chances of developing a useful marketable product. The projects must be reciprocal to avoid one-way capacity building by the bioprospecting partner. According to McAfee in the conference on "Development as if Equity Mattered" Georgetown, Guyana September 24-26, 2001, any new bioprospecting projects should therefore move away from extractive research and shift towards participatory research in which the need to share benefits with indigenous communities is fundamental (Insanally 2003). This would necessitate Amerindian involvement from the beginning of the project and will require the draft IPR and ABS protocols prepared by IIC to be agreed and sanctioned according to the laws of Guyana.

In 2007 the Government of Guyana established a national policy on ABS and has enacted relevant national legislative and administrative measures to uphold the national policy through the EPA Guyana to enable agreements to work properly. The poor success by companies such as Shaman Pharmaceuticals and Phytopharm to commercialize new pharmaceuticals or botanical medicines from traditional medicines or ethnomedicine to date, have made the use of traditional knowledge and ethnomedicine in the prospecting for bioactive compounds from flora and fauna samples less appealing (Firn 2003, Harvey and Gericke 2011; Kingston 2011). In addition, the renowned Instituto Nacional de Biodiversidad (INBio) of Costa Rica had a vibrant bioprospecting programme. After over 20 years of existence with more than twenty agreements with academic institutions and industry, the most prominent being the commercial bioprospecting research collaboration agreement

with Merck & Co. from 1991 to 2008, INBio's work has so far yielded over 27 patents (Lesser and Krattiger 2007), and several lead bioactive compounds, nutraceuticals and biological control microbes (Gámez 2007). However, INBio's bioprospecting efforts has failed to produce a blockbuster drug or to market a product to date (Conniff 2012). Similarly, the International Cooperative Biodiversity Group (ICBG) Projects in Suriname (1993), and Madagascar and Panama (1998), have yielded promising lead compounds. For example, (a) ipomoeassins A-F from the Suriname plant *Ipomoea squamosa* with potent antiproliferative activities, (b) a depsipeptide coibamide A from *Leptolyngbya* sp. from Panama with a unique selectivity profile in NCI 60 cancer cell lines and (c) schweinfurthins from the Madagascar plant *Macaranga alnifollia* with strong antiproliferative activity have shown potential. Nevertheless, they too are yet to be developed into commercial drugs (Kingston 2011), indicating the pitfalls of bioprospecting projects.

Despite this, IIC should consider the use of traditional knowledge and ethnomedicine in its search for new and useful bioactive compounds with market potential for two major reasons: (i) IIC, from its inception has engendered a good and long standing working and congenial relationship with the Amerindian communities within and bordering the Iwokrama forest thereby facilitating collaborations, and (ii) IIC is expected to demonstrate how tropical forests can be used in a sustainable manner to provide economic benefits to the people of Guyana and the world in general through research, training and the development and dissemination of technologies according to the Iwokrama Act. IIC is therefore presented with a good opportunity to be the first to show how traditional knowledge and ethnomedicine can be used effectively to select and commercialise new bioactive compounds into useful products or pharmaceuticals in the post CBD era. These points are even more relevant given the crisis being experienced now from resistant bacterial infections which completely changes the societal dynamics regarding bioprospecting for novel antibiotics.

Bioproduct Selection & Development By the turn of the twenty-first century most pharmaceutical companies either considerably scaled down or eliminated their investment in bioprospecting as a means of identifying new lead compounds (Conniff 2012; Harvey and Gericke 2011; Firn 2003). Developing countries with untapped pristine biodiversity resources with a high potential of new bioactive products are unable to access and utilize these resources owing to the lack of financial and technological capability to do so, thereby making bioprospecting almost nonexistent. Such biodiversity rich countries are therefore confronted with the dilemma to either 'exploit' their biological resources for short term profit or ascribe a monetary value to their biodiversity for its sustainable use and profit, an issue about which consensus is divided (Castree 2003; Lesser and Krattiger 2007; Harvey and Gericke 2011). However, the steady rise of antibiotic resistance infections caused by multi drug resistant microbial pathogens since the discovery of penicillin and the first recorded and recent emergence of a colistin-resistant strain of *E. coli* carrying the colistin-resistance gene, mcr-1 in the United States of America (McGann et al. 2016), has engaged worldwide attention for new antibiotic leads in recent times

even more than before (Lewis 1995, ECDC/EMEA Joint Technical Report 2009; Overbye and Barrett 2005; Braine 2011; Garner and Brown 2015). Natural products, an underexploited source of potentially novel leads with new modes of action, may be discovered through bioprospecting initiatives (Koehn 2008a, 2008b; Li and Vederas 2009; Nobili et al. 2009; Xiong et al. 2015). There has never been a better time to reconsider the work undertaken in this Iwokrama Bioprospecting project, and to develop it further with the lessons learned given the urgency and the highly increased awareness of the problems of multi drug resistant bacteria.

Should IIC decide to further develop the 110 lead fungal extracts in future from the present project, the repatriation of the 100 viable fungal strains held in long term storage at CABI from which the extracts were obtained would be required, since these fungal strains held at the lab in Guyana may no longer be viable. However, the Guyana strains require checking for viability as soon as possible as indeed do the CABI strains. A diverse collection of products should be carefully selected to study to enable IIC to improve the probability of developing a useful product to market. The selection of products can usefully be divided into two categories:

Category 1; Essential oils, resins and waxes, nutraceuticals, teas, health and so called "well-being" products, cosmetics, herbal or food supplements, and repellants requiring a short time for product development; and

Category 2; Bio-pesticides, food ingredients, botanical medicines and bioactive lead components for pharmaceuticals and which require a longer time to develop.

In making this distinction, the category 1 products that do not need the same strict regulatory testing as category 2 products and could be commercialized quickly and provide immediate financial support to IIC. Whereas the category 2 products could be commercialized over a longer period funded by resources obtained from the category 1 products.

8.5 Iwokrama Post 2003

By the time Iwokrama's initial bioprospecting project finished in March 2003, the project provided IIC with a solid start. Experience, technical knowledge, laboratory facilities, local, regional and international collaborators, skilled local staff and sufficient lead extracts were also obtained. These deserve further development and possible commercialisation by a collaborator with adequate funds to advance its programme to the next stage. Unfortunately, bioprospecting activities at IIC also ended in March 2003, since new donors or private sector funding was not procured. Iwokrama was unable to capitalize on the momentum generated by the initial project owing to financial constraints.

All staff hired under the CSUBIF project left Iwokrama in search of alternative employment. The bioinventory aseptic lab and the bioprospecting lab established at the University of Guyana was formally handed over to the University shortly after the project terminated through the establishment of a MOU between IIC and UG. The UG was given custodial responsibility of the facility with the understanding

that IIC will resume bioprospecting activities, should funding become available. Completion dates for the two remaining and active major donor funded programmes (ITTO, December 2003 and CIDA, March 2004) was imminent. IIC was at the crossroads of being an organization that was primarily funded by external donors through its various programmes, to one that was expected to be self-financing. Unable to realize the goal of becoming financially self-reliant by the end of its donor funded programmes, activities at the IIC was reduced considerably over the next 5 years and IIC made a radical shift in focus to short term commercial projects such as reduced impact and sustainable logging.

An acting Director General was reserved to oversee a short transition period before a new Director General was appointed in January 2005 to manage the IIC for a 3 year term where major emphasis was placed on sustainable income generating activities and business development through the sustainable use of forest resources. Following the resignation of the Director General in October 2007, IIC appointed the CEO, Mr. Dane Gobin shortly thereafter to manage its operations. Between 2005 and 2014, IIC was actively engaged in nine large projects that complemented its research goals and among these were sustainable timber operations. Phase I logging activities in the Iwokrama forest commenced in 2008 and continued until 2012, to give sufficient time to prepare for phase 2 logging activities to commence from the third quarter of 2015 following finalization of a contractual agreement in 2014.

Three main research themes exemplify the work currently being carried out by IIC: (i) Environmental Resilience, (ii) Human, Social and Cultural Capital and (iii) Ecosystem Service Values. These three themes were carefully selected to establish a harmonious partnership among (a) sixteen local communities within and bordering the Iwokrama Forest, (b) scientists, and (c) sustainably managed business operations to reinforce Iwokrama as a global leader in forestry management. Oversight for onsite research themes is the responsibility of the Iwokrama Science Committee established by IIC in 2009 in accordance with Article 14 of the Iwokrama Act 1996. The committee includes representatives from (a) Newcastle University, UK, (b) UG and (c) UWI, amongst other institutions.

IIC is currently managed by its CEO and staff in Georgetown and at the Iwokrama River Lodge and Research Centre with support and strategic policy guidance of IIC's International Board of Trustees. Financial support is provided by the Government of Guyana, the Commonwealth Secretariat, the Commonwealth Foundation and the Commonwealth Forestry Association. The three broad research themes are currently achieved through seven work programmes: (i) community development, (ii) conservation and monitoring, (iii) science and research, (iv) sustainable timber operations, (v) eco-tourism, (vi) learning services and (vii) information and communication.

The Learning Services programme has only recently become a promising area of revenue generation and the sustainable timber operations programme is only expected to become a significant contributor to revenue earnings when phase 2 operations commences in the latter half of 2015, although ecotourism continues to be a profitable activity for the Centre. IIC is likely to continue to experience major funding challenges in the short term to meet its core annual costs owing to current

international financial volatility and reduced donor subsidies. In light of this reality, IIC issued a Request for Proposals representing a wide range of scientific research from private and public companies, institutions and universities, donors as well as other organisations engaged in tropical forest conservation and research in 2014, in order to initiate and catalyse the revenue generation potential of IIC's Research and Science Programme. IIC is seeking scientific research proposals on sustainable forest management, climate change, hydrology/geochemistry interactions, ecosystems services, community impacts, biodiversity and *bioprospecting*. Proposals are also being actively sought from pharmaceutical companies interested in developing products from medicinal plants with the anticipation of long term income generation for the Centre. At present IIC is collaborating with UG and other institutions to develop another bioprospecting project building on the work that was done in the earlier project. This is crucial, amongst other things, in light of the current crisis caused by the lack of novel antibiotic leads and shortage of compounds to control multi drug resistant pathogenic bacteria.

8.6 Conclusions

In the current author's opinion, IIC's bioprospecting-bioinventory project was over optimistic and ambitious in its design considering the (a) capricious and risky nature of bioprospecting, (b) large investment in time and development costs required to successfully commercialize a product and (c) legal and ABS issues involved in commercializing a product developed from the Iwokrama Forest. The over optimism in the initial design of the project can be attributable to several reasons: (a) it was one of the few low impact and sustainable projects with the potential of earning significant financial rewards for the Centre at a time when IIC was expected to become financially self-sufficient and less dependent on donor funding, (b) IIC regarded this as a rare opportunity to initiate its bioprospecting programme and to achieve a great deal within the limited timeframe and resources allocated, given the shortage of donors and private sector organizations willing to fund bioprospecting, (c) the lack of interest worldwide by large pharmaceutical companies in bioprospecting as a viable means of identifying new bioactive lead compounds, and (d) an inadvertent response to conform to donor funding requirements and expectations in an effort to secure limited but vital funding for the project. Iwokrama's bioprospecting project almost met 50 % of the original project objectives, and resulted in the identification of more than 25 host plant extract leads and 110 fungal extract leads worthy of further development. The objectives on commercialisation of bioactive compounds and the establishment of pilot ventures to generate revenue for IIC were near impossible from the beginning in the absence of any IPR and ABS protocols prior to 2003.

The combined performance of Iwokrama's bioinventory and bioprospecting projects is a much better picture though, having achieved five out of the seven objectives of the project with an overall success rate of 70 %. Linking the bioinventory activities with the bioprospecting project was therefore a prudent decision that

enabled both components to work in unison and achieve better success overall. Nevertheless, the bioprospecting project may have achieved more, with greater efforts placed on identification, purification and bioactivity of compounds of interest, if the time and financial support for the project was focused less on bioinventory aspects. This was a matter of judgment: time and resources would still have to be committed to the selection, collection and identification of plants and fungi. In any case, the project still would not have been able to lease extracts, commercialize bioactive compounds and establish pilot ventures as explained before and the bioprospecting project would have only been able to achieve a 50 % success rate at best. Further, if the project was simply centered on bioprospecting, the EC and other donors may have been more reluctant to fund such a high risk project and it may not have materialized in the first place. It is a matter of obtaining the correct balance.

The bioprospecting project, after only 33 months of research, performed admirably in comparison to other bioprospecting initiatives. However, compared to the International Cooperative Biodiversity Group (ICBG) projects in Suriname (1993), and Madagascar and Panama (1998), and the much publicized INBio of Costa Rica project, more could have been done. Technical backstopping, support and extract analyses provided by the consortium of collaborators was essential to the success of the Iwokrama programme overall.

In its future bioprospecting initiatives, Iwokrama should aim to achieve the following:

- secure funding of at least 10 years for future bioprospecting projects to give enough time to transform genetic resources into potentially marketable products
- procure funding from a variety of multiple financial bodies such as national, regional and international donors, private sector and academic institutions and other groups engaged in bioprospecting. Iwokrama could use private sector funding principally to advance natural product research, and the development and commercialisation of useful bioproducts. Funding from donor agencies should be reserved for short term aspects of the project such as capacity building, outfitting the national bioprospecting lab at UG with basic analytical instruments, and maintenance of laboratory infrastructure
- work with either the previous consortium of partners or UG together with new specialist service providers such as regional and/or international academic institutions, industrial groups, and biotechnology and small pharmaceutical companies, capable of conducting modern natural products research and development, as well as business and marketing companies to market products
- re-fit the bioprospecting lab at UG with basic analytical instruments to assist with preliminary extraction, isolation, purification and analysis of bioactive compounds
- establish a set of routine bioassays and long term storage facilities for fungal strains, flora and fauna specimens at the bioprospecting lab at UG; and undertake the repatriation of the 100 viable fungal strains held in long term storage at CABI

- upgrade the reference herbarium at the Iwokrama Field station that will serve as a reference collection of all biological specimens collected from the Iwokrama forest
- ensure that from the establishment of any new bioprospecting project, there is continued strong participatory involvement of stakeholders of those Amerindian communities with legal rights to the Iwokrama forest as has been practicing from inception. Ensure that traditional knowledge is employed in the selection of flora and fauna, in accordance with the Amerindian Act 2005, Iwokrama Act 1996, Iwokrama's Policy on IPR, Access to Genetic Resources and BS. Guyana's National Policy on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization, 2007 should be adhered to.
- identify and conduct research on a diverse array of biological resources ranging from raw and semi purified natural products such as exudates, essential oils, and herbal food supplements to biopesticides.
- in particular, emphasis needs placing on novel pharmaceuticals with emphasis on antibiotic lead compounds to tackle multi drug resistant bacteria in light of the increase in antibiotic resistant infections worldwide.

Finally, IIC should actively seek out and engage the attention of national, regional and international donors, natural products researchers and private sector companies to re-establish its unique programme on bioprospecting that involves an integrated and strong partnership among Amerindian stakeholders, scientists and businesses to realize lasting ecological, social and economic benefits to the peoples of Guyana and the world. Iwokrama is therefore poised to be the first to demonstrate to the world how traditional knowledge and ethnomedicine can be used effectively to select and successfully transform new natural products from the Iwokrama forest into marketable products or pharmaceuticals.

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References

Arnold AE, Maynard Z, Gilbert GS (2001) Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity. Mycol Res 105(12):1502–1507

Baines G, Warner R (2000) Guyana 1999 – Iwokrama Rain Forest Programme – Project evaluation report GEF ASSISTANCE TO THE IWOKRAMA RAIN FOREST PROGRAMME Project Number GUY/92/G31/A/1G/99 Draft Report of the Final Project Evaluation Mission Final Project Evaluation, GUY/92/G31/A/1G/99. https://www.academia.edu/2085038/Guyana_1999_-_Iwokrama_Rain_Forest_Programme_-Project_evaluation_report. Accessed 26 June 2015

Bergstrom JD, Dufresne C, Bills GF, Nallin-Omstead M, Byrne K (1995) Discovery, biosynthesis and mechanism of action of the zaragozic acids: potent inhibitors of squalene synthase. Annu Rev Microbiol 49:607–639

Bhatnagar I, Kim SK (2010) Immense essence of excellence: marine microbial bioactive compounds. Mar Drugs 8(10):2673–2701

Blackwell M (2011) The fungi: 1, 2, 3... 5.1 million species? Am J Bot 98(3):426–438

Braine T, (2011) Race against time to develop new antibiotics. In: Bulletin of the World Health Organization 89:88-89 doi:10.2471/BLT11.030211.http://www.who.int/bulletin/volumes/89/2/11-030211/en/. Accessed 11 July 2015

Butler MS (2004) The role of natural product chemistry in drug discovery. J Nat Prod 67(12):2141–2153

Butler MS (2008) Natural products to drugs: natural product-derived compounds in clinical trials. Nat Prod Rep 25(3):475–516. doi:10.1039/b514294f

Butler MS, Robertson AAB, Cooper MA (2014) Natural product and natural product derived drugs in clinical trials. Nat Prod Rep 31(11):1612–1661

Cannon PF (1996) Filamentous fungi. In: Hall GS (ed) Methods for the examination of organismal diversity in soils and sediments. CABI, Wallingford, pp. 125–143

Cannon PF, Hawksworth DL (1995) The diversity of fungi associated with vascular plants: the known, the unknown and the need to bridge the knowledge gap. In: Andrews JH, Tommerup IC (eds) Advances in plant pathology, vol 11. Academic Press, London, pp. 277–302

Cannon PF, Reid A, Simmons CM (2002) Diversity and host preference of leaf endophytic fungi in the Iwokrama forest reserve, Guyana. Mycologia 94(2):210–220

Castree N (2003) Bioprospecting: from theory to practice (and back again). Trans Inst Br Geogr 28(1):35–55. doi:10.1111/1475-5661.00076

Chin Y-W, Balunas MJ, Chai HB, Kinghorn AD (2006) Drug discovery from natural sources. AAPS J 8(2):E239–E253

Clarke HD, Funk VA, Hollowell T (2001) Using checklists and collections data to investigate plant diversity I: a comparative checklist of the plant diversity of the Iwokrama forest, Guyana. In: BL Lipscomb (ed) SIDA, Botanical Miscellany, no 21 Botanical Research Institute of Texas, Forth Worth, pp 1–86

Clay K (1988) Clavicipitaceous fungal endophytes of grasses: coevolution and the change from parasitism to mutualism. In: Pirozynski KA, Hawksworth DL (eds) Coevolution of fungi with plants and animals. Academic Press, London, pp. 79–105

Clay K (1990) Fungal endophytes of grasses. Annu Rev Ecol Syst 21:275–297

Conniff R (2012) A bitter pill. Conservation 13(1):18–23

Cordell GA, Colvard MD (2005) Some thoughts on the future of ethnopharmacology. J Ethnopharmacol 100(1-2):5–14

Domsch KH, Gams W, Anderson T-H (1993) Compendium of soil fungi, 2nd edn, 2 vols. Academic Press, London

Dreyfuss MM, Chapela IH (1994) Potential of fungi in the discovery of novel, low-molecular weight pharmaceuticals. In: Gullo VP (ed) Discovery of natural products with therapeutic potential. Butterworth-Heinemann, Boston, pp. 49–80

Durgante FM, Higuchi N, Almeida A, Vicentini A (2013) Species spectral signature: discriminating closely related plant species in the Amazon with near-infrared leaf-spectroscopy. For Ecol Manag 291:240–248

Engstrom MD, Lim BK, Reid FA (1999) Guide to the mammals of Iwokrama. http://www.iwokrama.org/mammals/. Accessed 26 June 2015.

European Centre for Disease Prevention and Control (2009) ECDC/EMEA joint technical report the bacterial challenge: time to react. A call to narrow the gap between multidrug-resistant bacteria in the EU and the development of new antibacterial agents. http://ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf. Accessed 14 Dec 2015

Firn RD (2003) Bioprospecting – why is it so unrewarding? Biodivers Conserv 12(2):207–216

Gámez R (2007) The link between biodiversity and sustainable development: lessons from INBio's bioprospecting program in Costa Rica. In: McManis CR (ed) Biodiversity and the law: intellectual property, biotechnology and traditional knowledge. Earthscan, London, pp. 77–90
Garner C, Brown D (2015) Spotlight on antibiotic research UK. Biochemist 37(3):36–37
Giddings L-A, Newman DJ (2013) Microbial natural products: molecular blueprints for antitumor drugs. J Ind Microbiol Biotechnol 40(11):1181–1210
Gilmour D (1999) Rainforest pioneer. Millennium trailblazers 3: David Cassell. People & the Planet/IPPF, UNFPA, IUCN 8(4):20
Guérin-McManus M, Famolare L, Bowles IA, Malone SAJ, Mittermeier R, and Rosenfeld AB (2011) Bioprospecting in practice: a case study of the suriname ICBG project and benefits sharing under the convention on biological diversity. Montreal: Report for Convention on Biological Diversity, 11 2001
Gutierrez RMP, Gonzalez AMN, Ramirez AM (2012) Compounds derived from endophytes: a review of phytochemistry and pharmacology. Curr Med Chem 19(18):2992–3030
Guzman-Trampe S, Rodriguez-Peña K, Espinosa-Gómez A, Sánchez-Fernández RE, Macias-Rubalcava ML, Flores-Cotera LB, Sánchez S (2015) Endophytes as a potential source of new antibiotics. In: Sanchez S, Demain AL (eds) Antibiotics: current innovations and future trends. Caister Academic Press, Norfolk, pp. 175–204
Hammond P (1992) Species inventory. In: Groombridge B (ed) Global biodiversity: status of the earth's living resources. Chapman and Hall, London, pp. 17–39
Harvey AL, Gericke N (2011) Bioprospecting: creating a value for biodiversity. In: Pavlinov IY (ed) Research in biodiversity – models and applications. Available via INTECH. http://www.intechopen.com/books/research-in-biodiversity-models-and-applications/bioprospecting-creating-a-value-for-biodiversity. Accessed 20 Dec 2015
Hawksworth DL (1991) The fungal component of biodiversity: magnitude, significance and conservation. Mycol Res 95(6):641–655
Hawksworth DL (2012) Global species numbers of fungi: are tropical studies and molecular approaches contributing to a more robust estimate? Biodivers Conserv 21(9):2425–2433
Insanally R (ed) (2003) The cropper foundation report on the conference "Development as if Equity Mattered". Georgetown Guyana September 24-26, 2001, Port of Spain, Trinidad and Tobago
Iwokrama International Centre for Rain Forest Conservation and Development (2015) News Archives. http://iwokrama.org/2014/07/iwokrama-issues-request-for-proposals-for-its-science-and-research-programme/. Accessed 16 June 2015
Kelley J, Cannon P, Simmonds M, Pingal R and Simmons C (2003) The Iwokrama programme: an approach to the sustainable exploitation of genetic resources. In: Biological resource centres and the use of microbes. Lima N, Smith D (eds) XXII ECCO Meeting Proceedings Book 185–195
Kingston DGI (2011) Modern natural products drug discovery and its relevance to biodiversity conservation. J Nat Prod 74(3):496–511
Koehn FE (2008a) High impact technologies for natural products screening. In: Petersen F, Amstutz R (eds) Natural compounds as drugs volume I, Progress in drug research, vol 65. Birkhäuser, Basel, pp. 175–210
Koehn FE (2008b) New strategies and methods in the discovery of natural product anti-infective agents: the mannopeptimycins. J Med Chem 51(9):2613–2617
Lesser WH, A Krattiger (2007) Valuation of bioprospecting samples: approaches, calculations, and implications for policy-makers. In: (Krattiger A, Mahoney RT, Nelsen L, et al.) Intellectual Property Management in Health and Agricultural Innovation: A Handbook of Best Practices, vols 1 and 2 Chap 9.4, pp. 861-876 MIHR: Oxford, U.K., and PIPRA: Davis, U.S.A. (accessed December 5 2015) Available online at www.ipHandbook.org
Lewis R (1995) The rise of antibiotic-resistant infections. FDA Consum 29(7):11–15
Li JW-H, Vederas JC (2009) Drug discovery and natural products: end of an era or an endless frontier? Science 325(5937):161–165

Lim BK, Engstrom MD (2001a) Species diversity of bats (Mammalia: Chiroptera) in Iwokrama forest, Guyana, and the Guianan subregion: implications for conservation. Biodivers Conserv 10(4):613–657

Lim BK, Engstrom MD (2001b) Bat community structure at Iwokrama forest, Guyana. J Trop Ecol 17(5):647–665. doi:10.1017/S0266467401001481

Lim BK, Engstrom MD (2005) Mammals of Iwokrama forest. Proceedings of the Academy of Natural Sciences of Philadelphia 154(1):71-108doi: http://dx.doi.org/10.1635/0097-3157(2004)154[0071:MOIF]2.0.CO;2

Lu G, Cannon PF, Reid A, Simmons CM (2004) Diversity and molecular relationships of endophytic Colletotrichum isolates from the Iwokrama forest reserve, Guyana. Mycol Res 108(1):53–63

Lv Y-L, Zhang F-S, Chen J, Cui J-L, Xing Y-M, Li X-D, Guo S-X (2010) Diversity and antimicrobial activity of endophytic fungi associated with the alpine plant Saussurea involucrata. Biol Pharm Bull 33(8):1300–1306

Márqueza SS, Bills GF, Herrero N, Zabalgogeazcoaa Í (2012) Non-systemic fungal endophytes of grasses. Fungal Ecol 5(3):289–297

McGann P, Snesrud E, Maybank R, Corey B, Ong AC, Clifford R, Hinkle M, Whitman T, Lesho E, Schaecher KE (2016) Escherichia coli Harboring mcr-1 and blaCTX-M on a novel IncF plasmid: fFirst report of mcr-1 in the USA. Antimicrob Agents Chemother. doi:10.1128/AAC.01103-16

Monette M, Simpson A, Masoom H, Liaghati Y, Mobarhan Courtier-Murias D, Fortier-McGill B, Soong R, KL Colson (2015) Characterization of environmental samples in their natural state using comprehensive multiphase NMR. In: 2015 Annual Meeting of the ASP, July 25th–29th, 2015, Copper Mountain, CO, USA

Mousa WK, Raizada MN (2013) The diversity of anti-microbial secondary metabolites produced by fungal endophytes: an interdisciplinary perspective. Front Microbiol 4:65

Newman DJ, Cragg GM (2007) Natural products as sources of new drugs over the last 25 years. J Nat Prod 70(3):461–477

Nobili S, Lippi D, Witort E, Donnini M, Bausi L, Mini E, Capaccioli S (2009) Natural compounds for cancer treatment and prevention. Pharmacol Res 59(6):365–378

Nisa H, Kamili AN, Nawchoo IA, Shafi S, Shameem N, Bandh SA (2015) Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: a review. Microb Pathog 82:50–59

O'Brien HE, Parrent JL, Jackson JA, Moncalvo J-M, Vilgalys R (2005) Fungal community analysis by large-scale sequencing of environmental samples. Appl Environ Microbiol 71(9):5544–5550

Omacini M, Chaneton EJ, Ghersa CM, Muller CB (2001) Symbiotic fungal endophytes control insect host-parasite interaction webs. Nature 409(6816):78–81

Overbye KM, Barrett JF (2005) Antibiotics: where did we go wrong? Drug Discov Today 10(1):45–52

Pingal R (2001) From tropical rain forest to pharmaceuticals and other bioactive compounds: the process of discovery. Paper presented at Iwokrama Brown Bag Lunch Seminar, Iwokrama Centre, Bel Air, Georgetown, Guyana 14 February 2001

Pingal R (2002a) Challenges in using biodiversity. Paper presented at the international workshop on national biodiversity systems: building inter-generational capabilities for conservation and sustainable use, Georgetown, Guyana, July 2002

Pingal R (2002b) Iwokrama's bodiversity prospecting programme. Paper presented at the 5th International inter-Guianas conference on the development of the Guianas: building capacity to meet the emerging challenges in the Guianas, University of Guyana, Turkeyen, Guyana, 22-25 October 2002

Pingal R (2002c) Physical and chemical properties of crabwood oil: requirements for export. Paper presented at the international workshop on critical issues in the market and sustainable and equitable use of crabwood oil in Guyana, Lake Mainstay Resort, Essequibo, Guyana, 23-24 November 2002

Pingal R, McTurk M (2002) Biodiversity prospecting in the Iwokrama forest. Paper presented at the Friends of Iwokrama Public Seminar, Iwokrama Centre, Bel Air, Georgetown, Guyana, 7 June 2002

Pingal R, Simmons C, Cannon P, Paterson R, Simmonds M, Kokubun T, Mootoo B, Maxwell A and Jacobs H (2001) Iwokrama's bioprospecting programme: Paper presented at the Caribbean Academy of Sciences 12th General Meeting and Conference, Georgetown, Guyana 4-9 June 2001

Pingal R, Simmons C, Cannon P, Paterson R, Simmonds M, Kokubun T, Mootoo B, Maxwell A and Jacobs H (2002) Bioprospecting for bioactive metabolites from endophytic fungi and plants of the Iwokrama forest. Paper presented at the 19th Mona Symposium on Natural Products and Medicinal Chemistry, the University of the West Indies, Mona, Jamaica, 7-10 January 2002

Rossman AY (1994) A strategy for an all-taxa inventory of fungal biodiversity. In: Peng C-I, Chou CH (eds) Biodiversity and terrestrial ecosystems, *a*cademia sinica monograph series no 14, Institute of Botany, Tapiei, p 1691-1694

Rossman AY, Tulloss RE, O'Dell TE, Thorn RG (1998) Protocols for an all taxa biodiversity inventory of fungi in a Costa Rican conservation area. Parkway, Boone

Saikkonen K, Gundel PE, Helander M (2013) Chemical ecology mediated by fungal endophytes in grasses. J Chem Ecol 39(7):962–968

Saurí J, Chan STS, Buevich V, Gustafson KR, Williamson T, Martin GE (2015) Structure elucidation of a proton-deficient natural product using LR-HSQMBC supported by DFT calculations. Paper presented at the ASP 2015 annual meeting, Copper Mountain, Colorado 25-29 July 2015

Shrivastava G, Ownley BH, Auge RM, Toler H, Dee M, Vu AK, Tobias G, Chen F (2015) Colonization by arbuscular mycorrhizal and endophytic fungi enhanced terpene production in tomato plants and their defense against a herbivorous insect. Symbiosis 65(2):65–74

Simmons C (2002a) Iwokrama's bioinventory/bioprospecting programme. Paper presented at the 5th international inter-Guianas conference on the development of the Guianas: building capacity to meet the emerging challenges in the Guianas, University of Guyana, Turkeyen, Guyana, 22-25 October 2002

Simmons C (2002b) Iwokrama – efforts towards the conservation and sustainable utilization of the Iwokrama forest. Paper presented at the Caribbean Herbs Business Forum, Montego Bay, Jamaica, 2 – 5 December 2002

Simmons C (2002c) Medicinal plants of the Iwokrama reserve. Paper presented at the Natural Products Institute University of the West Indies, Mona Campus 6 – 7 December 2002

Simmons C, Cannon PF (2002) Diversity and host preference of leaf endophytic fungi in the Iwokrama forest reserve, Guyana. Paper presented at 7th International Mycological Congress, University of Oslo, Norway, 11 – 17 August 2002

Simons L, Bultman TL, Sullivan TJ (2008) Effects of methyl jasmonate and an endophytic fungus on plant resistance to insect herbivores. J Chem Ecol 34(12):1511–1517

Smith PG, Kerry SM (1996) The Iwokrama rain forest programme for sustainable development: how much of Guyana's bat (Chiroptera) diversity does it encompass? Biodivers Conserv 5(7):921–942

Sneader W (1996) Drug prototypes and their exploitation. Wiley, Chichester 788 pp

von Arx JA (1980) The genera of fungi sporulating in pure culture, 3rd edn. Lubrecht & Cramer, Vaduz

Xiong M, Guo Z, Han B, Chen M (2015) Combating multidrug resistance in bacterial infection by targeting functional proteome with natural products. Nat Prod Res 29(17):1624–1629

Zhao J, Shan T, Mou Y, Zhou L (2011) Plant-derived bioactive compounds produced by endophytic fungi. Mini-Rev Med Chem 11(2):159–168