# TRANSACTIONS
## of the
# NATIONAL ACADEMY OF SCIENCE AND TECHNOLOGY
## Republic of the Philippines

1989
Volume XI

Published by
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Bicutan, Taguig, Metro Manila
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*Trans. Nat. Acad. Sci. & Tech. (Phils.)*
*1989.11:1-3*

# WELCOME ADDRESS[1]

Dioscoro L. Umali
*President*
*National Academy of Science and Technology*

Kagalang-galang na Ceferino Follosco, Kalihim ng Kagawaran ng Agham at Teknolohiya ng Pilipinas

Excellencies . . .

Mga dalubhasa ng Agham . . .

Kapwa ko Academician . . .

Mga kasama at kaibigan

Lahat kayo ay magiliw at malugod kong tinatanggap sa taunang pulong ng Akademia ng Agham at Teknolohiya.

Malabis kaming nagpapasalamat sa inyong pagdalo.

Isang araw napag-usapan ng mga kaklase ng anak kong bunso tungkol sa propesyon ng kanilang mga magulang. Ang sabi ng anak ko, *"Sayang ang aking ama. Nabibilang siya sa sayang na mga tao. Siya ay SA YANGTIST."*

Kung talagang tutuusin, ang sayang ay ang ating Bansa. Sagana ang ating bansa sa likas na kayamanan. Mayaman ang ating lupain at sagana sa ulan at araw; dati'y sagana rin ang ating mga gubat at sagana ang ating mga ilog, lawa at dagat. Ngunit bakit 69 na porciento ng ating mamamayan ang naghihirap at nagugutom? Of this number 80 percent are from the rural areas.

They suffer from the lack of food and shelter, poor health, and inadequate education. The problems of the urban poor: the slum dwellers, squatters, and scavengers of Smoky Mountain is just an extension of rural poverty.

Poverty has less tangible but equally destructive features. The poor are isolated as they are often remote from center of power and government. They are neglected. They are not organized so they lack power. Often they are manipulated. As a consequence, their access to land, water, technology, and other productive support services is at best tenuous. They are the first to suffer from natural calamities, from social unrest or political upheaval. As we all know, the root of insurgency is poverty.

To a large measure, this deplorable situation is mainly due to our failure to fully use the magic tools of science. Our present means to produce food, cloth-

[1] Remarks of D. L. Umali during the Opening Session of the *Annual Scientific Meeting of the National Academy of Science and Technology* at the Hotel Nikko Manila Garden on July 12, 1989.

ing, building materials, medicine and other essential products of life are inadequate to meet the demand of our increasing population.

History has shown that science and technology have played a most impressive role in the promotion of human welfare and in the progress of civilization.

The Philippines has lagged behind in harnessing modern technology to improve the quality of life and the purchasing power of our people for various reasons. It is very encouraging that the DOST under the leadership of Secretary Follosco, has adopted an action plan that emphasizes the importance of spreading the benefits of modern technology to our people.

Today, integrating traditional and advanced technologies open vast opportunities for our country to improve the economic competitiveness and consumer acceptance of the products of small and village industries and alieviate poverty in the rural areas.

Traditional technologies evolved over the years from the crucible of economic survival. They also form a part of our people's cultural pattern. Traditional technologies are a way of life and a source of pride not just a means of livelihood.

Traditional technologies are used because they require less energy and lower cost inputs; are environmentally compatible; can be managed easily; and are well-adapted to subsistence conditions.

However, traditional agriculture is often characterized by low productivity; drudgery; incompatibility with modern processing and consumer needs; use of less productive crop varieties and breeds of animals, and to field and storage losses.

Although advanced technologies have not been widely adopted, they have some obvious advantages. Among these are high productivity; efficient and diversified labor use; and permanent systems for sustained high yields. Their main disadvantages are high capital and input demand; specialization; vulnerability, and high mechanization which may lead to environmental damage and unemployment.

The challenge confronting us is to seek possible ways to achieve a beneficial blend of emerging and traditional technologies for development.

Emerging technological advances can provide new prospects to accelerate the development process. However, new technologies replacing older technologies can create hardship, particularly for unskilled labor and illiterate women, and for others who depend on traditional crafts for their livelihood.

The real challenge then to us is to find appropriate ways to integrate and apply traditional and emerging technologies, combining their strengths while minimizing their weaknesses.

Therefore, the criteria for adopting new technologies should include not only those that are normally referred to as economic viability: income- and employment-generating capacity, ecological soundness, etc. It must be imbued with a social philosophy that new technology must not have a built-in seeds of social discrimination. Modern technology must help improve the quality of life of the poor. The integration strategy should be based on accelerated economic growth

through modernization of the agricultural, industrial, and service sectors of the economy; coupled with self-reliance and social justice.

New technologies must recognize women as equal partners in development and provide opportunities for them to be such whether within or outside the context of formal education.

Unschooled or traditional rural populations should be regarded as potential innovators rather than passive receptors of new technologies. The enhancement of indigenous creativity and human skills must be encouraged with actual or real incentives.

Community participation in the innovative process must be recognized as a key element in the successful utilization of new technologies. Appropriate social and/or political or cultural mechanisms should be developed to make this participation a reality.

Opportunities to use indigenous resources must be maximized to help pave the way toward the realization of production by the masses, and less mass production with little or no regard for effective labor utilization. Economic development should aim at labor diversification and generation, not labor displacement.

Support mechanisms like credit, storage, marketing, insurance, etc., must be established to facilitate the adoption of new or emerging technologies. They must be communicated effectively and reflect prevailing personal and social values and norms or mores (including superstitions).

Technology must be continually updated and improved to meet the changing needs of society and the challenges of nature.

To those of us who have been given the gift of scientific training, alleviating poverty is the challenge to our technical, economic, and social ingenuity.

*Trans. Nat. Acad. Sci. & Tech. (Phils.)*
*1989.11:5-6*

# INTRODUCTION OF GUEST SPEAKER

Dioscoro L. Umali
*National Scientist and President*
*National Academy of Science and Technology*

Bago ko ipakilala si Secretary Follosco gusto kong batiin ang ating mga panauhin na nagbigay dangal sa ating sesyon. Natutuwa kami at nandidito si Undersecretary Lantican ng DOST. Asst. Secretary Ancog, si Asst. Secretary Tansinsin, Ginoong Mijares, Ginoong Orillo at ang ating mga national scientists, at si Ginoong Santos at Lagmay.

Our guest speaker today who is simple, very energetic and dynamic was born in Malabang, Lanao del Sur. His motto is for excellence. His outstanding scholar, his being always in number one. He finished his elementary course in 5 years, can you imagine that, as valedictorian; his high school also as valedictorian. He completed his Bachelor of Science in Mechanical Engineering as Magna Cum Laude and his B.S. in Electrical Engineering as Summa Cum Laude. These records have been surpassed only by his unbroken record of completing a master of science degree in agricultural engineering as Fulbright Smithmoon Scholar in only eight months with a straight A. He topped the professional mechanical engineer's board exam in 1984 for his honors while being very active student leader as President of school organizations and editor of school paper.

He was awarded most outstanding professional mechanical engineer by the Professional Regulation Commission and as most outstanding agricultural engineer by the Philippine Society of Agricultural Engineers in 1981. Before joining in July 1986 in six agro-industrial firms he was actively involved with the Philippine Chamber of Commerce and Industry as Vice-President. On his international activities he has been a Consultant and expert of United Nations Agencies such as UNIDO, ESCAP and so forth. In government, he was first appointed Undersecretary of Department of Trade and Industry with responsibility for regional operations, domestic trade and small business and product standards.

In recognition of his leadership and performance, President Aquino designated him to be the Secretary of the Department of Science and Technology (DOST) effective April 7, 1989 with instructions to accelerate the development of science and technology in the Philippines so as to enable us to attain an NIC status by the year 2000.

As a prolific writer, he has written more than 100 technical papers on productivity, science and technology, and industrialization. Leading a very active life and believing in effective management and full utilization of time, he is the President

of the Philippine Foundation for Science and Technology Incorporated, Philippine Society of Mechanical Engineers, Philippine Society of Agricultural Engineers, Association of Management to Industrial Engineers of the Philippines and many others.

As a person, he is what we call the magic of leadership. As you know, so many wars have been lost due to indecision. He has the ability to make things happen on time, never believes in half measures. Although he may appear to top person but his heart is very compassionate and enviewed with the deep social philosophy. He cares for the poor as human beings. He is a hard task master, but he can afford it because he works the hardest in the office, he is the earliest and he leaves the office the latest.

He believes that the basic element for leadership is to set example. On week-end he visits the outlying areas, the barrios and the villages to be with the masses and to learn from them.

Ladies and gentlemen, let me give you a scholar, professional civic leader, former business leader, internationalist, writer, farmer and now government democrat and a dutiful and affectionate husband and father to his children. Siya ay isang maginoong tao. He believes that the President is our national freedom but he double believes that in spirit there are masters, ang mamamayan and he is first accountable to them. Ang sabi nila ang tumpak gawad ganap na scientist, member of KMU, Secretary Follosco.

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*1989.11:7-13*

# KEYNOTE ADDRESS

Ceferino L. Follosco
*Secretary*
*Department of Science and Technology*

For a while, I thought Dr. Umali will include in his introduction that I am a shining example of an engineer.

Well, President Umali, past President Campos, Academician Soriano, distinguished national scientists, and academicians. Excellencies of the diplomatic corps from Israel, India and the United Kingdom, fellow crusaders in Science and Technology, friends, ladies, and gentlemen.

I feel quite nervous, when I appear before the so-called national scientists and academicians and even in our MANCOM meetings when I'm with almost all Ph.D's and directors. I did not pursue my Ph.D. although I had a chance because at that time I was already working and married, and so I had to rush home. My chance of taking up the Ph.D. was there but I thought I would be a businessman. I did become a businessman and I said to myself, I probably don't need it anymore.

It's always a great pleasure to interact with scientists who are the Divine Creators' special workers in this youth's laboratory of our earthly existence. They give Glory to God's creation by enriching and preserving them and perhaps immortalizing them for the next brave new world yet to come. More than ever, it is at this point in our history that the National Academy of Science and Technology, plays its vigorous role in nation building. The Academy is the highest scientific recognition body in the country. Scientific, because it conducts activities that are equal in prestige to those in other countries. Our academy is among the world's greatest and excellent body inspired by no less than such venerable institutions as the Royale Society of London, the Academy's counterparts in the U.S., USSR and France and many other countries.

We appreciate very much the NAST's efforts as our active partner in many of our activities including the active participation of your President, Vice-President and many of your members. I also would like to thank them for always taking time to attend in our Management Committee meetings which are held twice a month and in our budget meetings where we make certain presentations to various committees of both Houses of Congress.

We further need the inputs of NAST in making the 15 leading edges yield their utmost productivity, in making them attune with the investment priorities for both local and foreign markets, and in making them synchronize with the 10-year NIC journey into the year 2000.

When the Philippine industrial revolution is programmed to attain its peak and sustain it, the 15 leading edges and the program on emerging technologies provide great vistas yet to be explored by all scientists. The highly-exciting field of bio-

technology had encouraged many to get involved with genetics engineering with the subconscious aim of finally being able to control the destinies of living things. Materials science and engineering have titillated the imagination about substitutions and generations of entirely new raw materials for the industries and laser. The 20th century search light which reveals humans could never be foreseen make our measures is also called the engine of basic measures.

To those whose industrial use run to hundreds and holds vast potentials for transforming matters, I know that the field of high technology can give you more attraction than the other disciplines. High-technology involves long-term research whose results would alter geopolitics. The developing nations of today would take control from the present powers. To the scientist, all this would mean as an honored place in history and material games.

President Umali has already pointed out the need for low technologies and at the same time, developing high technologies. I would always say, let us promote appropriate technology. Since our Ambassador from India is here, I would mention that in 1978 it took 40 scientists just to define what is appropriate technology in a forum we attended in New Delhi. They defined it in just one sentence: "appropriate technology is that technology that considers economic, technical, social and cultural factors including the resource endowments to the country." That's all that they did for one week.

Unfortunately, I was in another workshop and I only caught up with it in the plenary. So, if you compute the performance word for word, the productivity is indeed very poor. I would like to mention to you what Bro. Andrew mentioned yesterday. He said I would like to be with engineers because they make very fast decisions, in one hour the meeting is finished. But he said, with scientists, especially the basic scientists, it takes much longer. He added that with lawyers and politicians, it takes too much time, and so I think we must have the proper balance of both the basic sciences and engineering or technology. They must have the proper mix. People have always regarded scientists as a special class of individuals. Indeed, they are. Despite the fact that life is constantly changing, scientists have managed to retain the fragment of their ivory tower image. In fact, in many of these fora, many retain the fragment of their ivory tower image. Their requests especially those coming from the Sanduguan, – the organization of farmers now launching a blockade when they met with us, said, "please do not bring with you your Ph.D.'s." It is probably because of this image that we must try to correct it; because they are the most learned people that you can find. And so I brought along Ph.D's with me and explained to them that they are the experts in the area they are looking for. Of course, this is 20th century and I know for a fact that scientists especially, in our neighboring NIC's have managed to come down from their ivory towers and have made themselves alert to the economic needs of their countries and of the rest of the world, without losing the essential scientific lifestyle, without yielding to materialism but holding fast their scientific code of honor. The scientist must be able to link with everyone and he should not confine himself to his own

environment alone. He must not only react with other scientists, the basic scientists, the technologists, the sociologists and even the politicians because in our country they are the ones who really lay down the policies for government. The country would like you to perform active roles in the development of the 15 leading edges namely: construction; electronics; instrumentation and controls; metals and engineering; textile industry; mining and minerals; process industry; food and field industry; energy; transportation; information technology; marine fisheries and oceanography; forestry and natural resources; agriculture and aquaculture and the emerging technologies. There are many subsectors under these and the task force has laid down the various areas where we would have comparative advantage. We are looking at the comparative advantages in these areas, and therefore, we would like to get your assistance in trying to make these leading edges really lead our economy. Your expertise and insights are much needed in fleshing out the details as well as the implementation of the major components of our S&T major strategies, namely: to modernize our economic sectors especially agriculture which has been pointed out by your President as very much behind now, and industry which has very low productivity levels, through technology transfer activities from both domestic and foreign technologies. We know that we are very weak. As scientists, we have a lot of research and development results. Unfortunately, many of these remain in the laboratories and in our shelves. We should change our measure of performance from the number of papers and results of R&D to the number of technologies transferred and their impact to the economy.

I know that this is not easy especially for the basic sciences but if we take a look at what we call the bottom line and in the case of the private sector, they look at projects. And to us, the contribution to the country whether in terms of economic health or whatever basic sciences you are in, are important for the development of the country and the people. And for this matter we have gotten one brand new Undersecretary, I think he is not here today but he will concentrate only on technology transfer activities including technology information where before this has not been probably given much attention.

Second, to improve our R&D capability especially adaptive R&D and basic researchers where our comparative advantages exist and there are so many of these, probably it will take time to be able to discuss this in the short time that we have.

Third, is to develop our S&T infrastructure including your own academy, R&D institutions and S&T manpower for both the government and the private sectors. We're very glad that one of your members, Fr. Nebres is going to take the challenges of handling the manpower development programs for the whole science and technology community. Your laboratories have become an extension of the government policy making in negotiating tables. Your science has no choice but to become relevant to the needs of your clientele, your experiments must take into account the nation's economy, politics and the people immediately. We realize that 60% of our people live below the poverty line. When you listen to them, especially

those who eat two square meals a day, you will note the big difference between the area where they live and where you are now.

When you go to the uplands of Porac, Pampanga and uplands of Bukidnon you will observe the very big difference. Then you go to Tondo. I think what is really needed for us people in S&T is to really work much harder in order to lead our country away from massive poverty. When you take care of the country's future scientific needs, you also pay attention to its basic needs: food, clothing, shelter, health care, education and means of livelihood. Being partners in nation building, you can also input towards alternatives so that we can have a better life for our people whether you are in the area of economic, socio-political, cultural or in areas of S&T infrastructure.

A scientist makes our society your specimen. What we can do is to save it, remove its cancers, help it, so it can live to its fullest. Truly, this country demands much more from its scientists. At this point in time, when it dreams of the NIC goal and embraces it to make possible, it has no choice but to demand for all the best that it brings: resources and manpower. I know that you normally would ask, what are the programs that we have in the S&T community? Well, I cannot answer that now. I noted from the program which will start after the breaktime, Fr. Nebres will talk about our 15 leading edges, leading to an NIC status to the year 2000. You will therefore tell us what have you done for the last 3 months that you have already been in the office. You know, I've done a lot of ad lib here because I always tailor my speeches to the audience. I thought that being with the highest science advisory body, I have to try to make a short report card to you. This is my first time to do this as a secretary of DOST. The private sector will make monthly reports of our progress because we are productivity-oriented and I thought that I should make a report to you regularly. Well, for the last 3 months especially the first month it was not really easy because I had to submit myself for confirmation in the Commission of Appointments. You all know that it is not an easy task. It turns your life upside down. I was quite fortunate and probably because of the importance of science and technology on that record. They say it was the fastest confirmation process ever done while after seven (7) days I submitted a thick volume of papers which took me two weeks to prepare including income tax returns and everything. But the committee members took only 30 minutes to deliberate the need and they did not even ask me personal questions. Instead, they were asking me already what was my program for S&T and in the plenary session I was very glad that it took only 20 minutes with Senator Salonga and all the Senators there. Of course, there was an objection and the objection came from a good friend of mine, Senator Pimentel when he said, "I object Mr. Chairman to the appointee. I do not see space around". But you know, I was out in the corner and he wanted me to sit in front, beside him. That was the only objection raised. And so often, you know, when every one raised their hands because the Chairman asked: any objections? And you know I got scared when Sen. Enrile raised his hand. I thought "patay na," opposition! But anyway, he said I did not repeat this because this is the only good

appointment made by the present administration. But that is not correct because all the appointments made especially to the later ones have been quite good. You know I feel nervous meeting with top scientists in here but I've never been nervous in confirmation meetings. We have gone through, and at the same time reviewed the budgets of the 33 groups within the DOST because we have 20 agencies and 13 regions. It was on that 2nd week that we were on a budget review. You know, it was not easy to go into a budget review internally and meeting with various groups outside. Many of them probably who were against DOST are here today. These people told me that the Department of Science and Technology is the department of *suka* and *toyo*. I listened a lot and tried to correct many of these impressions. I have been in a very massive information campaign, talking to various groups – talking to physicists, chemists, computer experts. So let me tell it to you briefly. I know I'm conscious of time and it says here that this session ends at 10:15. I'm sure the organizers, the leaders, are asking me to delay a little so the merienda will be ready. So let me just spend a little more time on this.

Well, first I believe in building a team because a team is very important. We need a team that is not only within the DOST but the whole scientific and technological community. We are concentrating on how to build a team and to demolish whatever conflicts are existing within the DOST. We have succeeded a little, although exactly not 100%. There are still some complaints but they are hoping we will be able to solve them. We've been able to talk to people who have been dismissed, people who have not taken their retirements for 1 1/2 years and all of these have been accelerated.

Second, we have improved on the organizational structure, we have decentralized our authorities to all the officials of the DOST. I was quite shocked when all the papers would be reaching me to sign including casuals, at one point in time, including overtime. We have decentralized the budget to all the regions instead of passing through to my office. We have filled up already the other positions (one Undersec, one Asst. Sec., Asst. Director) I have set my targets that we will complete the vacancies of the department where there exists about eight directors to be filled up, and therefore be filled up. I hope not later than the next 2 months.

Third, we've been able to generate millions of pesos in our savings. In projects that we have redirected there were probably very little inputs/outputs that have been accomplished. We've been able to save our own expenses and we are diverting the savings to more important areas or activities including R&D or grants-in-aid. That is why, we have asked many groups already to submit projects that are relevant to our priority areas. We are also expecting that some of the savings will be used to modernize some of our systems in DOST, like for example, buying a fax machine. I know you are shocked because you don't have one. We are also buying a telex machine. You know, sometimes it's a shame when they tell: "Could you let me know what your fax number is?" Well, I just pretend and answer, "sorry I forgot the number." You know you don't have to admit that you are so backward because they will say you are so backward in your technology. We're trying to put up a

direct dialing system so we don't have to go to the operator. But you know many of these are important and we hope we can have enough water also within our compound. We have talked to the MWSS already; we discovered that there is a 30-inch pipe line along our building complex that's busted. You know, we believe in productivity and we will do a lot of savings all over and we will spend it where it will count.

Fourth, I mentioned to you about budget. Quite fortunately, that's the first hurdle we have been able to make and that is the executive branch of government. The DBM has already given us 24% increase in the locally-funded budget as against their imposed ceiling of 4% and some even suffered a 2% decline like agriculture. And so, with 24% I'm quite happy although it took a lot of trouble not only for our own people presenting their programs but also with the cabinet and the President. That is why my former boss, a good friend Secretary Concepcion, was saying you got your budget at my expense, and I said why? You know there was this cabinet meeting and he presented the export figure of the Philippines. He said we are going to meet eight billion dollars and that is 25% increase over that of last year.

When you hear him talk about it emotionally, you right away clap your hands as the cabinet clap their hands and said: This must be an opportunity for me and I said it's very good and we must congratulate the Department of Trade and Industry about it because their goals have been attained, but I think we should also compare them to Thailand. It earned 15 billion dollars last year against 8 billion only in the Philippines and Taiwan's 60 billion. So, what we need really is a massive science and technology work to be able to catch up with them. And not only that: in one-on-one talk with the President and in that diagloue, we were able to get increased budget.

I wanted to ask for more but probably we did not have all the resources to be able to get more. We have yet to prove our credibility.

Fifth, I would like to talk about foreign assistance.

In terms of multilateral and bilateral assistance, we should be able to get in 1990 about 70% increase of today's counting but we will get much more than that because we are making efforts to work harder for both bilateral and multilateral assistance and I'm very glad that some of our friends from diplomatic fora are here. I've already met with them and I'm sure they are going to help us as they have indicated to me when we get with them. In fact, already, we have gotten 150 million pesos to beef up our laboratory facilities coming from Japan. We have already met many groups including the World Bank for science and technology program for the Philippines and yesterday I started to meet, following the advice of our Ambassador from Israel, Department of Commerce, and tap many of these programs of the US that will try to finance some of our programs.

Sixth, in financing, you should no longer worry about the commercialization of your research results. The DBP under its third window is going to give unlimited amount for technology projects. The TLRC has already done so and both these organizations (DBP and TLRC) have signed up an agreement so that low-cost funds

will be given for the commercialization of these technologies. And for the collateral-conscious we have added another thing to this: that for those who cannot put up their collateral among the researchers and inventors, we should, at the DOST guarantees the non-collateralized portion of the loans, and so, for the good projects they should have no problem in funding.

Seventh, on incentives, the science and technology bill which we have prepared will be approved by the Senate and the House. I know that about 7 years ago, I was the Chairman of the team that prepared the science and technology bill together with former Sec. Arizabal and former National Security Director Noel Soriano. That was even under the old administration when we prepared that bill. Even before the passage of that we have already gotten the BOI 1989 Incentives Program Investment Priorities Plan.

First is the modernization of industry. Technology that will be introduced in the areas that we have pin-pointed will be given tax-and duty-free importation of equipment or tax credit for local equipment.

Second, is the inclusion of science parks and technology incubators under the 1989 IPP. I'm 99 percent sure that this will be approved by the President.

Because of this, we have many interested parties like the Diliman Community Park and the Canlubang Park. On technology incubation, we expect the first one in Iligan or in Cagayan, and so we need incentives for it because this will be run by the private sector.

Third is for R&D activities done by corporations. We expect it to be an area of productivity that will be given incentives and we are hoping that within a few weeks' time, the President will approve the 1989 IPP and these three areas will be included.

# PLENARY PAPERS

*Trans. Nat. Acad. Sci. & Tech. (Phils.)*
*1989.11:15-27*

# BUILDING FOR THE YEAR 2000: REPORT OF THE PRESIDENTIAL TASK FORCE ON SCIENCE & TECHNOLOGY DEVELOPMENT

Fr. Bienvenido F. Nebres, S.J.
*Vice-Chairman, Presidential Task Force on S & T Development*

Yesterday, July 11, there was an all-day forum at the Department of Science and Technology Executive Lounge in Bicutan on the Report of the Presidential Task Force on Science and Technology Development. I know that many of you were there so I shall not redo that presentation. In this talk, then, I would like to focus on three aspects of the report and follow-up:

1) Content and overall assumptions and philosophy of the work of the Task Force.

2) Aspects of the Task Force report which are of special importance to the scientific community.

3) Structure of follow-up and implementation.

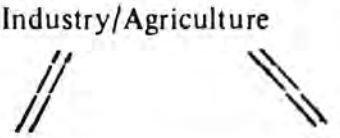
## I. Content and Overall Philosophy and Assumptions of the Task Force

In the very first meetings of the Task Force, the Executive Committee (Chairman and two vice-chairman) presented the following overall guiding framework:

A. The output of the Task Force should be a concrete, programmable plan, not another "wish list".
*Comment:* In a couple of the discussion of the Task Force, the question was raised as to how this Task Force plan relates to the NEDA five-year plan. Some of us pointed out that at least the S&T portion of the NEDA plan is not a plan at all. It is a collated "wish list" from various groups and agencies with no clear commitment with regard to resources and implementation.

B. There should be a "carrier" for this plan, i.e., persons, institutions, who not only do the planning, but will also take (at least partial) responsibility for the implementation.
*Comment:* One of the phenomena of our culture, which needs some study and reflection, is our habit of forming resolutions committees and presenting resolutions at the end of a conference without designating responsibility for seeing to it that the resolutions are carried out. In fact, it is rare that the next conference reports on the record of implementation of the previous conference's resolutions.

C. This "carrier" should be mainly a *linked network* of the following:




Science & Technology Institutions (R & D institutions in government private sector, academe) ═══ S & T Manpower (Academe)

The point of view taken by the Task Force is that the first key moves in developing a Science and Technology Plan have to come from leaders in government and the private sector. They have to answer the following questions (perhaps with the help of academics and scientists, but they have to give answers together with the requisite commitment to resources and implementation):

– What is to be our path of industrialization going into the next century?
– What are our priorities and policies in agriculture?
– What path are we to pursue in developing aquatic and marine resources in terms of our forests and forest products?

It is true that initiative and invention in S&T may actually be the "push" factor for plans and decisions in industry and agriculture. But if we are in the process of developing overall plans and priorities, it is difficult to make decisions on investing in laboratories and manpower training without some idea of the priorities of government and the private sector.

It should also be noted that the Task Force focused mainly on the needs of industrialization and productivity. The Task Force realizes that this is only part of our S & T concerns:

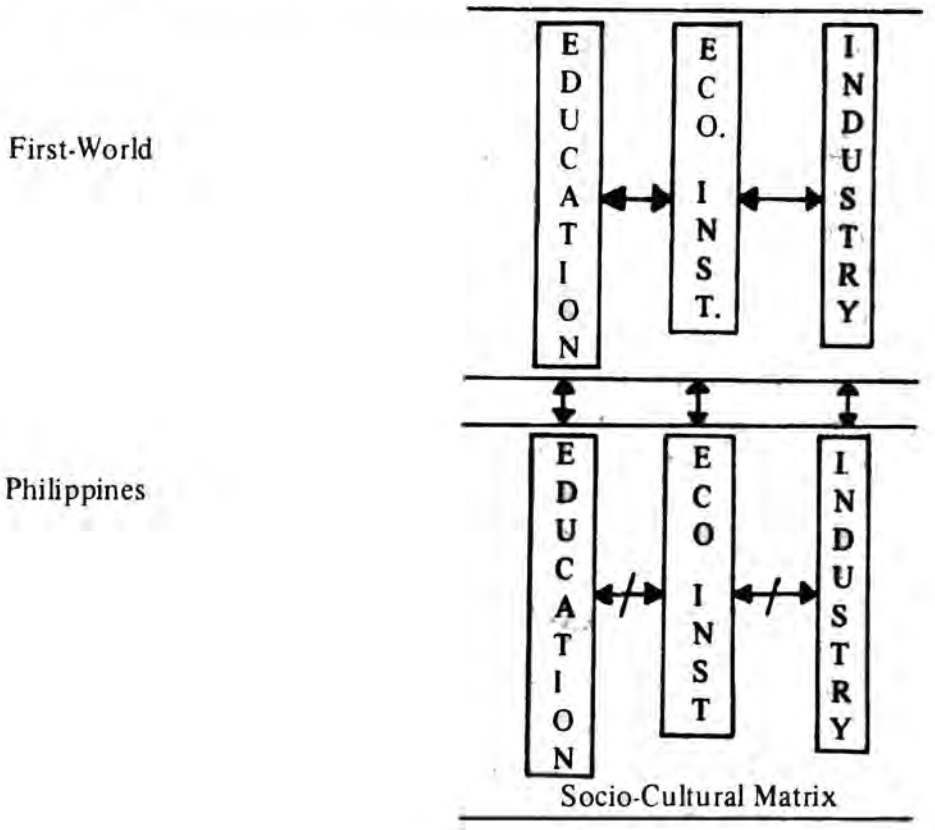
"Many have asked and many more will be asking why important S&T areas such as environment, health, and hazard management are not discussed in the report. The focus of the Task Force was specific: to dialogue with industry and agriculture and to identify the S&T areas needed for us to move to NIC status by the year 2000, then to work out the manpower, S&T infrastructure, policies, and organizational structure needed to make rapid progress in these identified S&T areas. The other concerns should be taken up soon in a different study and report." (Task Force Report, p. 4).

A second point of view which needs emphasis is the concern of the Task Force that the process of work and implementation stress and strengthen linkage between industry/agriculture – S & T institutions in government, private sector, academe – academic institutions. To help us understand the concern which underlies this preoccupation with linkage, I would like to refer to a section of a paper I gave in 1985 entitled: "A Framework and Premises for the Development of Science and Technology in the Philippines Today".

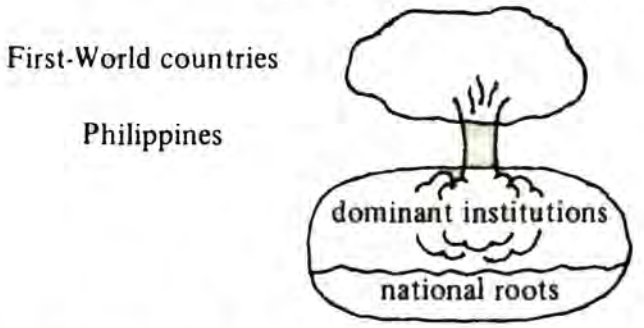
"(An earlier paper) points out several problematic observations about science and technology in the Philippines:

- very low government investment in science and technology
- lack of integration/complementarity in S & T
- expensive fiascos like the Bataan nuclear power plant
- lack of coordination between the different institutions and agencies
- low priority in agriculture
- little linkage between education and industry.

I would like to locate the primary cause of these problems in the history of our social and cultural institutions. I note two types of relationships between institutions. The first I would call vertical, that is the relationship between similar institutions, like banks, in different societies. The second I would call horizontal, that is, the relationship between different institutions in the same country. I would also add a third relationship of rootedness, that is, the integration of these institutions within the socio-economic-cultural matrix that underlies the given society.




To understand our situation, we should note that the history of our social, industrial, educational institutions in the Philippines has been guided almost exclusively by vertical relationships. For example, to understand our school system it is less necessary to understand the social and cultural situation in the Philippines than it is to understand the American school system and to note the adaptations that have been made here. The same can be said about the major hospitals like big medical centers and the Heart Center.




If we were to picture the development of institutions like that of a tree, where the institutions are represented by the leaves, branches, fruits (that are the visible developments in society) then we would picture our institutions like upside-down trees. They are rooted not so much in the socio-cultural matrix of the Philippines as in the socio-cultural matrix of model countries abroad. This whole pattern of development of institutions according to vertical relationships has produced what we call the modernized sector, of which Makati is the main exponent.

What are the consequences of this development guided mainly by vertical relationships:

(1) The development of our institutions is directed by their sources abroad, not by complementary institutions or needs in our society. For example, we come back with our PhDs from the United States or Europe and we want to teach immediately what we learned there, whether or not it has a serious relevance to the Philippines today.

(2) The horizontal relationships between different institutions are either non-existent or undeveloped. For example, the relationship between research and development in universities and industry. Several times in the past, it was pointed out to me by our scientists in PIPAC (Philippine Institute for Pure and Applied Chemistry) that they have the expertise to do a lot of chemical analysis for companies, but these companies have these analyses done in their mother institutions abroad. Senator Diokno once gave an example of the shoe industry, we find that it also is guided by vertical relationships. That is, most of the components that they use for manufacture are imported and the best shoes are exported.

This concentration on vertical relationships and the neglect of horizontal relationships as well as of rootedness in our national situation is, I think, at the heart of the problem that we are discussing today. There is a lack of fit between our social, financial, cultural, political institutions because their growth has not been guided by complementary needs, but rather by the effort to make vertical fit between our institutions and similar institutions abroad. This overemphasis on vertical fit has resulted in a lack of rootedness (even in uprooting, as we may see in brain-drain). It has also resulted in a lack of relationship and complementarity between the different institutions in our society. It should be no surprise to us then that these institutions are all out of joint and that it is extremely difficult to establish linkage among them. This for me defines the task for the future, that is, to develop better horizontal fit and a better rootedeness in the national situation."

The above long reference presents my analysis and point of view on the importance of insisting on linkages.

## II. Important Aspects of the Task Force Work and the Task Force Report

The above considerations guided the composition, process and report of the Task Force.

The composition of the Task Force reflects the concern that the directions of S & T planning fit into a larger national plan. Thus the role given to key government departments and to the private sector. The composition also reflects the concern for linkage. Thus the almost even representation from government, private sector, and academe.

The process, which gave the first round to sectoral technical panels, reflects the concern that we need to know where different industry/agriculture sectors wish to go, before we can rationally plan for S & T needs.

At the same time, the Task Force was painfully aware that there were fundamental problems that need attention no matter what priorities may eventually emerge from the sectoral technical panels. These have to do with:

- a better career environment for scientists, engineers, and technicians
- manpower development
- building needed S & T infrastructure.

Thus, parallel to the work of the sectoral technical panels, the Task Force gave much of its attention to these concerns.

As I said at the beginning, I do not intend to repeat the detailed discussion held yesterday at PCIERD, but to give some idea of the summary results from the sectoral technical panels, let me cite some portions of the report. Let me note also that the Task Force report is simply a summary (not always adequate) of a voluminous report from the sectoral technical panels. It is also important to note that the level of detail and adequacy of the reports from the different panels is uneven.

### A. Some Potential High Growth Sectors Recommended for Development

(1) *Electronics*

The main thrust of the industry is to increase the value added of electronic products by increasing the local inputs in the form of components and engineering content, and to increase exports of non-semiconductor products. The three areas of concern are:

a) *Semiconductor Electronics*

Development of IC design capability in custom or application of specific, integrated circuits utilizing current technologies

b) *Consumer Electronics*

Development of a wide range of consumer electronics products to include toys, clocks and watches, video/audio accessories and other such products within the capabilities of the electronics and support industry

c) *Computers, Industrial Electronics, Telecommunications and Others*

i) Increase in the local content of locally assembled/manufactured industrial and commercial products in areas such as computers and computer peripherals, telecommunication equipment, industrial control and instrumentation

ii) development of the use of automation such as computers, controllers, instrumentation, networks and similar areas in domestic manufacturing industries

(2) *Process Industries*

The country remains heavily import-dependent for its chemical requirements. Hence, the significant contributions of this sector to the economy may be measured in terms of utilization of indigenous raw materials, production of import substitute chemicals, and export of high-value added chemical products. The process sector has the following products as leading edges, including their technological requirements:

a) *Coconut Processing*

i) Development of downstream oleochemical products

ii) Development of coir industry and coco-food products

b) *Sucrochemicals/Fermentation Products*

i) Diversification of sucrochemical products

ii) Improvement of process efficiency in alcohol production

c) *Polymers*

) Development of polymer products from oleochemicals and sucrochemicals

ii) Improvement and adaptation of polymer acquired technology from foreign sources to local conditions

d) *Acids and Bases*

i) Development of pollution control equipment for factories producing acids as well as those using these materials

ii) Development of heat recovery systems and utilization of non-conventional fuels as a source of heat

e) *Agrochemicals (Fertilizers and Pesticides)*

i) Technology transfer of biofertilizer and biopesticides, e.g., rhizobia, azospirillum

ii) Development of organic and inorganic fertilizers and pesticides from locally available raw materials

f) *Industrial Gases*

i) Development of an appropriate container for industrial gases

ii) Improvement of product purification technology

g) *Industrial Salt*

i) Development of production of soda ash and bicarbonate from concentrated brine

ii) Development of the manufacture of sulfates, sulfites, and sodium metal and cyanides from locally produced industrial salt and recovery of other chemicals

h) *Fine Chemicals*

i) Adaptation of foreign technology through joint-ventures for the manufacture of active substances from indigenous sources in the pharmaceutical industries

i) *Plastics Industry*

i) Improvement of the quality of plastic products manufactured in the country

ii) Diversification of the range of products manufactured

iii) Reduction of external technological dependence through local development, adaptation, and innovation of production technologies and applications

j) *Coal-based Ammonia and Urea*

i) Development of coal as feedstock for industrial chemicals, e.g., urea, ammonia

(3) *Energy*

In order to reduce the country's vulnerability to the international fluctuations in energy supply and prices, a two-pronged energy supply/demand strategy is necessary. First, the country should develop indigenous energy sources and second, utilize energy resources at the least cost and in the most efficient manner. In view of the importance of energy in our economic development and the availability of various indigenous resources, the panel classified the leading edges for economic development into three groups:

a) *Conventional Energy*

(Oil and Gas, Geothermal, Coal, Hydro, Nuclear)

i) Continued development of the country's indigenous energy resources

ii) R & D in the following areas: geology and geophysics, chemistry and geochemistry, reservoir engineering, computer applications and environmental studies in the geothermal field

iii) quality upgrading and characterization of local coal

b) *Non-Conventional Energy*

(Biomass, Solar, Small Hydro, Wind)

i) Development of manufacturing capability in the fabrication of energy-related equipment and parts

ii) Development of adaptation capability in the acquisition of technology to certain local conditions

c) *Energy Conservation and Utilization*

i) Development of waste heat recovery and utilization technologies

ii) Development of local capabilities in the design, fabrication and installation of conservation equipment

(4) *Agriculture and Aquaculture*

The objective of the sector is to improve the food availability and nutrition of the people, reduce imports through local production of import substitutes, increase traditional and non-traditional exports, and (a) agriculture-fiber (abaca, sericulture, ramie), crops (coconut, coffee, cacao, rubber, sugarcane), fruits and nuts, feed grains (corn, soybeans), livestock (hogs, poultry, beef cattle), vegetables, ornamentals, spices, (b) aquaculture-prawns and shrimps, seaweeds, milkfish, tilapia, groupers, seabass, marine mollusks (clams, oysters, mussels) and freshwater aquarium fishes. Common features of R & D are:

a) Development of integrated pest-management systems using pest and disease-resistant varieties, better cultural management techniques, and biological, botanical and chemical pesticides

b) Varietal improvement for high yield, stress tolerance, resistance to pests and disease, adaptability and better crops

c) Improvement of genetic characteristics of livestock

(5) *New and Emerging Technologies*

A fourteenth sector may be the NEW AND EMERGING TECHNOLOGIES. These are technology areas not presently developed but which

could have a substantial impact on the national economy and the global marketplace. Of the five areas identified as first priority by the PCASTRD for the country, two, namely: microelectronics, instrumentation and control and information technology have been discussed above. The other three areas are:

a) Biotechnology
b) Materials Science and Engineering (including new energy sources such as photovoltaics)
c) Laser Technology

B. *Needed Modernization of S & T Infrastructure*

(1) *Electronics*

*S & T Infrastructure*

a) Establishment of an Electronics Research and Service Foundation
b) Establishment of Printed Circuit Board (PCB) prototyping facilities (accepting 5-10 boards) with computer grade density, double-sided plated through holes
c) Establishment of environmental testing facilities

(2) *Process Industries*

*Industry Infrastructure*

a) Refurbishing of existing fertilizer plants
b) Rehabilitation of four other fertilizer plants aside from the PHILPHOS plant
c) Restructuring of the salt industry

*S & T Infrastructure*

a) Establishment of modernized and efficient facilities for the chemical industry
b) Replacement of obsolete/antiquated distillery equipment
c) Establishment of a sucrochemical R & D center which will be a joint venture of the government and the private sector
d) Provision of necessary financial support or assistance in sourcing of funds to implement R & D projects
e) Establishment of a Plastic Research and Development Unit (PRDU) at the Industrial Technology Development Institute (ITDI)
f) Establishment of an ammonia plant with about 2,000 ton/day capacity

(3) *ENERGY*

*S & T Infrastructure*

a) Creation of an applied research center for geothermal energy
b) Creation of a coal technical body
c) Establishment of an energy conservation center
d) Creation of a Philippine Energy Foundation to develop, implement, finance and monitor the various S & T plans and programs, incentives and promotion activities, regulation, and institutional linkages for the energy sector.

(4) *AGRICULTURE AND AQUACULTURE*

*S & T Infrastructure*

a) Strengthening research-management program to enhance technology-planning development and utilization process in the region
b) Strengthening applied communication programs in order to bring research-based technologies and information closer to the target clientele
c) Strengthening the information and data-management systems
d) Strengthening the technology, utilization and commercialization program
e) Rationalizing and strengthening the agencies in agriculture and fishery systems
f) Upgrading/expanding post-production facilities

(5) *New and Emerging Technologies*

a) *Biotechnology:* The most immediate need is to develop and strengthen the biotechnology programs in several institutions
b) *Materials Science and Engineering:* The main need is for semiconductor materials characterization and testing. A building to house all the equipment will have to be constructed and this can be a center for all research activities in the area of material science and engineering.
c) *Laser Technology:*
    i) The acquisition of facilities and equipment needed for the rapid and accurate testing and characterization of lasers and laser systems, and for the faster and reliable collection and processing of spectroscopic information
    ii) The establishment and maintenance of optical shops with coating facilities at both the Ateneo and NIP, a machine shop capable of high-precision work, and a glass-blowing

shop, side by side with the training of skilled technicians to man shops.

iii) The establishment and maintenance of a laboratory with facilities and equipment for epitaxial growth (LPE, MBE, or CVD) of semiconducting crystal compounds, with clean room and other facilities for device processing.

C. *Manpower Development*

The report provides some details from the different sectoral panels on their needs. But it may be sufficient to cite the overall goals and directions of manpower development to support S & T priorities and the needs of R & D institutions.

Required by the economy for development over the period 1989-1992 is manpower with definite skills and expertise in S & T. The following are the targets of the S & T manpower sector:

1. To increase by 50% the present 8,000 R & D professionals over the period 1989-1992. This means producing quality scientists and engineers for R & D according to the following ratios: PhD:MS:BS = 1:2:5. That is, for every PhD produced, there should be two MS and five BS graduated correspondingly. Twice as many technicians must be trained to achieve the desired ratio of one R & D professional for every two R & D technicians.
2. To designate key institutions to conduct/support massive S & T faculty/staff development/training programs in order to upgrade their capability in the natural sciences, agriculture, and engineering.
3. To designate key secondary schools in the different provinces and regions and upgrade laboratory facilities and initiate science-honors programs in these schools.
4. To promote the inclusion of scientists and technologists outside of DOST in an improved and expanded scientific career system; to develop other incentives for a scientific career.

III. *Structure of Follow-up and Implementation*

A major concern of the Task Force was the mechanism for validation of its work in consultation with key sectors, a continuing process of correcting and improving the plan, and a structure for implementation. After the presentation to the President on March 27, 1989, most of the discussion focused on these needed mechanisms and structures.

The most important result of the discussion was the decision of the President, embodied in an administrative order dated April 4, 1988, to establish the Science and Technology Coordinating Council (STCC). Its composition is similar to that of the Task Force and its mandate is contained in Section 2 of the Administrative Order:

*Section 2:* The Council shall:

a) recommend appropriate systems and procedures for the effective implementation of the report of the Presidential Task Force on Science and Technology, hereinafter referred to as the Task Force.
b) coordinate the science and technology activities of departments, agencies, private sector organizations and the academe to accelerate science and technology utilization in accordance with the report of the Task Force;
c) monitor the implementation of the recommendations of the Task Force and the results thereof;
d) recommend measures to update, revise and enhance the Science and Technology Plan based on the report of the Task Force;
e) recommend mechanisms, structures, and measures to link technology sources, intermediaries and users to hasten transfer of technology, develop the countryside, attain high productivity and increase export potential;
f) constitute national, regional, sectoral and other subcommittees as may be necessary; and
g) perform such other functions as may be assigned by the President.

Note also Section 6:

*Section 6:* The Council shall submit quarterly reports to the President and such other reports that may be required from time to time.

This provision allows ongoing dialogue with the Chief Executive on crucial S&T concerns.

What is to be done now? For this body, composed mainly of the scientific community, I would like to end with the recommendations on *Human Resources and Scientific Infrastructure Development:*

1. Identify and strengthen key secondary schools in each province and region and upgrade facilities and initiate science-honors programs in these schools.
2. Establish a PhD Engineering Program and provide support infrastructure such as laboratory facilities and offering of a more attractive compensation package for engineers.
3. Strengthen the higher level scientific manpower programs in different universities and consortia and give priority to the upgrading of their facilities.
4. Support the implementation of the Science Education Development Plan.
5. Revive, improve, and expand a National Scientific Career System and develop other incentives to enhance careerism and professional growth among scientists and other personnel involved in S & T.

6. Develop a program to provide incentives to Filipino scientists abroad to return to a scientific career in the Philippines.
7. Strengthen existing and develop new national centers of excellence for science and technology.
8. Promote needed competence in technology.

I am Vice-Chairman of the STCC with specific responsibility for academic institutions. What we need to do is to hammer out specific plans, programs, proposals, especially in these areas of scientific manpower and scientific institutions. I hope that the work of the Task Force and the establishment of the STCC has helped in providing a better environment for our plans and programs. The challenge for us then is to help build these concrete programs and institutons.

# MATHEMATICAL, PHYSICAL AND ENGINEERING SCIENCES

*Trans. Nat. Acad. Sci. & Tech. (Phils.)*
*1989.11:31-43*

# DESIGN AND PERFORMANCE EVALUATION OF A BATCH-TYPE RICE HULL GASIFIER STOVE[1]

Alexis T. Belonio
*Agricultural Engineering Department*
*College of Agriculture*
*Central Philippine University, Iloilo City*

**ABSTRACT**

A batch-type rice hull gasifier stove was designed and its performance as a potential alternative fuel saving device for cooking was evaluated.

The stove is a single-burner with a double-core, down-draft type reactor. Gas is generated in the system by using a suction blower driven by a 90-watt electric motor.

Performance evaluation showed that the stove has a start-up time of 4 to 9 min and a total operating period of 0.98 to 1.25 hour per batch with corresponding rice hull consumption of 1.96 to 2.72 kg. About 1.2 to 4.0 liters of water can be boiled in the stove within 10 to 34 min while 0.7 to 1.0 kg rice can be cooked within 16 to 22 min.

Analysis showed that the stove has a gasification rate of 95 to 143 kg/m^2-hr, a fire zone rate of 0.80 to 1.02 cm/min, and an average burning and thermal efficiencies of 21 and 10%, respectively.

The stove entailed an operation cost of P0.94/hr and a payback period based on electric, charcoal, and LPG stoves of 0.42, 1.30, and 3.35 years, respectively.

## Introduction

Most families in the rural areas are now facing the problems of using conventional energy sources of fuel for cooking owing to the continuing increase in the prices of kerosene, liquified petroleum gas (LPG) and electricity. Firewood and charcoal are the best alternatives, but their decreasing availability (GATE/GTZ, 1984) is a problem.

Agricultural waste like rice hull is a biomass fuel that is a potential alternative source of energy which could replace fossil fuel, wood and charcoal. But using wood and charcoal would gradually deplete our forest (Belonio, *et al.*, 1989).

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[1]Paper presented during the 11th Annual Scientific Meeting of the National Academy of Science and Technology, July 12, 1989 at the Hotel Nikko Manila Garden, Makati, Metro Manila.

Rice hull is abundant in most localities and can be found at the back of most rice mills and/or along the sides of highways where it is being dumped and burned to avoid accumulation of bigger volumes. The improper disposal and burning of this waste product resulted in a waste of millions of calories.

Several attempts have already been done to develop low-cost devices utilizing rice hull as fuel for heating, but none has gained wider acceptance due to the problem of operation (Beagle, 1978; GATE/GTZ, 1984; and Stickney, *et al.*, 1987). Gasifying rice hull for domestic use has a potential application (Stickney, *et al.*, 1987). Through gasification, one can operate a stove conveniently similar to an LPG stove while utilizing electrical energy at very minimal cost.

This study was conducted to design and evaluate the performance of a batch-type rice hull gasifier stove.

## DESIGN CRITERIA AND BASIC DESCRIPTION OF THE DESIGN

The following criteria were considered in the design of the batch-type rice hull gasifier stove:

1. Continuity of operation – capacity to operate continuously for one hour with sufficient energy to provide heat for cooking.
2. Convenience of operation – non-intensive attendance during use.
3. Safety in operation – freedom from possible leakage of undersirable gases.
4. Portability – ease of transfer from one place to another, if desired.
5. Economy – low initial and maintenance cost.

Based on these criteria, a double-core, down-draft type reactor was designed in such a way that it is directly coupled into the burner to simplify operation. A chimney was also provided to safely discharge undesirable gases.

### *The Batch-Type Rice Hull Gasifier Stove*

The batch-type rice hull gasifier stove as shown in Figure 1 consists of the following major components: 1) reactor, 2) suction blower, 3) burner, 4) tar collector, and 5) T-chimney. The design specifications of these components such as the diameter and length of the reactor, and airflow rate and static pressure requirement of the suction blower were computed using the equations presented in Appendix 1:

**Reactor.** The reactor is a double-core cylinder made of a cold-roll black iron (B.I.) sheet gage No. 20. The inner and outer cores have a diameter of 15 and 20 cm, respectively, allowing 2.5 cm annular space for the passage of gas. Its length of 65 cm ensures continuous operation for one hour and its conical shape bottom with a clip-type door makes discharge of char easy. A screen covering is provided to protect the operator from touching the reactor. Directly beneath the inner core, a flat grate made of 3 mm diameter round bar spaced 6 mm apart is attached to hold

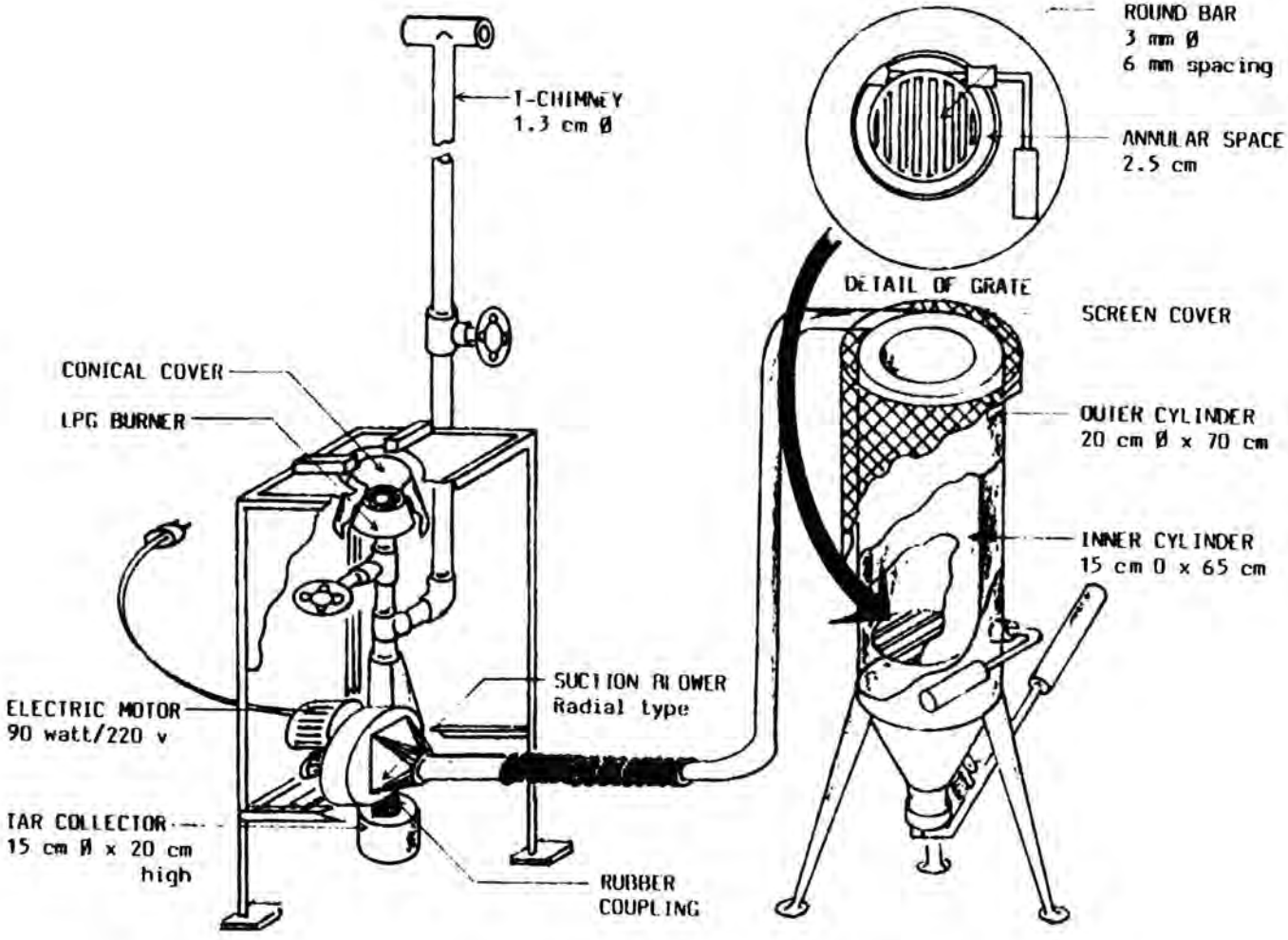



Figure 1. Technical drawing of the batch-type rice hull gasifier stove.

the fuel. This grate can be tilted to facilitate the removal of char. Three identical legs are welded to support the reactor.

**Suction Blower.** A backward-type high static pressure blower made out of B.I. sheet gage 18 sucks gas from the reactor. Its impeller has eight blades each with a diameter of 15 cm and a width of 5 cm. A 90-watt sewing machine electric motor drives the blower.

**Burner.** A standard LPG-type (7.5 cm D) burner stove with a 1.3 cm diameter gate valve regulates the flow of gas. Directly above the burner, a conical cover provides proper combustion and minimizes heat loss.

**Tar Collector.** Condensing tars on the piping of the stove during operation are collected in a tar container beneath the blower. This container at the lowest most portion of the stove collects tars from the pipings. It has a design capacity of 3.5 liters sufficient to collect tars for a one-week operation. To minimize the leakage of gas, a thermal resistant rubber tubing is used as coupler.

**T-Chimney.** A 1.3 cm diameter B.I. pipe is used as chimney. It is connected to the pipe installed between the blower and the burner to easily divert and discharge undesirable gases during operation. A gate valve controls the flow of gas.

## METHODOLOGY OF PERFORMANCE EVALUATION

### *Materials and Equipment*

Rice hull with moisture content of about 13% which was obtained from a rubber roll type rice mill was used as fuel in testing the performance of the stove.

The weight of fuel used and the corresponding char produced during gasification were measured with a 10-kg capacity spring-type balance. At each test, the time required to finish a run was recorded with a stop watch.

In boiling water and in cooking rice, a standard-sized aluminum casserole (8 cm D) was used as container. Initial and final temperatures of water were taken using a dial-type bimetallic thermometer (150 C max 2 C acc). Ambient air temperature and relative humidity were taken with a sling-type pyschrometer (43 C max 1 C acc).

The energy content of rice hull and char after the performance evaluation was determined on a 1341 Plain Jacket Oxygen Bomb Calorimeter.

### *Performance Evaluation Procedure*

The performance of the stove was evaluated in actual condition at Brgy. Balicua, Tubunga, Iloilo during the month of December 1987. The unit was installed in an open shade to allow free passage of air.

Operation was started by initially loading two handfuls of rice hull at the reactor occupying approximately 5 cm depth serving as primer. The fuel was ignited by dropping burning pieces of paper and then switching on the blower. After proper ignition was attained, i.e. approximately 3/4 of the total area occupied

by rice hull was burning, the reactor was fully filled with rice hull. Successive loadings of fuel were made at different intervals until the reactor was filled with the accumulated char.

The time required to generate combustible gas at the start and at 10-min lag period between operations was taken during the tests to determine the start-up time of the stove. The amount of rice hull fuel consumed in each run was recorded to determine the gasification rate of the stove. The depths of char between successive loadings were also measured to monitor the rate of the fire zone in moving along the reactor. The corresponding amount of char produced was also measured after each run.

Boiling time test was conducted by subjecting 1.2, 2.0, and 4.0 liters of water. The corresponding amount of water evaporated during this test was taken to evaluate the thermal efficiency of the stove. Cooking time test, on the other hand, was carried out by cooking 0.7, 0.9, and 1.0 kg rice (IR-64) with different volumes (1.12 – 1.50 liters) of water used. Ambient air temperature and relative humidity were also recorded in each run.

Analysis of the energy content of rice hull fuel used and the char produced during the study was also conducted to determine the burning efficiency of the stove.

## Results and Discussion

*Operating Performance*

Series of test runs were conducted in evaluating the performance of the stove. The ambient air temperature and the relative humidity during the tests ranged from 27 to 29 C, and 70 to 90%, respectively.

As illustrated in Figure 2, flammable gases are generated in the reactor through the suction created by the blower wherein the air upon contact with the burning rice hull at the fire zone is converted into gases, primarily carbon monoxide (CO). These gases leave the reactor through the annular space between the inner and outer cylinders. As clearly shown in the figure, the outlet is positioned tangentially to the reactor to allow uniform heating as well as separation of fly ashes. Primary air, on the other hand, is provided in the burner for proper combustion through a conical cover directly installed above it. Tar is separated from the gas by condensation while passing through the pipings.

Results showed that immeditely after igniting rice hull in the reactor, about 4 to 5 min is required before combustible gas can be generated(Figure3). Note that the undesirable gases produced during the start-up of the stove are diverted outside the shed by closing the valve at the burner while opening the valve at the chimney. Switching off the stove for 10 minutes between operations requires a longer time of about 4 to 9 minutes before gas can be re-generated.

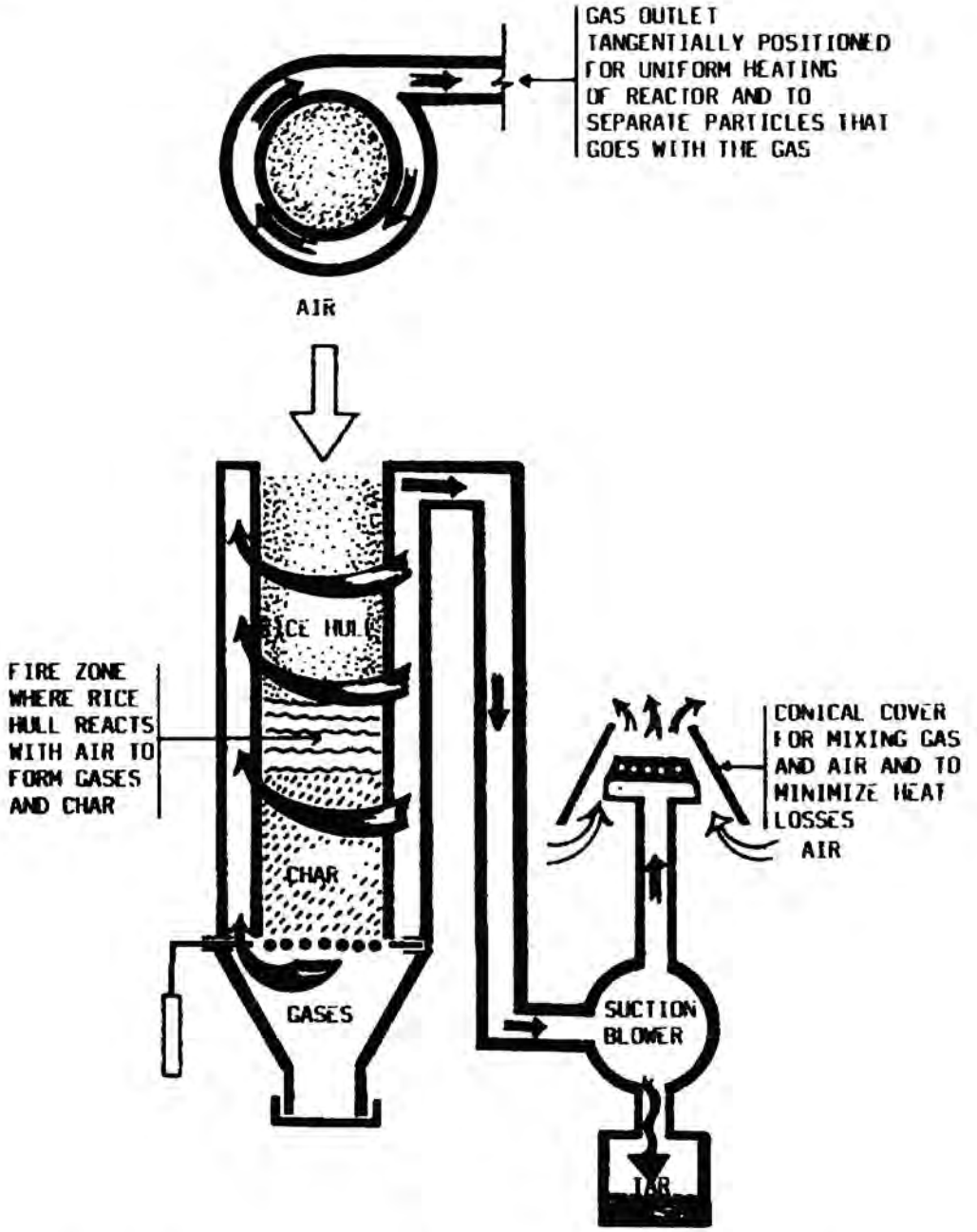
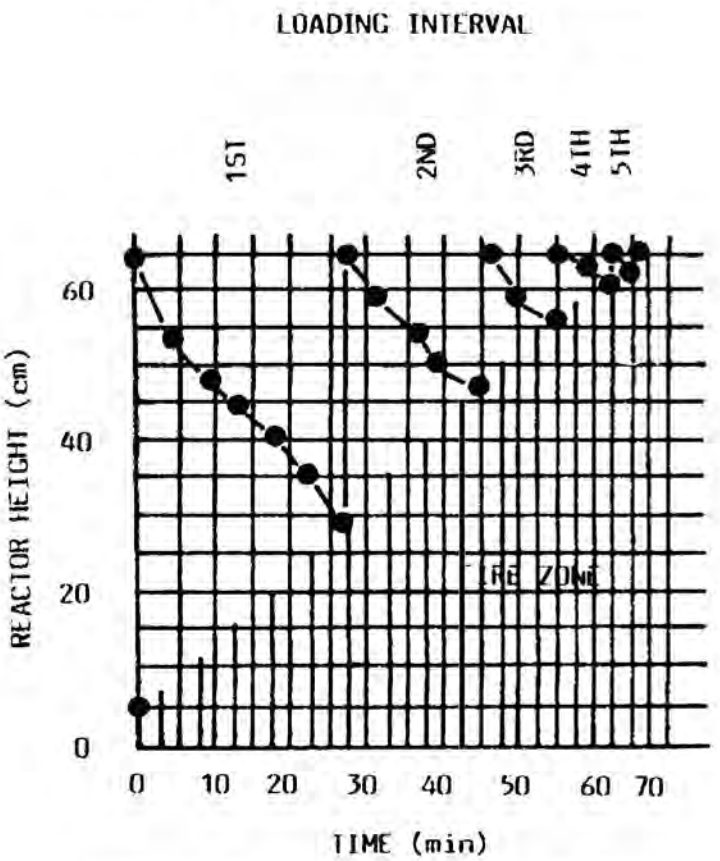



Figure 2. Illustrative example of the gas generation of the rice hull gasifier stove.

It can be further observed in Figure 3 that in continuous operation of the stove, successive loadings of rice hull is necessary before completing a batch. The need to successively load fuel in the reactor basically can be attributed to the decrease in the volume of rice hull which is converted into char upon burning. In each batch, about 5 to 6 loadings are required. Loading time interval, as shown in the same figure, decreases with time.




| | START-UP TIME (min) |
|---|---|
| At the start of operation | 4 - 5 |
| At 10-min Lag period | 4 - 9 |

Figure 3. Operating performances of the rice hull gasifier stove.

Performance test results for the three separate runs showed that the stove can be continuously operated within 0.98 to 1.25 hr. (Table 1). The corresponding weight of rice hull consumed and char produced ranged from 1.96 to 2.72 kg and 0.53 to 1.04 kg, respectively. The computed gasification rate which is the ratio of the rate of burning rice hull per unit area of the reactor of 0.0047 square meter range from 95 to 143 kg/m^2-hr. This result is also within the range of the standard gasification rate of rice hull gasifiers which is 100-210 kg/m^2-hr (Kaupp, 1984). The fire zone rate which is the time required for the fire zone to reach the top most portion of the reactor, on the other hand, ranged from 0.8 to 1.02 cm/min.

Table 1. Operating performance of the batch-type rice hull gasifier stove on three different runs

| Run | *Operating Period*[1] *(hr)* | *Weight of Fuel Consumed (kg)* | *Weight of Char Produced (kg)* | *Gasification Rate*[2] *(kg/m^2-h)* | *Fire Zone Rate*[3] *(cm/min)* |
|---|---|---|---|---|---|
| 1 | 0.98 | 1.96 | 0.53 | 114 | 1.02 |
| 2 | 1.08 | 2.72 | 1.04 | 143 | 0.93 |
| 3 | 1.25 | 2.09 | 0.55 | 95 | 0.80 |

[1] Measured from the time after a combustible gas is produced until the the reactor is completely filled with char.

2 $\frac{\text{weight of fuel used}}{\text{area of the reactor x time of operation}}$

3 $\frac{\text{length of reactor}}{\text{time of operation}}$

*Boiling and Cooking Time*

Results of boiling and cooking time tests as indicated in Table 2 revealed that the stove can boil 1.2 to 4.0 liters of water initially from 28 C within 10 to 34 minutes and cook 0.7 to 1.0 kg rice with 1.12 to 1.50 liters of water within 16 to 22 minutes. It was observed during the test that the more water is boiled and the more rice is cooked, the longer is the time required in operating the stove. However, for typical rural families, results of tests on boiling and cooking time indicated that the unit can sufficiently provide the needed heat requirement per operation.

Table 2. Summary of the results of the boiling and cooking time tests of water and rice in the stove*

| *Volume of Water and/or Weight of Rice* | *Boiling/Cooking Time (min)* |
|---|---|
| 1.2 to 4.0 liters of water | 10-34 |
| 0.7 to 1.0 kg of rice (IR-64) with 1.12 to 1.50 liters of water | 16-22 |

*Averages of nine (9) runs conducted in Barangay Balicua, Tubungan, Iloilo during the month of December 1987.

*Burning and Thermal Efficiency*

In determining the burning efficiency of the stove, the percentage heat liberated method as mathematically expressed below was used:

$$BE = \frac{HErh - HEc}{HErh} \times 100$$

where:

BE – burning efficiency, %
HErh – heat energy content of rice hull, kcal/g
HEc – heat energy content of char, kcal/g

Based on the heat energy content of rice hull and the char obtained in the bomb calorimeter tests which averaged 3.620 kcal/g for rice hull and 2.876 kcal/g for char, the burning efficiency for the rice hull gasifier stove was 21%. The process of gasifying fuel which only converts rice hull into char rather than into ash gave a relatively lower burning efficiency for the stove.

The thermal efficiency of the stove was computed using the equation:

$$TE = \frac{W\ Cp\ (T2-T1) + We\ Hfg}{Wf\ HVF} \times 100$$

where:

TE – thermal efficiency, %
W – weight of water, kg
Cp – specific heat of water at constant pressure, kcal/kg-C
T2 – final temperature of water, C
T1 – initial temperature of water, C
We – weight of water evaporated, kg
Hfg – latent heat of vaporization, kcal/kg
Wf – weight of fuel consumed, kg
HVF – heating value of fuel, kcal/kg

With the average amount of rice hull fuel consumed of 2.26 kg and a corresponding amount of heat utilized of 818.12 kcal (combining both the sensible and latent heat during heting and evaporation of water) gave a thermal efficiency for the stove of 10%. Similarly, the process of gasifying rice hull as well as the use of uninsulated metal sheet which radiated some heat during the operation gave a relatively lower

thermal efficiency for the stove. However, the result obtained can still be considered valid since it is in the range of efficiencies of various charcoal (2.5 – 12%) and open firewood (3 – 11%) stoves (Appovecho Institute, 1984).

Table 3. Cost analysis of operating the stove

| | |
|---|---|
| Investment Cost (IC) | ₱1,500.00 |
| Fixed Cost (P/yr) | |
| Depreciation[a] | 675.00 |
| Interest on Investment[b] | 75.00 |
| Repair and Maintenance[c] | 45.00 |
| Total | 795.00 |
| Variable Cost (P/yr) | |
| Fuel[d] | 43.20 |
| Electricity[e] | 172.80 |
| Total | 216.00 |
| Total Operating Time (hr/yr)[f] | 1080 |
| Operating Cost (P/hr) | 0.94 |
| Payback Period (Yr) | |
| Electric Stove[g] | 0.42 |
| Wood Charcoal Stove[h] | 1.30 |
| LPG Stove[j] | 3.35 |

[a]Straight line method at 10% salvage value and 2 yr life span (LS)
[b]5% of IC
[c]3% of IC
[d]P 0.25/sack (10 kg/sack), 1 hour operation at 1.8 kg/hr
[e]90 watt-hour at P 1.80/kw-hr
[f]3 hours/day operation.
[g]P 720.00/unit, 2.24 kw, 220 v, 40% eff., and 1 yr LS
[h]P 25.00/unit, 2 kg/hr at P 4.00/kg, 6% eff., and 0.5 yr LS
[j]P 775.00/unit (including tank), 1.3 months/tank at P 91.60/tank, 40% eff., and 2 yr LS.

## Economic Considerations

The ability of the rice hull gasifier stove to produce flammable gas out of rice hull with the minimum use of electricity (PO.16/hr solely for driving the blower) is an economic advantage.

With an initial investment of ₱1,500 for the stove and a combined cost of ₱0.20/hr, for hauling fuel and electricity the unit entailed an operating cost of ₱0.94/hr only.

The stove can be paid after 0.42, 1.3, and 3.35 years of operation compared to electric, wood charcoal, and liquified petroleum (LPG) gas stoves, respectively.

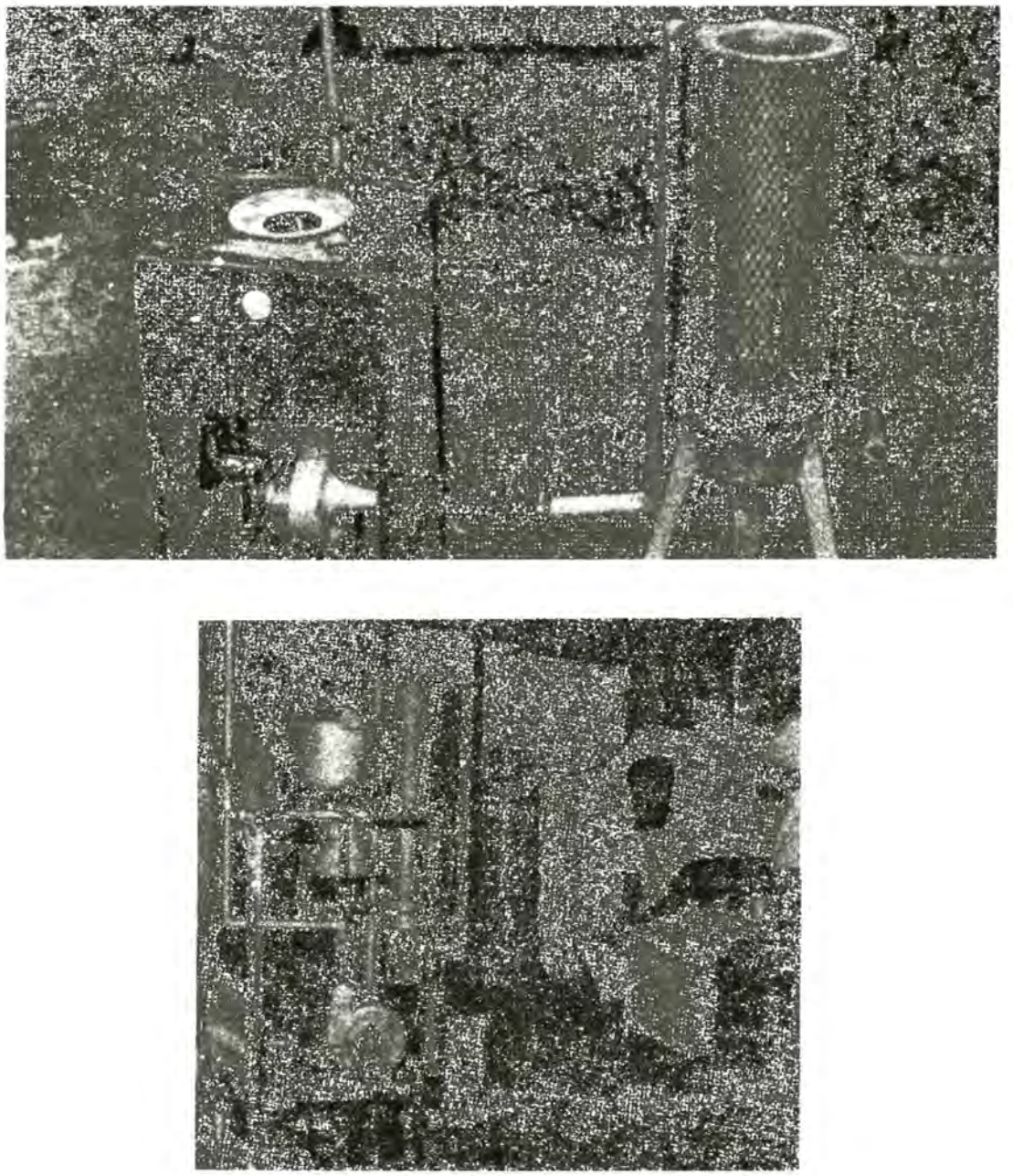


Figure 4. Photographs showing the rice hull gasifier stove: (a) Pictorial view, and (b) during operation.

## Concluding Remarks

The stove is a potential alternative fuel-saving device which produces combustible gas out of rice hull. It can be conveniently operated continuously for an hour with sufficient amount of heat for cooking.

The stove is more economical to use compared with electricity and wood charcoal stoves.

Further modifications in the design to improve the efficiency and to reduce the initial cost of the stove are recommended for future study.

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Appendix 1. Equation used in the design of the batch-type rice hull gasifier stove.

A. NOMENCLATURE

| *Symbols* | *Notations* |
|---|---|
| AFR | airflow rate requirement, m3/min |
| D | diameter of the reactor, m |
| Dg | gas density, kg/m^3 |
| Eb | burner efficiency, % |
| Eg | gasifier efficiency, % |
| FZR | fire zone rate, m/min |
| GFR | gas flow rate, m3/min |
| HVG | heating value of gas, kcal/m^3 |

| | |
|---|---|
| Qd | energy demand, kcal/hr |
| L | length of the reactor, m |
| sp | static pressure requirement of char, cm of water/m depth of char |
| SA | stoichiometric air requirement, kg of air/kg of fuel |
| SGR | specific gasification rate, kg/m^2-hr |
| SP | total static pressure requirement of the blower, cm of water |
| T | operating time, min |

B. EQUATIONS

a) Gas Flow Rate

$$GFR = AFR = \frac{Qd}{Eb \times HVG}$$

b) Weight of Rice Hull

$$WRH = \frac{GFR \times DG}{0.32 \times SA \times EG}$$

c) Diameter of the Reactor

$$D = \left[ \frac{1.27 \times WRH}{SGR} \right]$$

d) Length of the Reactor

$$L = FZR \times T$$

e) Static Pressure Requirement

$$SP = sp \times L$$

by rice hull was burning, the reactor was fully filled with rice hull. Successive loadings of fuel were made at different intervals until the reactor was filled with the accumulated char.

The time required to generate combustible gas at the start and at 10-min lag period between operations was taken during the tests to determine the start-up time of the stove. The amount of rice hull fuel consumed in each run was recorded to determine the gasification rate of the stove. The depths of char between successive loadings were also measured to monitor the rate of the fire zone in moving along the reactor. The corresponding amount of char produced was also measured after each run.

Boiling time test was conducted by subjecting 1.2, 2.0, and 4.0 liters of water. The corresponding amount of water evaporated during this test was taken to evaluate the thermal efficiency of the stove. Cooking time test, on the other hand, was carried out by cooking 0.7, 0.9, and 1.0 kg rice (IR-64) with different volumes (1.12 – 1.50 liters) of water used. Ambient air temperature and relative humidity were also recorded in each run.

Analysis of the energy content of rice hull fuel used and the char produced during the study was also conducted to determine the burning efficiency of the stove.

## Results and Discussion

### *Operating Performance*

Series of test runs were conducted in evaluating the performance of the stove. The ambient air temperature and the relative humidity during the tests ranged from 27 to 29 C, and 70 to 90%, respectively.

As illustrated in Figure 2, flammable gases are generated in the reactor through the suction created by the blower wherein the air upon contact with the burning rice hull at the fire zone is converted into gases, primarily carbon monoxide (CO). These gases leave the reactor through the annular space between the inner and outer cylinders. As clearly shown in the figure, the outlet is positioned tangentially to the reactor to allow uniform heating as well as separation of fly ashes. Primary air, on the other hand, is provided in the burner for proper combustion through a conical cover directly installed above it. Tar is separated from the gas by condensation while passing through the pipings.

Results showed that immeditely after igniting rice hull in the reactor, about 4 to 5 min is required before combustible gas can be generated(Figure3). Note that the undesirable gases produced during the start-up of the stove are diverted outside the shed by closing the valve at the burner while opening the valve at the chimney. Switching off the stove for 10 minutes between operations requires a longer time of about 4 to 9 minutes before gas can be re-generated.

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# TOPOLOGICAL AND ALGEBRAIC STRUCTURES OF $R^{n+1}$ INDUCED BY RELAXED TRAJECTORIES*

Edgar E. Escultura**
*College of Science, University of the Philippines*
*Diliman, Quezon City*

## ABSTRACT

This paper belongs to a collection of papers that attempt, to probe for the first time, the algebraic, geometric and topological structures of $R^{n+1}$ using the sequential compactness and completeness property of generalized curves of which relaxed trajectories form a special class. The paper itself covers the algebraic and topological part.

The highlight of the topological part is the introduction of Extended Bundle and its application to space science. It is shown that an extended bundle can be approximated by a bundle and that it possesses some properties of a bundle, such as uniform boundedness. The introduction of a new metric and, therefore, a new topology on of $R^{n+1}$ using respect to trajectories, already yield some important results, such as the continuity of that distance function and the completeness of $R^{n+1}$ in this metric. However, this part is still wide open for exploration. For example, if we identify two trajectories that yield the same value for the cost functional as equivalent, then we can study the space of equivalence classes of trajectories. A new algebra of trajectories can also be built on these equivalent classes.

Among the highlights of the algebra part are Theorems 7, 8, 9 and 10.

Theorem 7 generalizes the Optimality Principle found on page 27 of Cesari [2] and includes the converse of that principle for sufficient trajectories. Theorems 8 and 9 are characterizations of chains of trajectories in terms of infinite series. Theorem 10 shows that the partial ordering of $R^{n+1}$ by means of trajectories induces a nice property for that space: completeness.

## Introduction

Young introduced the idea of bundle of trajectories [17]. A bundle is the set of trajectories of bounded flight times that meet a compact set in (t, x)-space. The key property of a bundle in this formulation is that of being sequentially compact

*With support from the Natural Sciences Research Institute, College of Science, University of the Philippines.

**Associate Professor of Mathematics, U.P., Diliman, Q.C.

and complete. This property is used to develop an existence theory for the optimal control problem. From the theory of ordinary differential equations, it follows that the elements of a bundle have a common extension on some compact interval T. It can be shown that a bundle is uniformly bounded and that the function $g(t, x, u)$ is also uniformly bounded on it [5].

In this paper we introduce the notion of extended bundle whose elements need not have bounded flight times. Nevertheless, like a bundle, an extended bundle is also uniformly bounded. It possesses properties that approximate the sequential compactness and completeness of a bundle, as well as the uniform boundedness of $g(t, x, u)$ on a bundle.

We will use the properties of an extended bundle to investigate some topological and algebraic structures in $R^{n+1}$. But first we set our basic premises.

*Relaxed Trajectories*

Let $g(t, x, u)$ be a vector-valued function with values in $R^n$ and suppose that g is continuous in $(x, u)$ for each t; measurable in t for each $(x, u)$; and bounded on bounded sets of $(t, x, u)$, where $t \in R$, $x \in R^n$ and u belong to a compact subset U of $R^m$, $m \leq n$. If $u(t)$ is a measurable function on a compact interval T with values in U, then the function $g(t, x, u(t))$ is measurable in t, being a composite of a measurable and a continuous function. A measurable function $u(t)$ on some compact interval T with values in U is called a *conventional control*. A *conventional trajectory* is a triple $C:(t, x(t); u(t))$, $t \in T$, where $u(t)$ is a conventional control and $x(t)$ is a solution, subject to an initial condition $x(t_o) = x_o$, of the differential equation,

$$\dot{x} = g(t, x, u(t)), \quad \text{a.e. } [t]. \tag{1}$$

The existence of such a solution is assured by a theorem of cartheodory's [1] in some neighborhood $(t_o, x_o)$ which, by subdivision, extends to any compact interval T. In that sense the solution is global.

Now, we assume further that g is Lipschitzian in x, in which case the solution of (1) is unique in some neighborhood of $(t_o, x_o)$. Thus uniqueness also holds along any compact interval T.

To achieve some completeness property for the space of trajectories, which is necessary for an existence theory and quite useful for topological, algebraic and even geometric investigation of $R^{n+1}$, we enlarge that space by embedding the control set U in the space of unit or probability measures V which is the closure of the convex hull of U. Our control function is now a function $v(t)$ with values in V. The function $v(t)$ is called a *chattering control*. For each t we denote by $g(t, x, v(t))$ the integral

$$g(t, x, v(t)) = \int_U g(t, x, u)\, dv(t) \tag{2}$$

where, for each t, v(t) is a unit measure on U. We consider an element $u \epsilon U$ as a unit measure on U concentrated at u. Thus a conventional control u(t) is a special case of a chattering control [16]. A *relaxed trajectory*, or simply a trajectory, is a triple C:(t, x(t); v(t)) satisfying the differential equation

$$\dot{x} = g(t, x(t); v(t)), \quad \text{a.e. } [t], \tag{3}$$

subject to some initial condition $x(t_0) = x_0$, where v(t) is a chattering control. It can be shown that subject to the above premises a solution x(t) of equation (3) is absolutely continuous [17]. If $C:(t, x(t); v(t))$, $t \epsilon T$, is a trajectory, $x(t)$, $t \epsilon T$, is called a representation of T and the set $[(t, x(t) \mid t \epsilon, T]$ is called the trace of C in $R^{n+1}$ or (t, x)-space. When there is no ambiguity, we may denote the trajectory C by C: (t, x(t)), $t \epsilon T$, or by its representation x(t), $t \epsilon T$, and refer to each of them as the trajectory C, provided we understand that there is some chattering control v(t) that generates C in accordance with equation (3). It is noteworthy that two control functions $v_1(t)$, $v_2(t)$, $t \epsilon T$, that generate the same trajectory, i.e., having common initial condition and flight time T and both satisfying equation (3), are equivalent in the sense that $v_1(t) = v_2(t)$, a.e., along T [17]. (For a modified formulation of relaxed trajectory see [10]).

We now define a notion of convergence of trajectories.

A sequence of vector functions

$$x_n(t), t \epsilon T_n, n = 1, 2, \ldots,$$

where the $T_n$ are compact time intervals all contained in some compact fixed time interval, will be said to *converge uniformly* to

$$x_0(t), t \epsilon T_0,$$

if, first, $T_0$ is a compact time interval whose extremities are the limit of those of $T_n$ and, second, for some choice of a compact interval T containing $T_0$ and all but a finite number of the $T_n$, there exist for large n, extensions of the functions of the form

$$x_n(t), t \epsilon T_n,$$

which tend uniformly to a corresponding extension to T of $x_0(t)$. ($T_0$ may reduce to a point).

A set of vector functions is called *sequentially compact* and *complete* if, given a sequence $x_n(t)$, $n = 1, 2, \ldots$, of vector functions in the set, there exists a subsequence that converges uniformly to a vector function in the set.

*Theorem 1.* (Young) A bundle is sequentially compact and complete and the set of simplicial trajectories in the bundle is dense in it [16].

(A simplicial trajectory is one that is generated by a simple conventional trajectory).

This theorem provides an automatic existence theorem for the solution of the optimal control problem [17]. For, since the dimensionality of the optimal control problem is at our disposal, we can replace g by $(f_0, g)$, where $f_0$ is the cost function, add one more component $z_0$ to x, and consider the differential equation

$$\dot{z} = (\dot{z}_0, \dot{x}) = (f_0(f, z, v(t)), g(t, z, v(t)) = \hat{g}(t, z, v(t)), \text{ a.e. } [t]$$

The first coordinate of z is subject to $\dot{z}_0 = f_0(t, z, v(t))$. Hence

$$z_0(t) = z_0(t_1) + \int_{t_1}^{t} f_0(s, z(s), v(s))ds,$$

or

$$z_0(t) = z_0(t_1) = \int_{t_1}^{t} f_0(s, z(s), v(s))ds.$$

Thus the minimum problem reduces to the search for a trajectory the difference of whose first component at the ends is minimum.

We summarize some of the related results proved in [10].

*Theorem 2.* The reachable set from a closed subset of $I \times R^n$, where I is a bounded interval, is uniformly bounded.

*Corollary.* The reachable set from a closed set whose initial times lie in a bounded time interval is closed.

*Theorem 3.* The reachable set from a bounded subset of (t, x)-space in finite time is bounded.

*Corollary.* The vector function g is uniformly bounded on the reachable set from a bounded set in finite time.

*Corollary.* The reachable set from a compact set in finite time is compact.

*Corollary.* The simplicial points are dense in the reachable set.

The *source* and *destination* of a trajectory are the initial and final points of that trajectory, respectively. A set B is the source of a family of trajectories if their sources lie in B.

*Extended Bundle*

We now consider a control system where, starting from a compact source B in (t,x)-space the representations of the trajectories either have final points in a bounded set D in $R^n$, called the target, or are asymptotic to D as $t \to \infty$. The Family trajectories that meet a compact set and are asymptotic to a bounded set form is called an *extended bundle.* Here, the point in motion may wander about

beyond any pre-assigned time t before going towards its destination or some trajectory may go beyond any pre-determined bound, i.e., given any finite ball in $R^n$, there may exist some chattering control that generates a trajectory whose representation has a point in common with the complement of the closures of the ball. We will identify the premises that would insure that those trajectories exhibit some properties of a bundle, or at least approximate some of its key properties, even if the flight times are unbounded.

But first we remark that this mathematical model approximates a gravitational system where the gravitational fields of certain bodies ultimately dominate the system and these bodies lie in a bounded set. The representation of every trajectory is either asymptotic to or intersects one of these bodies and does not escape that particular object. A special case of this setting is one where the gravitational field of one body ultimately dominates that system [10].

Let B be a compact source and O a $\delta$ -neighborhood of the bounded set D in $R^n$. A $\delta$ -neighborhood O of the bounded set D is the set in $R^n$ defined by

$$O = \bigcup_{x \in D} \{ y \mid \| x - y \| < \delta \}$$

where $\delta > 0$ is fixed.

Let M be the closure of O. We consider the case where M is disjoint from the components of B in $R^n$, the other case being simpler.

Since B is compact, the initial times of the trajectories with source B lie in some interval [a, b], where a and b are, respectively, the least and greatest time components among the elements of B. Now, we shall mark the destination of the initial segment of each trajectory at the last time it intersects the complement of O. We shall call that point the final time of impact on M. We shall consider those trajectories that are asymptotic to some subsets of D. They are useful for soft landing those that simply intersect D have bounded flight times and belong to a bundle. They lead to crash landing.

If the initial trajectories belong to a bundle then, since their "final segments" (with unbounded flight times) are contained in M, they are uniformly bounded and hence the entire family of asymptotic trajectories (with unbounded flight times) are uniformly bounded.

Do these initial segments of the trajectories (along with those that simply intersect D) form a bundle? No, since there is no upperbound for the time of impact on M. The point in motion may goof around beyond any specified time and bound before turning back to M.

There is no loss of generality in assuming that no time of impact on M lies in [a, b]. We can simply make B small enough so that $b$ would be close enough to $a$ and $[a, b]$ would miss every-final time of impact on M.

Now, what conditions will insure the uniform boundedness of the trajectories? The key is to force those initial segments into a bundle (bounded flight time) with suitable assumptions. We now identify those assumptions.

If our formulation is to have any practical application we must require that there is a limit to the cost integral, i.e., the integral

$$\int_C f_o \, dt$$

is bounded by some number L, uniformly in C. This is part of our premises. The requirement that $f_o > 0$, a.e., along any trajectory is not sufficient, for we can contrive a scenario where this is satisfied yet the time of flight need not be bounded. For example, if the cost integral $f_o$ is dominated by a function of the form $\frac{1}{e^t}$ then the cost integral along any trajectory C: (t, x(t)), $t_1 \leq t \leq t_2$ satisfies the inequality

$$\int_{t_1}^{t} f_o \, ds \leq \int_{t_1}^{t} \frac{1}{e^s} ds = -\frac{1}{e^t} + \frac{1}{e^{t_1}}$$

which remains bounded as t becomes positively large. i.e., as t remains unbounded. This also means that the point in motion can wander around for an indefinitely long period without exceeding cost limitations.

With this observation we now identify those conditions that would insure uniform boundedness of the flight times. We state them in the hypothesis of the theorem below.

But first let us introduce another concept. Given a bundle, which meets and is determined by some compact set K in (t, x)-space, we shall call a subbundle any of its subsets. Thus a subbundle also meets K. Therefore, any sequence belonging to a subbundle has a convergent subsequence but the uniform limit belongs to the bundle, not necessarily to the subbundle. Still it belongs to the closure of the subbundle. In this sense we also say that the closure of a subbundle is sequentially compact and complete.

*Theorem 4.* Suppose the cost functional $\int_c f_o \, dt$ along the relevant trajectories (originating from B) is uniformly bounded by some number L and $f_o$ is bounded away from 0, i.e., $f_o \geq \epsilon$ for some $\epsilon > 0$, then the initial segments of the extended bundle belong to a subbundle.

*Proof.* Without loss of generality, we assume that all the extended trajectories have been extended to an initial time *a*. (This follows from the existence theorem for differential equation (3), a theorem due to Caratheodory [1]).

Let F be the family of initial segments of the extended bundle with corresponding final impacts T, where T is a variable positive real number. Then the set of real numbers

$$E = \left\{ \int_a^T f_0 dt \quad | \quad C: (t, x(t)),\ t \in [a, T] \text{ belongs to } F \right\}$$

is uniformly bounded above by L. Therefore E has a supremum t. Since M is compact, it determines the bundle whose flight times are bounded by t. Therefore the initial segments of the extended bundle on flight times contained in [a, t] belong to this bundle and, therefore, form a subbundle. #

In the proof of the Theorem the family T forms a subbundle.

*Corollary.* Let $\delta > 0$. Then there exists a subbundle whose destinations lie in the $\delta$ -neighborhood of D.

*Proof.* Take O to be the $\frac{\delta}{2}$ -neighborhood of D and apply the Theorem. #

It is in the sense of this corollary that we can say that an extended bundle can be approximated by a subbundle. Since the closure of a subbundle is sequentially compact and complete, it contains a minimal trajectory. In view of this we state a more useful corollary.

*Corollary.* Let $\delta > 0$. There exists a minimal trajectory among the asymptotic trajectories, whose destination lies in a $\delta$ -neighborhood of the target D.

*Application*

In a space project, due to limitations of technology, the trajectory sought is a relative minimum because the path may be prescribed to pass through a set of points. Even if the path between two consecutive points is minimal, the entire chain of trajectories, which form a trajectory, need not be minimal.

For example, in the Euclidean plane the segments AB and BC are, respectively, the shortest distances from A to B and from B to C (see Figure 1). But the polygonal line ABC from A to C (via B) is not the shortest distance.

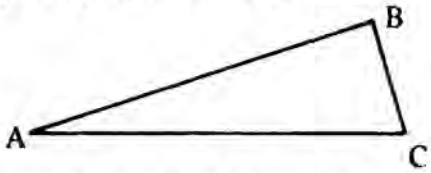

Figure 1

In an actual flight for soft landing on the moon, there are a number of phases. The first involves shooting for a space station in orbit around the earth or taking an orbit around the earth. The next phase, from this station or orbit, is concerned with taking the vehicle to an orbit around the moon. Usually the service module remains there after the second phase which involves firing the lunar vehicle towards a point on an arc of an orbit low enough for safe free fall under lunar gravity.

Taking an orbit around the earth or around the moon is really taking a trajectory asymptotic to the desired orbit and entering an $\epsilon$ -neighborhood of it at the proper injection angle (angle of the velocity vector with the tangent to the desired orbit). In such a project the trajectory is at least sectionally smooth, the result of a multi-stage power rocket and so such injection angle makes sense. The rapid braking action involved in this type of project, however, pushes the analysis

towards consideration of infinite chains which will be the subject of a future paper.

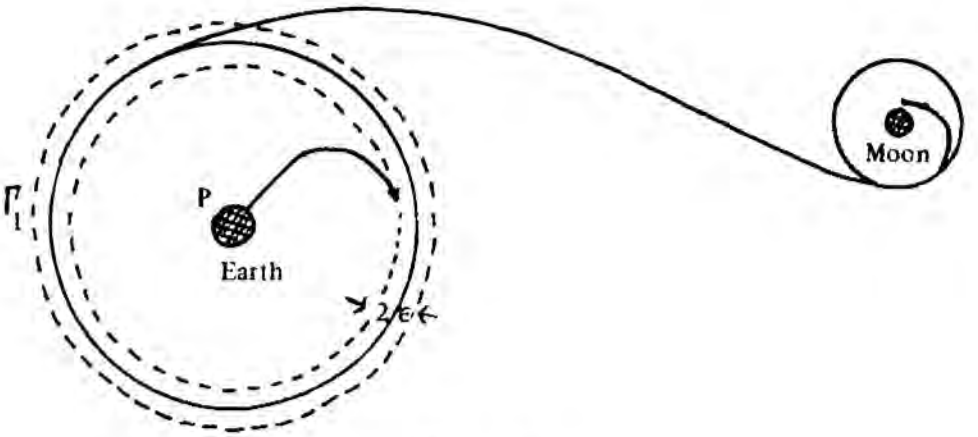


Figure 2

Take a cross-section K of the closure of the $\epsilon$ -neighborhood of the desired orbit $\Gamma_1$ of Figure 2. There is certainly a subbundle of the bundle determined by K which emanates from the point P and which reaches K at the proper injection angle. (The injection angle is generally very narrow and unless the trajectory reaches the vicinity of the desired orbit within this angle that orbit will be missed). That closure of the subbundle is sequentially compact and complete and, therefore, contains a relative minimum. The requirement of an injection angle further reduces the minimum to a relative one). A future paper will work out an algorithm for approximating the minimal trajectory, if known. Finding the minimal trajectory requires application of the Pontrjagin's Maximum Principle. The author has worked out a strong Pontrjagin's Maximum Principle [10] with very weak requirement at the ends. However, the Pontrjagin's Maximum Principle still needs to be investigated for applicability to probabilistic motion, especially the motion of an elementary particle in quant in mechanics.

The analysis for the succeeding phases is the same. In the last phase, it suffices to take the vehicle to a point above the target and low enough for a safe free fall under the action of lunar gravity. This last phase also involves an arc of a low orbit and the proper injection angle. For reasons of safety, the injection angle is much narrower here. An error could mean landing crash or getting lost in space.

We conclude this section with a few corollaries that would show some properties of extended bundle in common with a subbundle.

Since the initial segments of an extended bundle belong to a bundle, they are uniformly bounded. And since the rest of the extended subbundle lie in M, which is bounded, we have the following corollaries.

*Corollary.* An extended subbundle is uniformly bounded.

*Corollary.* The functions g and $f_0$ are both uniformly bounded on an extended subbundle.

*The Cost Metric*

This section is being introduced here as part of our topological analysis. But we need the notion of partial ordering (t, x) – space and so we refer the reader to page 31 of the next section for formal definition of $p \leq q$, where p and q are points of (t, x) – space).

We introduce a function $\rho$ from the Cartesian product of (t, x)-space with itself into the extended real numbers as follows. If p and q are points of (t, x)-space and $p \leq q$, we define $\rho(p, q) = \inf I(C)^*$ among all trajectories C from p to q. Since $f_0 > 0$, a.e., $\rho(p, q) \geq 0$. Note that C could be degenerate (could reduce to a point), in which case I(C) = 0. If p and q are not comparable, we write $\rho(p, q) = \infty$.

The function $\rho$ is a directed distance function, i.e., except for symmetry, it satisfies the following:

(a) $\rho(p, q) \geq 0$ for each pair p, q;
(b) $\rho(p, q) = 0$ if and only if p = q; and
(c) if $p \leq q \leq r$, then $\rho(p, r) \leq \rho(p, q) + \rho(q, r)$.

To have symmetry, we note first that

$$\rho(p, q) = \rho(q, p)$$

and set

$$\rho^*(p, q) = \inf |I(C)|$$

for all trajectories C connecting p and q. Then we have a genuine metric on certain types of control systems called $\rho$-homogenous.

A control system is *$\rho$-homogeneous* if, given p, q, r in (t, x)-space such that $p \leq q \leq r$, then $\rho(p, r) \geq \rho(p, q)$ and $(p, r) \geq \rho(q, r)$. It is clear that if the control system is $\rho$-homogeneous, then $\rho^*$ is indeed a metric on any connectible subset of (t, x)-space, i.e., it satisfies the requirement for a metric:

i) $\rho^*(p, a) \geq 0$ for each pair p, c
ii) $\rho^*(p, o) = 0$ if and only if p = q;
iii) $\rho^*(p, q) = \rho^*(q, p)$; and
iv) $\rho^*(p, r) \leq \rho^*(p, q) + \rho^*(q, r)$.

We shall call $\rho^*$ the cost metric.

---

*I(C) is the curvilinear integral along C: $(t, x(t), v(t)),\ t \in [t_1, t_2]$ $\int_{t_1}^{t_2} f(t, x(t), v(t)dt,$

+This notion was introduced in [3].

A set $\Omega$ in (t, x)-space is *connectible* if, given p, q $\epsilon$ $\Omega$, there exists at least one trajectory joining p and q. Thus $\rho^*$ induces a metric topology on any connectible set. We may identify a basis for a connectible set by means of a neighborhood system.

Let r be a point of a connectible set K and let $\delta$ be a positive real number. The star $\delta$-neighborhood of r is the set

$$(4) \qquad K^*_{(r,\,\delta)} = \left\{ p \in K \mid \rho(r, p) < \delta \right\}$$

Note that $p \in N^*_{(r,\,\delta)}$ implies p and r are comparable and since $f_0 > 0$, a.e., every point between them lies in the neighborhood. We may now construct a neighborhood system on a connectible set K of (t, x)-space as follows:

For each point $\rho \in K$, there is a neighborhood system with the following properties:

a) $\eta_r \neq \phi$
b) $N \in \eta_p$ implies $p \in N$.
c) $N_1, N_2 \in \eta_p$ implies there exists N such that $p \in N \subset N_1 \cap N_2$.
d) If $N \in \eta_p$ and $q \in N$ then there exists $N' \in \eta_c$ such that $N' \subset N$.

The totality $\eta$ of all neighborhood systems $\eta_p$ for all $p \in K$ defines a topology for K of which $\eta$ is a basis. With this metric topology the structure of k can be more fully investigated.

An extended real-valued map f on (t, x)-space is termed *star continuous* at r if, given $\epsilon > 0$, there exists a $\delta > 0$ such that $|f(p) - f(r)| < \delta$ whenever $p \in N_{(r,\,\delta)}$.

Let F be any connectible set. We define the set

$$F^{(-)} = \left\{ p \in R^{n+1} \mid p \leq q \text{ for every } q \in F \right\}$$

Consider the map $\rho^*(p, F) = \inf_{q \in F} \rho^*(p, q)$, where p is a variable point of (t, x)-space and F is a fixed connectible subset. The following theorem expresses the fact that this distance function is continuous.

*Theorem 5.* If the control system is $\rho$-homogeneous and F is any closed connectible subset of (t, x)-space, then the restriction of $\rho^*$ to $F^{(-)}$ is star continuous.

*Proof.* The theorem is trivial if $F^{(-)} = \phi$. Let $r \in F^{(-)}$, $\epsilon > 0$ and p be a point in some $\delta'$ -neighborhood $N_{(r,\,\delta')}$ of r. For each $q \in F$, either $r \leq p \leq q$ or $p \leq r \leq q$. It suffices to prove our assertion for one case, say, the first inequality.

By the triangle inequality, we have

$$\rho^*(r, q) \leq \rho^*(r, p) + \rho^*(p, q).$$

Since F is closed, there exists $s \in F$ such that

$$\rho^*(p, F) = \rho^*(p, s).$$

Hence

$$\rho^*(r, s) \leq \rho^*(r, p) + \rho^*(p, s),$$

But $\rho^*(r, F) \leq \rho^*(r, s)$. Therefore,

$$\rho^*(r, F) \leq \rho^*(r, p) + \rho^*(p, F),$$

Since the system is $\rho$-homogeneous, it is clear that

$$\rho^*(r, F) - \rho^*(p, F) \geq 0.$$

Therefore,

$$|\rho^*(r, F) - \rho^*(p, F)| = \rho^*(r, F) - \rho^*(p, F) \leq \rho^*(r, p).$$

Take the smaller of $\delta^1$ and $\epsilon$ for $\delta$. Then $\rho^*(r, p) < \delta$ implies $|\rho^*(r, F) - \rho^*(p, F)| < \epsilon$. #

*Corollary.* For each point q, $\rho^*(p, q)$ is star continuous and monotone decreasing on any pairwise comparable subset of $q^{(-)}$.

*Corollary.* If F is any connectible set, then $\rho^*(p, F)$ is star continuous and monotone decreasing on any pairwise comparable subset of $F^{(-)}$.

If A is a connectible set-and p a point on some connectible set containing A, then we can define the distance from p to A as

$$\rho^*(p, A) = \inf_{q \in A} \rho^*(p, q)$$

The diameter of a set A is defined as

$$D(A) = \sup_{p, q \in A} \inf \rho^*(p, q)$$

If A is connectible, then D(A) is finite. Otherwise, it is infinite. If A is a maximal pairwise comparable set, D(A) is called its length.

In view of the completeness property of [p, q] with the order topology, p and q are the minimum and maximum of [p, q], respectively. It is clear that $\rho^*([p, q]) \leq D([p, q])$.

*Theorem 6.* Let M be a maximal pairwise comparable set with minimum and maximum p and q, respectively. Then M, with the usual topology, is homeomorphic to the unit interval.

*Proof.* Let $p = (t_o, X_o)$ and $q = (t_1, t_2)$. For each $t \in [t_o, t_1]$, there exists a point $(t, x_t) \in M$ and some trajectory C with source p and destination $(t, x_t)$. The set of all such points, being pairwise comparable, is linearly ordered by $[t_o, t_1]$. Consider the map $f(t) = (t, x_t)$ which is defined for each $t \in [t_o, t_1]$. Since $t' \neq t''$ implies $(t', x_{t'}) \neq (t'', x_{t''})$, the function f is one-one. Let $\epsilon > 0$ and $t \in [t_o, t_1]$. For any $t' \in [t_o, t_1]$, $\rho^*(f(t), f(t')) = \inf \left| \int_t^t f_o(s, x(s), v(s))ds \right|$ along all trajectories connecting f(t) and f(t'). Since $f_o$ is bounded on M, there is some $\alpha$ such that

$$| f_o(s, x(s), v(s)) | \leq \alpha.$$

Take $\delta = \frac{\epsilon}{\alpha}$. Then

$$\rho^*(f(t), f(t')) \leq \alpha | t-t' | < \alpha \frac{\epsilon}{\alpha} = \epsilon$$

whenever $| t-t'| < \delta$. The inverse of f can be shown to be continuous as well being the projection of f(t) on the t-axis. Thus M is homeomorphic to $[t_o, t_1]$ and hence to $[0, 1]$. #

The set $F^{(-)}$ may be called the backward set of F. This has a perfectly symmetric relationship with the forward set $F^{(+)}$ defined as follows. If F is a connectible set we define the set

$$F^{(+)} = \left\{ q \in R^{n+1} \mid p \leqslant q \text{ for every } p \in F \right\}$$

All the statements about $F^{(-)}$ are true, with slight modification, for the set $F^{(+)}$ as well.

In the next section we will prove, using the results there and those of this section, a theorem which says that every maximal connectible subset is complete in the $\rho^*$ metric. #

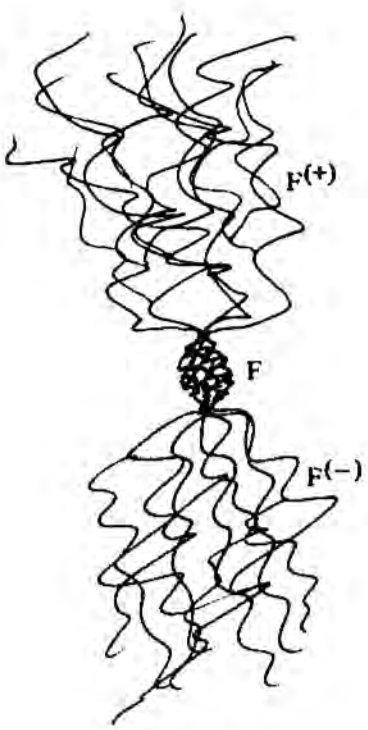


Figure 3

*Some Algebra on $R^{n+1}$*

In this section we introduce a binary operation on relaxed trajectories and and study chains of trajectories and their convergence.

We first introduce a binary operation or switching of trajectories.

Let $C_1$: $(t, x_1(t); v_1(t))$, $t_1 \leq t \leq t_2$ and $C_2$: $(t, x_2(t); v_2(t))$, $\bar{t}_1 \leq t \leq t_2$, be two trajectories that collide (intersect) such that the destination of $C_1$ coincides with the source of $C_2$, i.e., $t_2 = \bar{t}_1$ and $x_1(t_2) = x_2(\bar{t}_1)$. We define the *join* of $C_1$ and $C_2$ as the trajectory $C$:$(t, x(t); v(t))$, $t_1 \leq t \leq t_2$, where

$$(5) \qquad x(t) = \begin{cases} x_1(t) \ , \ t_1 \leq t \leq t_2 \\ x_2(t) \ , \ t_2 \leq t \leq \bar{t}_2 \end{cases} \qquad v(t) = \begin{cases} v_1(t), t_1 \leq t \leq t_2 \\ v_2(t), t_2 \leq t \leq \bar{t}_2. \end{cases}$$

We denote the join of $C_1$ and $C_2$ by $C = C_1 * C_2$. Note that join is a closed operation since $v(t)$ is measurable if $v_1(t)$ and $v_2(t)$ are. It is also an associative but not commutative binary operation. Thus switching or the join operation introduces some algebra on certain families of colliding trajectories.

The cost integral is additive on the join of two trajectories, i.e., if we define $I(C)$ by

$$(6) \qquad I(C) = \int_C \Gamma_o$$

and if $C = C_1 * C_2$ then

$$(7) \qquad I(C) = I(C_1 * C_2) = I(C_1) + I(C_2).$$

It is clear that the cost integral I is additive on a finite chain, i.e., the join of a finite number of trajectories.

A trajectory that joins two given compact sets will be called a *sufficient trajectory*. If C is a trajectory defined on some interval, a segment of C defined on some subinterval $[t_1, t_2]$ will be denoted by $C[t_1, t_2]$.

The following is proved in [10] but since that is still in press we reproduce the proof here. It is a generalization, and includes the converse, of the Principle of Optimality in [2].

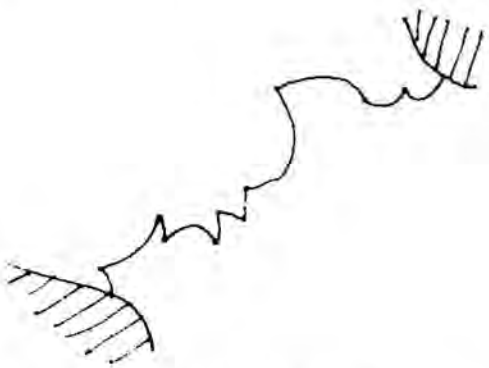

Figure 4. A sufficient finite chain of trajectories.

*Theorem 7.* A sufficient trajectory is minimal if and only if it is a finite join of segments from sufficient minimal trajectories.

*Proof.* The first part is trivial since every trajectory is a finite join of its segments (though this is not unique). We need only prove the converse: Any sufficient finite join of segments from sufficient minimal trajectories is a minimal trajectory.

It suffices to prove the theorem for the join of two segments from sufficient minimal trajectories since if there were N such trajectories involved then, by induction, the join of N-1 segments from sufficient minimal trajectories will be a segment of some sufficient minimal trajectories so that its join with the Nth segment reduces it to such a case. (See Figure 6).

Let C be the join of two segments of sufficient minimal trajectories $C_1$:$(t, x_1(t); v_1(t))$, $t_1 \leq t \leq t_2$ and $C_2$: $(\bar{t}, x_2(t)); v_2(t))$, $\bar{t}_1 \leq t \leq \bar{t}_2$ which collide at $(s, x_1(s))$, i.e., $x_1(s) = x_2(s)$ and $s \in [t_1, t_2] \cap [\bar{t}_1, \bar{t}_2]$ then

$$C = C_1[t_1, s] * (C_2[s, \bar{t}_2].$$

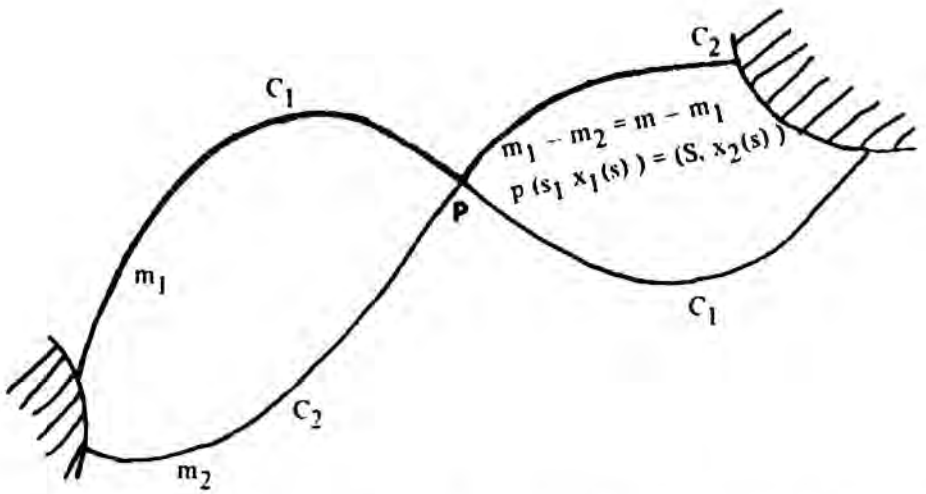


Figure 5. Sufficient minimal trajectories $C_1$ and $C_2$ collide at the point P at which the initial segments of $C_1$ and $C_2$, respectively, to form a sufficient minimal trajectory.

Let $I(C_1[\bar{t}_1, s] = m_1$, $I(C_2[\bar{t}_1, s] = m_2$ and let the minimum most integral be m, i.e., $I(C_1) = I(C_2) = m$. We want to show first that $m_1 = m_2$. For, suppose $m_1 < m_2$, say. Then

$$I(C) = I(C_1[t_1, s] * C_2[s, t_2]) = m_1 + (m - m_2) = m_1 + m - m_1 = m,$$

a contradiction since m is the minimum cost integral among sufficient trajectories and C is sufficient. Therefore $m_1 = m_2$ and it follows that $I(C_1[s, t_2]) = I(C_2[s, \bar{t}_2])$ so that $I(C) = m_1 + m - m_1 = m$. #

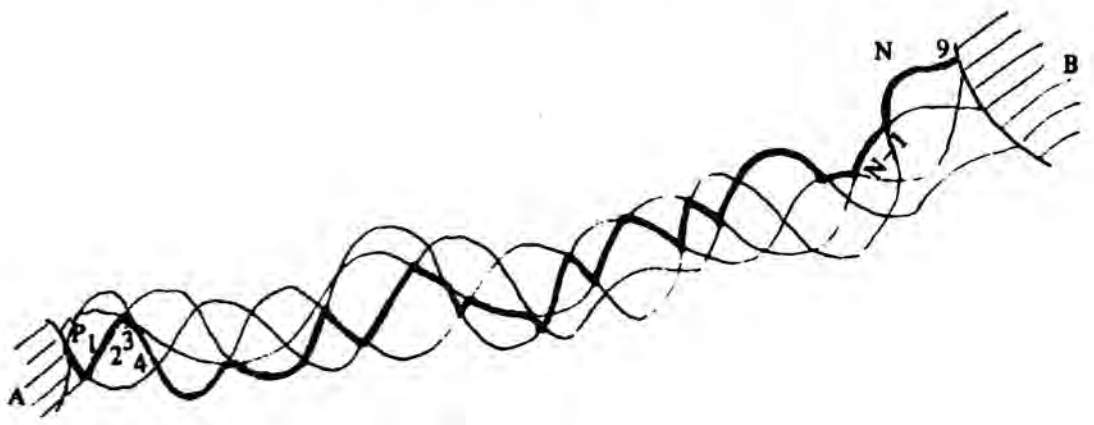


Figure 6. Projection in $R^n$ of a sufficient finite chain.

Figure 1 is a counter-example to show that the requirement "sufficient" in Theorem 7 is necessary. Neither AB nor BC in that figure is sufficient.

*An initial segment* $C_o : (t, x_o(t)), t_1 \leq t \leq t_2$ of a trajectory C: (t, x(t)), $t_1 \leq t \leq t_3$, where $t_1 \leq t_2 \leq t_3$, is a trajectory such that $(t, x_o(t)) = (t, x(t))$ on the common time of flight $[t_1, t_2]$. We construct the following joins of segments from sufficient minimal trajectories $C_1, C_2, \ldots C_n, \ldots$:

$$C_{11} = C_1\,[t_1, t_2]$$

$$C_{22} = C_1\,[t_1, t_2] * C_2\,[t_2, t_3]$$

---

$$C_{nn} = C_1\,[t_1, t_2] * C_2\,[t_2, t_3] * \ldots * C_n\,[t_n, t_{n+1}]$$
$$C_{n+1}\,[t_{n+1}, t_{n+2}] * \ldots$$

$$C_{nn}\ldots = C_{nn}^* = C_1[t_1, t_2] * C_2[t_2, t_3] * \ldots *$$
$$C_n\,[t_n, t_{n+1}) * C_{n+1}[t_{n+1}, t_{n+2}]^* \ldots .$$

From here on we denote by m the minimum, among all sufficient trajectories, of the cost integral I(C). The following theorem appears in [10]

*Theorem 8.* a) $I(C_{nn}\ldots)$ converges and $I(C_{nn}\ldots) = \lim_{n \to \alpha} I(C_{nn}) \leq m$.

b) If for some $\epsilon > 0$, $f_o \geq \epsilon$, a.e., along any trajectory, then $C_{nn}$ converges to an initial segment C of some sufficient minimal trajectory and for each n, $C_{nn}$ is an initial segment of C.

Proof of (a). Let n take the values n = 1, z, . . . , then we have an increasing join of segments from $C_1, C_2, \ldots$ If $C_{nn}$ hits the target at some point $(t_{N+1}, x(t_{N+1}))$ the conclusion follows from Theorem 7. Suppose that is not the case. Then we have an infinite "series" or chain of trajectories,

$$C_{nn}\ldots = C_1\,[t_1, t_2] * C_2\,[t_2, t_3] * \ldots * C_n\,[t_n, t_{n+1}] * \ldots,$$

of which $C_{nn}$ is a fundamental segment. Then $I(C_{nn})$ is also a fundamental segment of the series of positive real numbers

$$(8)\quad I(C_{nn}\ldots) = I(C_{nn} * \ldots) = I(C_1\,[t_1, t_2]) + I(C_2\,[t_2, t_3]) + \ldots + I(C_n\,[t_n, t_{n+1}]) + \ldots.$$

$I(C_{nn})$ is monotone increasing and bounded by m and from Calculus $I(C_{nn}\ldots) = \lim_{n\to\infty} I(C_{nn}) \leq m$. Equality holds if the limit of $C_{nn}$ is sufficient.

Proof of (b). By hypothesis and the conclusion in (a), $I(C_{nn}) \leq m$, and we can apply the argument in the first section by setting the set M as a a compact target, to establish an upperbound for the times of flight of the set $\{C_{nn}\}_{n=1, 2, \ldots}$. Therefore, the sequence $\{C_{nn}\}_{n=1, 2, \ldots}$ belongs to some bundle determined by the point $(t_1, x_1(t_1))$ and $\{C_{nn}\}_{n=1, 2, \ldots}$ has a convergent subsequence. By construction any subsequence of $\{C_{nn}\}_{n=1, 2, \ldots}$ inherits the property that for each $\ell \leq n$, $C_{\ell\ell}$ is an initial segment of $C_{nn}$. We will, without loss of generality, denote this convergent subsequence by $C_{nn}$ and the trajectory to which it converges by C. $\{C_{nn}\}_{n=1, 2, \ldots}$ has the interesting property that for each $\ell \leq n$, $C_{\ell\ell}$ is already in place and lies in C. It is only the destination of $C_{nn}$ and its shrinking (to a point) neighborhood that chase their limit, say q, which is clearly the destination of the limiting trajectory C. Since for each n, the destination of $C_{nn}$ lies on a sufficient minimal trajectory, q must be in some sufficient minimal trajectory $C_o$. The join of C and the upper segment of $C_o$ is a sufficient minimal trajectory (by Theorem 7). Now, C is clearly an initial segment of $C_o$ and, for each n, $C_{nn}$ is a fundamental segment of C. #

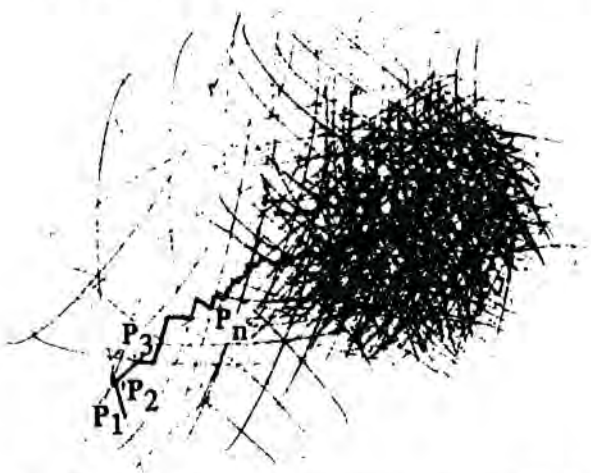


Figure 7. Projection in $R^n$ of a chain of trajectories formed by segments of trajectories.

*This idea was originally introduced in [3].

If a chain converges, that means it approaches some trajectory C. It also implies that I $(C_{nn} \ldots)$ converges and approaches the limit I (C). However, it is possible for I $(C_{nn} \ldots)$ to converge without $C_{nn}$ doing so. If the cost function is small the fundamental segment I $(C_{nn})$ of the series of cost integrals on the segments may converge yet $[C_{nn}]_{n=1, 2, \ldots}$ may not lie in a bundle if the times of flight are unbounded. However, we can force the formation of a bundle by requiring that $f_o$ be bounded away from zero. Thus if I $(C_{nn} \ldots) = \lim_{n \to \alpha}$ I $(C_{nn})$ converges, it must be bounded by some number $\alpha$. Therefore, the times of flight must be bounded, otherwise the inequality

$$\int_{t_1}^{t_{n+1}} \epsilon \, dt \leq \int C_{nn} f_o < \alpha,$$

will be contradicted since the left side diverges with $t_{n+1}$. Conversely, if $\{C_{nn}\}_{n=1, 2, \ldots}$ converges, I $(C_{nn} \ldots)$ converges. Thus we have the following theorem.

*Theorem 9.* If $f_o$ is bounded away from zero along any trajectory then the sequence of initial chains $\{C_{nn}\}_{n=1, 2, \ldots}$ converges if and only if I$(C_{nn} \ldots)$ converges.

*Corollary.* Subject to the hypothesis of Theorem 9, the infinite chain $C_{nn} \ldots$ converges if and only if the cost integral converges on it.

*Complete Lattice Property of $R^{n+1}$*

We next partially order the points of (t, x)-space by means or trajectories*, taking a rectangular coordinate system for $R^{n+1}$ whose origin is the point $(0, \overline{0})$.

Let p, q be points in (t, x)-space. The point p is said to be comparable with q if there is a trajectory C whose source is p and whose destination is q. In this case we say that p precedes q and write $p \leq q$. We may also write $q \geq p$. Clearly, the relation $\leq$ partially orders (t, x)-space and this ordering is induced by R.

A subset of (t, x)-space is said to be pairwise comparable if any two points of it are comparable. If $p \leq q$ but $p \neq q$ we write $p < q$ or $q > p$. It is clear that any pairwise comparable subset of our space is linearly ordered. Let $p \leq q$ and define

$$< p, q > = \{ r \mid p \leq r \leq q \}$$

A pairwise comparable subset of $< p,q >$ containing p and q is said to be maximal if it is a largest pairwise comparable subset of $< p, q >$. We will denote to such a subset by [p, q] and there may be more than one of them in the set $< p, q >$. Note that $p, q \in [p, q]$.

*Theorem 10.* Under the partial ordering $\leq$, (t, x)-space forms a complete lattice.

*Proof:* The partial ordering already provides a lattice structure on (t, x)-space. We need only prove that if a non-empty subset of (t,x)-space is bounded above (below) then it has a least upper bound (greatest lower bound) in (t,x)-space.

Let $A \neq \phi$ be a subset of (t,x) - space bounded above by some number q and define the following set of real numbers:

$T = \{t \quad x_t \text{ s.t. } (t, x_t) \leq q \text{ and } \underline{a} \in A \text{ implies } \underline{a} \leq (t, x)\}$. Then $T \neq \phi$, since the time component of q belongs to T, and the time at any element of A is a lower bound for T. Let s = inf T. We shall prove that there exist $x_s \in R^n$ such that $(s, x_s)$ is the least upper bound for A.

First we prove that $(s^-, x_s)$ is an upper bound for A. There exists a sequence of real numbers $t_j$, $j = 1, 2, \ldots$ such that $t_j \leq t_{j-1}$ and $t_j \to s$, and a sequence of points $x_j$, $j = 1, 2, \ldots$ in $R^n$ such that $\underline{a} \leq (t_j, x_j) \leq q$ for some $\underline{a} \in A$. The sequence of trajectories $C_j$ with sources $(t_j, x_j)$ and destination of clearly belongs to a bundle whose times of flight are bounded below by s and bounded above by the time at q. There is, therefore, a subsequence of points $(t_i, x_i)$ of $(t_j, x_j)$ as well as a corresponding subsequence of trajectories $C_i$ with sources $(t_i, x_i)$ and destination q that converge to some trajectory $C_2$. Its initial time is clearly s and we label its source as $(s, x_s)$ which is clearly a point of (t,x) - space.

Now at any point $\underline{a} \in A$ there is another sequence of trajectories with source $\underline{a}$ and destination $(t_1, x_1)$, $i = 1, 2, \ldots$ that also belongs to a bundle and whose times of flight are bounded by the times at $\underline{a}$ and q. Therefore, there exists a subsequence of trajectories that converges to some trajectory $C_1$ whose source and destination are $\underline{a}$ and $(s, x_s)$, respectively. Therefore, $\underline{a} \leq (s, x_s)$. The point $(s, x_s)$ is indeed the least upper bound for if there were some point $(t, x_t)$ such that $(t, x_t) < (s, x_s)$ then $t < s$, contradicting the choice of s.

The proof for the greatest lower bound is similar. #

*Corollary.* The set $\langle p,q \rangle$ and $[p,q]$ are complete lattices.

It is clear from the proof, and even on geometrical grounds, that the least upper bound (greatest lower bound) need not be unique. That is expected because we have a partial ordering.

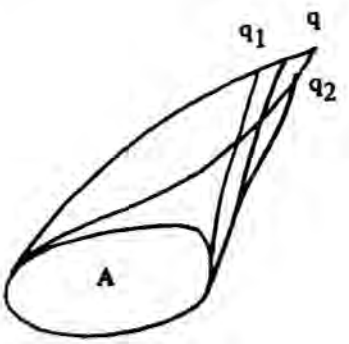


Figure 8

What is the implication of this theorem in practice? There might be a number of them. But one is quite obvious: If a point is reachable from any point in some set B, then there is one point that is reachable from B at the earliest time.

Finally, combining the results of the last two sections the following theorem should be quite obvious.

*Theorem 11.* Every maximal connectible subset of (t,x) - space is complete in the $\rho^*$ metric. #

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# MINIMIZING THE MAXIMUM DIFFERENCE BETWEEN INTEGER LABELS OF ADJACENT VERTICES IN A GRAPH

Severino V. Gervacio
*MSU-Iligan Institute of Technology*
*Tibanga, Iligan City 9200*

**ABSTRACT**

In 1962, F. Harary proposed the following problem. Given a graph G of order n, how can its vertices be labeled with the positive integers 1, 2, . . . , n such that the maximum difference between labels of adjacent vertices is a minimum?

We shall use the term *density* to denote the minimum of the maximum difference between adjacent labels in a graph of order n whose vertices are labeled with 1, 2, . . . , n. In this paper, we give formulas for densities of some classes of graphs as a function of their orders. In general, lower and upper bounds of the density of a graph in terms of graph parameters other than order are obtained. A note on the behavior of density with respect to the maximum degree of a graph is also included.

## Introduction

In this study all graphs considered are finite, undirected, loopless and without multiple edges. If G is a graph, V(G) denotes its vertex-set and E(G) denotes its edge-set. The graph G is written as the ordered pair $G = \langle V(G), E(G) \rangle$.

The symbol [x] denotes the greatest integer not exceeding x and the symbol [x] denotes the smallest integer not less than x.

An *integer label* of a graph G is an assignment of distinct integers to the vertices of G. More formally, we define an integer label of G to be a one-to-one mapping $\lambda: V(G) \to Z$, where Z is the set of all integers.

Let $\lambda$ be an integer label of a graph G, and let $a, b \in Z$ such that $a \neq 0$. We define the mapping $a\lambda + b$ by $(a\lambda + b)(v) = a\lambda(v) + b \quad v \in V(G)$. The following lemma is easy to prove.

LEMMA 1. Let $\lambda$ be an integer label of a graph G and let $a, b \in Z$ such that $a \neq 0$. Then $a\lambda + b$ is also an integer label of G.

Let $\lambda$ be an integer label of G. If $e = [a, b] \in E(G)$, we define the $\lambda$-*span* of e to be the positive integer $e_\lambda = |\lambda(a) - \lambda(b)|$. The *divergence* of $\lambda$ in G is define to be $G_\lambda = \max[\, e_\lambda : e \in E(G)\,]$. Finally, we define the *density* of G, denoted by p(G), to be the minimum $G_\lambda$, as $\lambda$ ranges over all the integer labels of G. We assume

that G has positive size (number of edges) in the definitions of divergence and density. For convenience, we define the density of a graph having size zero to be zero.

LEMMA 2. Let G be a graph. Then there exists an integer label $\lambda$ of G such that $\lambda$IV(G)) consists of consecutive integers and $G_\lambda = \rho(G)$.

Proof: Let $\phi$ be an integer label of G such that $G_\phi = \rho(G)$. If $v_1, v_2, \ldots, vn$ are the vertices of G, we may assume, without loss of generality, that $\phi(v_1) < \phi(v_2) < \ldots < \phi(v_n)$. If $\phi(V(G))$ does not consist of consecutive integers, we shall show how to construct one which does. Assume that $\phi(v_k)$ and $\phi(v_k)$ and $\phi(v_{k+1})$ are not consecutive integers, and let the positive difference between them be t. Define the integer label $\phi'$ by $\phi'(v_i) = \phi(v_i)$ if $i \leq k$ and $\phi'(v_i) = \phi(v_i) - t + 1$ otherwise. Obviously, the $\phi'$-span of any edge is less than or equal to its $\phi$-span. Hence, the divergence of $\phi$i is less than or equal to that of $\phi$. But $\phi$ has the minimum divergence. Therefore, $\phi'$ has the same divergence as $\phi$. Note that $\phi'(v_k)$ and $\phi'(v_{k+!})$ are consecutive integers already. This procedure may be repeated a sufficient number of times until we obtain the desired integer label $\lambda$.

Using Lemma 1, we easily deduce the following corollary.

COROLLARY. If G is a graph of order n, then there exists an integer label $\lambda$ of G such that $G_\lambda = \rho(G)$ and $\lambda(V(G))$ consists of 1, 2, . . ., n.

We define a *natural integer label* of a graph G of order n to be an integer label $\lambda$ such that $G_\lambda = p(G)$ and $\lambda(V(G))$ consists of the integers 1, 2, . . ., n.

This study deals mainly with the problem of finding natural integer labels of graphs — a problem proposed by Harary in 1962.

LEMMA 3. Let G be a graph and H a subgraph of G. Then $\rho(H) \leq p(G)$.

Proof: Let $\lambda$ be a natural integer label of G. Then the restriction $\lambda|_H$ of $\lambda$ to H is an integer label of H whose divergence is less than or equal to $\rho(G)$. It follows $p(H) \leq p(G)$.

LEMMA 4. Let G be a graph whose connected components are $G_1, G_2, \ldots, G_k$. Then $g(G) = \max\{\rho(G_i)\}$.

Proof: Observe that if $\lambda$ is an integer label of a graph H, then the mapping $\lambda + b$, where b is any integer, is also an integer label of H with the same divergence as $\lambda$. Furthermore, if $\lambda(V(H))$ consists of consecutive integers, then $(\lambda + b)(V(H))$ also consists of consecutive integers. Let $\lambda_1$ be a natural integer label of $G_1$. In view of our observation, we can find an integer label $\lambda_2$ of $G_2$ whose divergence is $\rho(G_2)$ and such that $\lambda 2(V(G_2))$ consists of consecutive integers the smallest of which is equal to the order of $G_1$ plus 1. We see therefore that integer labels $\lambda_i$ can be found such that the divergence of $\lambda_i$ is $\rho(G_i)$ and that the union of all $\lambda_i(V(G_i))$ is $\{1, 2, \ldots, n\}$, where n is the order of G. The mapping $\lambda$ whose restriction to $G_i$, for each i, is $\lambda_i$ is then an integer label of G whose divergence is equal to $\max\{\rho(G_i)\}$. Therefore $\rho(G) \leq \max\{\rho(G_i)\}$. Now, since each $G_i$ is a subgraph of G, $\rho(G_i) \leq \rho(G)$. It follows that $\max\{\rho(G_i)\} \leq \rho(G)$. Therefore, $\rho(G) = \max\{\rho(G_i)\}$.

In view of the preceding lemma, we need to consider only the problem of finding natural integer labels of connected graphs.

The formulas in the next lemma are easy to derive. Part (d) may be established with the help of the idea presented in the proof of Theorem 3.

LEMMA 5.

(a) $\rho(P_n) = 1$ for all paths $P_n$ with $n \geq 2$.

(b) $\rho(C_n) = 2$ for all cycles $C_n$ with $n \geq 3$.

(c) $\rho(K_n) = n-1$ for all complete graphs $K_n$ with $n \geq 2$.

(d) $\rho(K_{m,n} = [(m+1)/2] + n - 1$ for all complete bipartite graphs $K_{m,n}$ with $m \geq n$.

COROLLARY. If G is a graph with maximum clique of order q, then $\rho(G) \geq q-1$.

Proof: This follows from Lemma 3 and Lemma 5 (c).

Note that this lower bound is attained by all paths of order greater than 2, and hence is a best possible lower bound.

**Bounds for density in terms of maximum degree or minimum degree**

THEOREM 1. Let G be a graph with maximum degree $\Delta$. Then $\rho(G) \geq [(\Delta+1)/2]$.

Proof: Since G has maximum degree $\Delta$ then G contains a subgraph isomorphic to $K_{\Delta,1}$ whose density, by Lemma 5, is $[(\Delta+1/2]$. By Lemma 3 (d), $\rho(G) \geq [(\Delta+1)/2)$.

By Lemma 5 (c), the density of $K_{1,n}$ is $[(n+1)/2]$. Since n is the maximum degree of this graph, we see that the lower bound given in Theorem 1 is best possible.

LEMMA 6. Let G be a graph of order n, v a vertex of G, and $N_v = \{\lambda: \lambda$ is a natural integer label of G with $\lambda(v)= n\}$. Then $G_\lambda \geq \deg(v)$ for each $\lambda \in N_v$.

Proof: Let $\deg(v) = d$ and let $v_1, v_2, \ldots, v_d$ be all the neighbors of v. Let $\lambda \in N_v$ and without loss of generality we assume that $\lambda(v_1) < \lambda(v_2) < \ldots < \lambda(v_d)$. Since $\lambda(v) = n$, then $\lambda(v_d) \leq n-1$ and $\lambda(v_1) \leq n-d$. Therefore the $\lambda$-span of the edge $[v, v_1]$ is at least $n-(n-d) = d$. Therefore $G_\lambda \geq d = \deg(v)$.

THEOREM 2. Let G be a graph with minimum degree $\sigma$. Then $\rho(G) \geq \sigma$.

Proof: Let $V(G) = \{v_1, v_2, \ldots, v_n\}$ and let $N_i = \{\lambda: \lambda$ is a natural integer label of G such that $\lambda(v_i) = n\}$. Then $N = \cup N_i$ is the set of all natural integer labels of G. Now, $\rho(G) = \min\{G_\lambda: \lambda \in N\} = \min\{\min\{G_\lambda: \lambda \in N_i\}: i = 1, 2, \ldots, n\} \geq \sigma$.

The *complement* of G, denoted by $\tilde{G}$, is the graph with vertex set V(G) and where two distinct vertices are adjacent if and only if they are not adjacent in G.

COROLLARY 1. Let G be a graph with maximum degree $\Delta$ and minimum degree $\sigma$. Then $\rho(G) + \rho(\tilde{G}) \geq n-1-(\Delta-\sigma-)$

Proof: The minimum degree of $\tilde{G}$ is $n-1-\Delta$. Thus, applying Theorem 2, we get the desired result.

If G is an r-regular graph, then $\sigma = \Delta = r$ and we get the following corollary.

COROLLARY 2. If G is a regular graph, then $\rho(G)+(\rho(\tilde{G}) \geq n-1$.

The lower bound for density given in Theorem 2 is also best possible since it is attained by the cycle $C_n$. In fact, if $n > 3$, the complement of the cycle $C_n$ also attains the same lower bound, namely $\sigma = n-3$.

**Bound for density in terms of stability number**

THEOREM 3. Let G be a graph of order n and stability number $\alpha(G)$. Then $\rho(G) \leq n - 1 - [\alpha(G)/2]$.

Proof: Let S be a maximum stable set in G, i.e., $|S| = \alpha(G)$. Let $\lambda$ be any integer label of G such that $\lambda(V(G)) = \{1, 2, \ldots, n\}$ and $\lambda(S)$ consists of the $[\alpha(G)/2]$ smallest labels and the $[\alpha(G)]$ largest labels, i.e., $\lambda(G) = \{1, 2, \ldots, [\alpha(G)/2], n, n-1, \ldots, n-[\alpha(G)/2]+1\}$. Then no edge can have a $\lambda$-span of $n-[\alpha(G)/2]$ or more. Hence, $\rho(G) \leq n-1-[\alpha(G)/2]$.

The upper bound for density given in Theorem 3 is best possible since it is attained by the graph $K_{1,n}$.

THEOREM 4. Let G be a graph with stability number $\alpha$ and minimum degree $\sigma$. Then $\rho(G) + \rho(\bar{G}) \geq \alpha + \sigma - 1$.

Proof: Clearly, the complement of G contains a clique of order $\alpha$. Hence, combining Theorem 2 and the Corollary to Lemma 5, we get the desired result.

**Bound for density in terms of diameter**

LEMMA 7. Let $P = x_1 x_2 \ldots x_k x_{k+1}$ be a path of length k and let $\lambda$ be an integer label of P such that $\lambda(x_1) = 1$, $\lambda(x_{k+1}) = n$ and $1 \leq \lambda(x_i) \leq n$ for each i. Then the divergence $P_\lambda$ is at least $[(n-1)/k]$.

Proof: For convenience, we let $\lambda(x_i) = a_i$. In case $\langle a_i \rangle$ is an increasing sequence, then the summation of all $\lambda$-spans is n-1. Otherwise, we consider the maximal monotonic subsequences of $\langle a_i \rangle$:

$$a_1 < a_2 < \ldots < a_{n1}$$
$$a_{n1} > a_{n1+1} > \ldots > a_{n2}$$
$$a_{n2} < a_{n2+1} < \ldots < a_{n3}$$
$$\vdots$$
$$\vdots$$
$$a_{np} < a_{np+1} < \ldots < a_n$$

In this case, we see that the sum of all $\lambda$-spans is $(a_{n1} - a_1) + (a_{n1} - a_{n2}) + (a_{n3} - a_{n2}) + \ldots + (a_n - a_{np})$. Clearly, this quantity is greater than $n - 1$. Thus, in all cases, the average $\lambda$-span is at least $[(n-1)/k]$. It follows that the maximum $\lambda$-span is at least $[(n-1)/k]$. Consequently, the divergence is at least $[(n-1)/k]$.

THEOREM 5. Let G be a connected graph of order n and diameter d. Then $\rho(G) \geq [(n-1)/d]$.

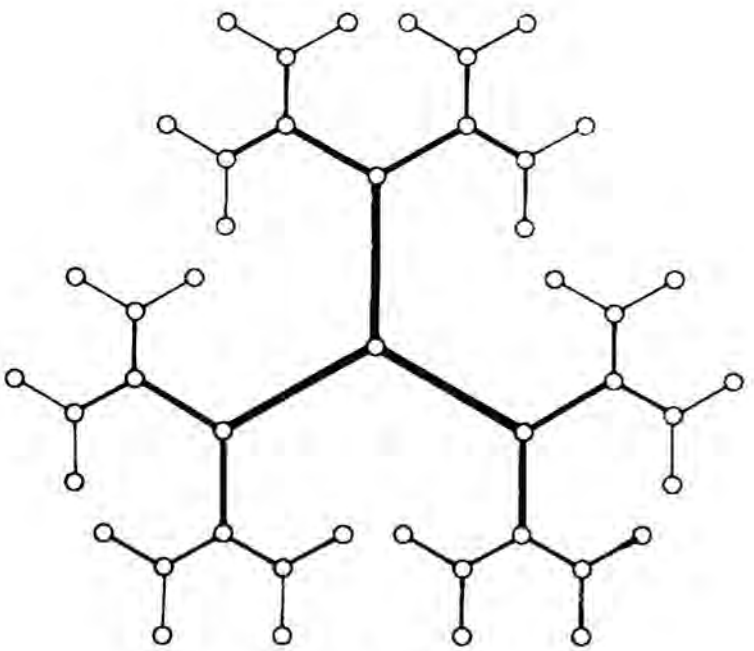
Proof: Let $\lambda$ be any natural integer label of G. Let u, v be the vertices with $\lambda(u) = 1$ and $\lambda(v) = n$. Let P be any shortest path joining u and v. Then by Lemma 7, there is an edge of P whose $\lambda$-span is at least $[(n-1)/k]$, where k is the length of P. But $k \leq d$ since d is the maximum length of a path in G. Hence $[(n-1)/k] \geq [(n-1)/d]$. It follows that $\rho(G) \geq [(n-1)/d]$.

Observe that the lower bound for density in Theorem 5 is attained by the path $P_n (n>1)$, the cycle $C_n$ $(n>2)$, and the complete graph $K_n (n>1)$.

**Further result**

This section aims to show that it is impossible to get an upper bound for density in terms of maximum degree only. Specifically, given a fixed $\Delta > 2$, a graph with maximum degree $\Delta$ may have an arbitrarily large density. We will illustrate this for $\Delta = 3$ by showing that given any positive integer N, there exists a graph G of maximum degree 3 whose density is greater than N.

We define $G_1 = K_{1,3}$. Recursively, we define $G_k$ as follows. Take as many mutually disjoint copies of $K_{1,2}$ as there are end vertices of $G_{k-1}$ $(k>1)$. To each end vertex of $G_{k-1}$, attach the central vertex of one $K_{1,2}$. The resulting graph is $G_k$. It is easy to see that $G_k$ has diameter 2k and simple mathematical induction will show that it has order $3 \cdot 2^k - 2$. As an example, the graph $G_4$ is shown below.



GRAPH G4

By Theorem 5, $\rho(G_k) \geq [(n-1)/2k] = [3(2^k-1)/2k]$. Hence, given any integer $N > 0$, we choose k to be any integer which is greater than 2-|n(N)/|n(2). Then $\rho(G_k) \geq [3(s^k - 1)/2k] > N$.

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# USE OF SIMULATION IN EVALUATING POTENTIAL YIELD AND WEATHER-RELATED VARIABILITY IN CROP PRODUCTION*

Felino P. Lansingan
*Institute of Mathematical Sciences and Physics*
*College of Arts and Sciences*
*University of the Philippines at Los Baños, College, Laguna*

**ABSTRACT**

Estimation of potential yield of a crop over an area and evaluation of its stability due to weather fluctuation are important concerns in crop production. The use of a crop growth model that provides a sound ecophysiological basis for evaluating crop yield and yield variability due to weather or other factors that are otherwise difficult to analyze through field experiments or on-farm trials is presented. Applications of simulation as a scientific tool in crop production research and development are illustrated by simulating crop behavior under different management situations and weather conditions.

## Introduction

Statistics on yields and areas under crops are often used as determinants of crop production. Reliable estimates of crop production are imperative in formulating and planning national programs to meet the food requirements of increasing population. Crop production data are used for such purposes as implementing policies on importation or exportation of agricultural products during deficit or surplus periods, formulating price policies (including subsidies) to encourage improved crop production, measuring the contribution of agriculture to the gross domestic product (GDP), and assessing agricultural productivity level (FAO, 1982).

The impact of weather on agricultural production is often evaluated in terms of crop yield stability or yield loss due to extreme weather conditions. However, assessment is usually based on subjective measurements through interviews or ocular inspection, or on empirical procedures, such as regression models or some yield loss indices. But the planning and decision-making applications of such important basic information require more improved methods and reliable and valid agricultural statistics, such as crop areas and yields as well as the associated variability due to weather fluctuations.

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## Estimation of Crop Yield

Several methods of estimating crop yields have been used to provide data on crop production components. One widely used method is eye estimation or "guesstimation" of expected yield per unit area, and also of areas under different crops. This method can provide reliable estimates only if the data collector is highly experienced and results are further validated by other means. Another method is by crop-cutting where a smaller area under a crop is harvested and the yield for a much bigger area is estimated by extrapolation. This method is very accurate but may be costly and time-consuming. An alternative method being used, particularly in developed countries, is the aerial estimation technique using aerial photographs and/or imageries. This procedure is used only for large areas where the cost of aerial photographs is relatively cheap or minimal. It requires experience and is expected to provide reliable estimates.

The use of yield prediction models has also become popular because of its accuracy and ease of application. The method is largely statistical, requiring historical data series on which to base or develop the model. There are quite practical reasons for moving from the purely experimental/statistical approach to a process-type approach. Just recently, the use of crop models (Penning de Vries, *et al.*, 1989) to simulate crop growth and also determine crop yield were proposed. Such process-based crop models may also be used to evaluate impacts of factors, such as weather fluctuations and/or biological constraints on crop production (see e.g. Penning de Vries, *et al.*, 1989; Lansigan, *et al.*, 1989).

## Evaluating Crop-Weather Relations

Weather is an important factor in crop production and greatly influences the stability of crop yields. Weather variables, such as rainfall, solar radiation, and temperature influence significantly the rate of crop growth. In the tropics, weather-related perturbations such as droughts, floods, typhoons, and strong winds account for significant loss in crop yields. Weather also indirectly exerts influence on biotic stresses to crop growth like pests, diseases, and weeds.

One commonly used indicator in evaluating crop-weather relations is yield performance of crop grown under weather conditions or climatic zones. The reliability of the assessment depends on the ability to replicate a weather condition under which the crop is grown, as well as in the ability to control other factors in the field excluding weather. Unfortunately, however, this is almost impossible to do.

There are two basic methods in evaluating crop-weather relations. In the first method, the statistical or correlation approach, empirical relations or functions are determined between yield and one or more weather variables usually by regression analysis. The form of the model may vary in the number of variables, type of weather data used, and crop being considered, and may apply only for a specific

location or region. Although statistical models can provide reasonable quantification of weather effects, they require a large set of data on crop yields and weather variables. Thus, their applicability may be quite limited. Moreover, since these models do not relate to the ecophysiological processes governing crop growth, they are less useful in research on crop production and on crop-weather interactions.

The second approach is by using crop simulation models. Since these models are based on crop physiology, they are applicable for any crop anywhere, provided the quantitative information on the processes required in the model are available. Construction of crop models uses the systems analysis approach whereby the key processes involved in crop growth are incorporated in the simulation models. However, the reliability of the models depends on the quantitative representation of the basic processes involved. Due to the large data sets required and iterative calculations to be handled, crop simulation models necessitate the use of computers with adequate storage memory. Moreover, modeling in the computer requires the use of an efficient simulation language.

This paper illustrates the potential uses of crop modeling and simulation in estimating crop yield and evaluating yield stability in crop production due to weather variability. The presentation is concerned with rice since it is an important crop, although the same approach may be used for other crops as well. Summary models for rice (e.g. RICEMOD, IRRIMOD, etc.) are already available and have been validated. Applications of such models in agricultural research particularly on rice-based cropping system will be presented by citing some of the research and simulation results that have already been done elsewhere.

## Crop Simulation Model

A crop simulation model is a simplified representation of a crop considering the processes governing crop growth. Such as model can be used to determine the crop behavior under a specific environmental condition. Several crop simulation models have already been developed and documented (see e.g. Penning de Vries *et al.*, 1989; McMennamy and O'Toole, 1983), and have been demonstrated to give satisfactory results during evaluation. Utilizing the systems approach, these models allow identification of those processes and interactions which are not yet sufficiently understood or quantified. Thus, they easily help define directions and goals for further research.

Figure 1 shows a relational diagram of a model for a crop growing under optimal conditions, i.e. no water stress, without soil nutritional deficiency, and no pest and disease problems. The relations among key crop growth processes such as photosynthesis, maintenance respiration, and assimilate partitioning are indicated, so are the effects of light and temperature on crop growth and development. Since the model is process-based, other process components may be introduced without changing its basic form. For instance, a crop growing under limited water may be

modeled by incorporating a soil water balance component into the basic crop model (see Figure 2). Likewise, the effects of pests and diseases on crop can easily be introduced in the basic model (see e.g. Rabbinge *et al*, 1989).

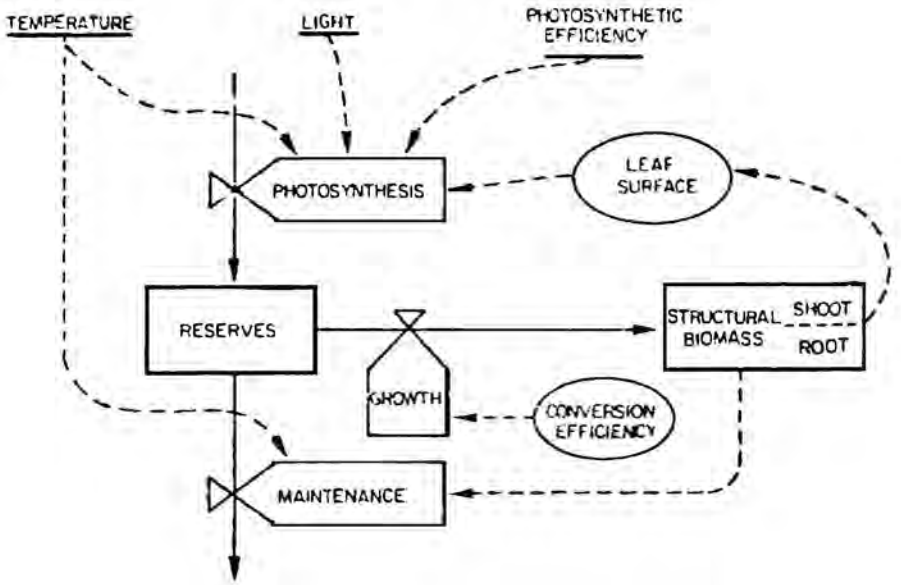


Figure 1. A Relational diagram of a model of a crop growing under well-watered condition and without nutritional or pest problems (Penning de Vries and van Laar, 1982). Light and temperature affect growth and development.

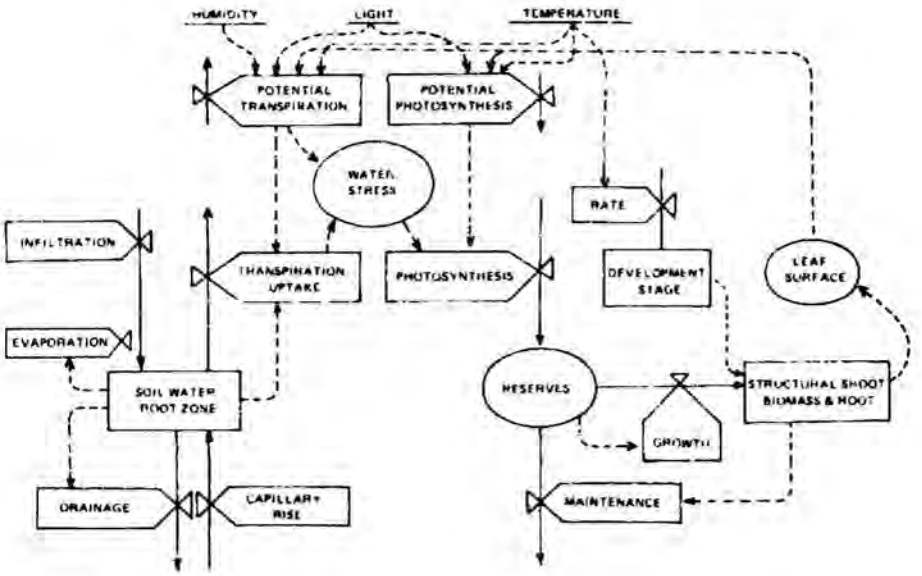


Figure 2. A relational diagram of key processes in crops growing with limited water. From: Penning de Vries *et al*, 1989.

Since the crop model considers the key ecophysiological processes affecting crop growth and development, many input data sets are required in the simulation. Aside from the crop data and weather data sets (either historical or generated), the model may require soils data (e.g. soil depth, number of soil layers, soil type, etc.). Although some quantitative information on the basic processes are available for some crops such as rice, description of the processes still need to be based on experimental data.

The dynamic rice growth model (Penning de Vries *et al.*, 1989) is a summary model that simulates the such processes as photosynthesis, respiration, and crop phenological development for time increments of one day (De Wit, *et al.*, 1978). One computer language that is widely used in crop simulation model is CSMP which has been adapted recently for IBM PC micro-computers or compatibles.

## Some Practical Applications of Crop Models

### *Determination of Optimal Cropping Calendar*

One useful application of a crop model in determining the best time to plant crop in a particular location. The optimal planting period can be approximated by simulating crop yields given, say, 20 years of weather data (Lansigan, *et al.*, 1987), and evaluating at which time periods the yields are relatively high and stable.

Figure 3 shows the simulated yields of a rice crop planted during different dates throughout the year under Los Baños condition based on 20 years historical weather data. It is noted that yields are high during the period of April-November which coincides with the wet season in the area.

### *Evaluating Weather-Related Yield Variability*

Weather-related variability in potential production can also be evaluated quantitatively using a crop model by determining crop performance under different weather conditions and management situations. Figures 4 and 5 show the simulated yields of rainfed rice and computed yield variabilities for Iloilo and Davao, respectively. The figures indicate the different behavior of crop yield under two different climatic regimes. Yield variability is relatively low during May-October in Iloilo; it is also low throughout the year in Davao.

### *Influence of Biological Constraints*

The effect of pests and diseases on crop yield can also be assessed by incorporating a pest model component in the basic crop model. For example, stemborer damage on rice crop has been simulated, and validation with experimental data in the field show good results (Xia, 1988).

On the other hand, yield reduction due to weeds has also been evaluated and simulations show satisfactory results (Legaspi, *et al.*, 1987). Crop-weed competition

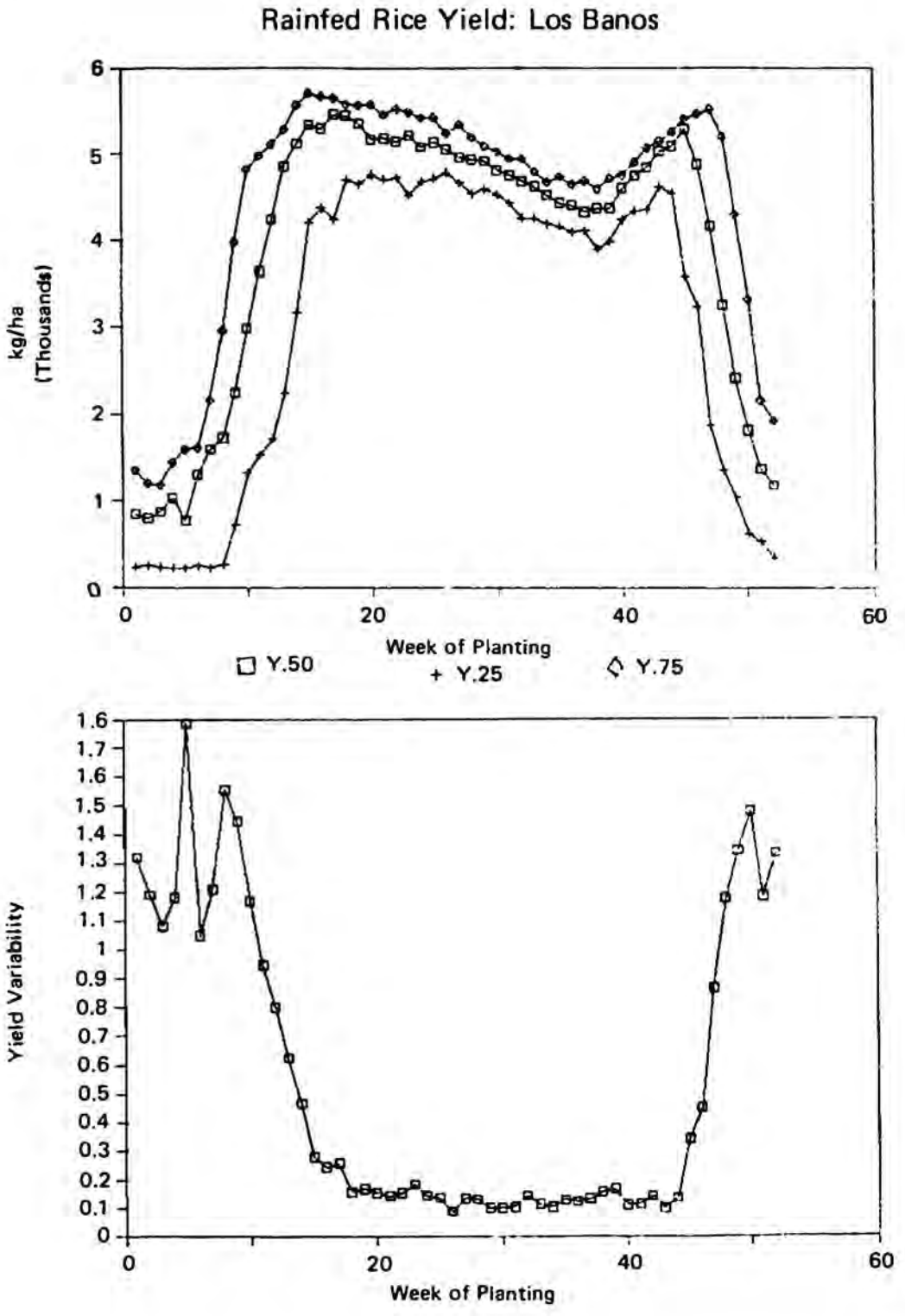


Figure 3. Simulated potential rainfed rice yields and associated variability under Los Baños conditions.

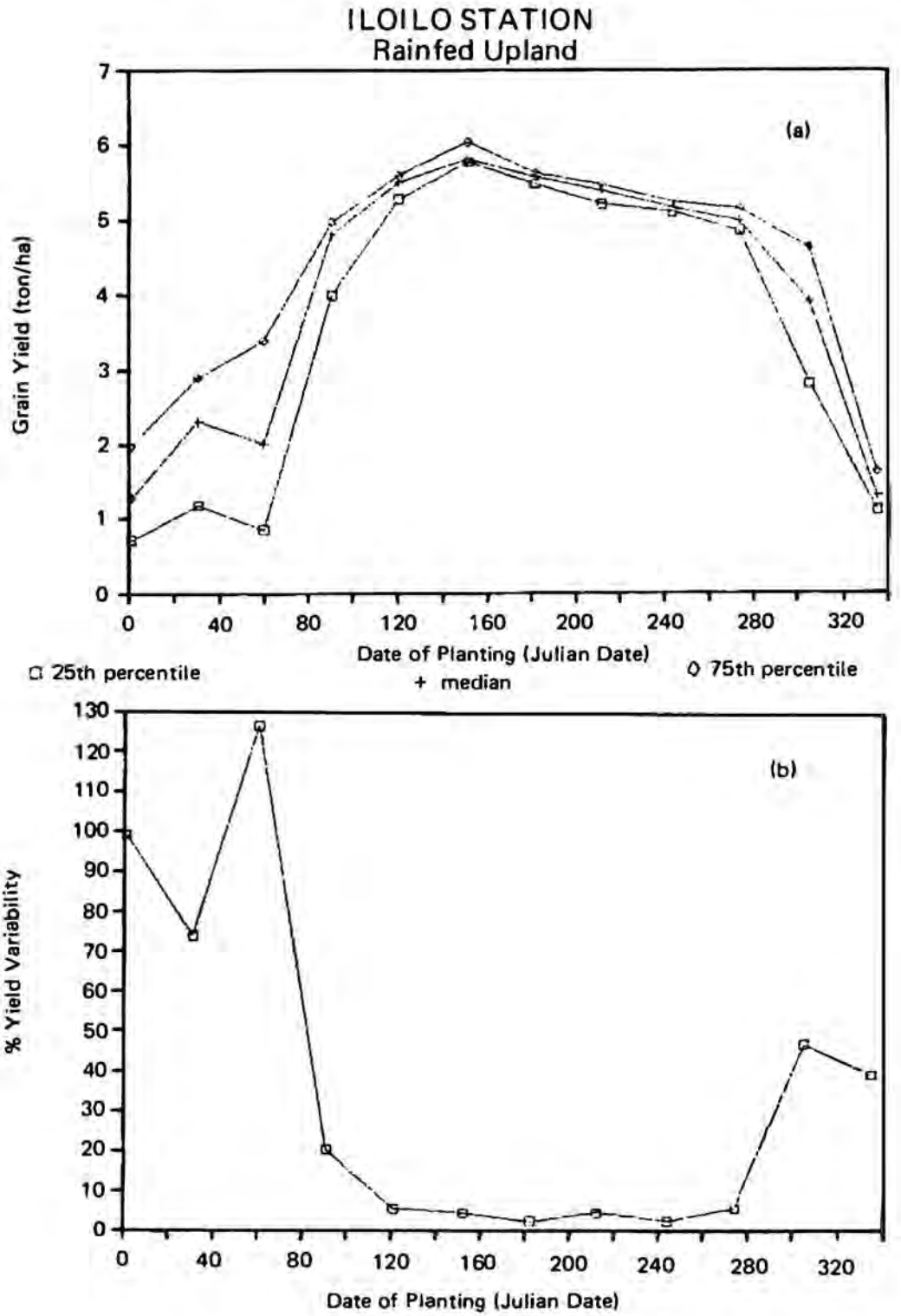


Figure 4. Simulated (a) median and quartile yields of rainfed rice; and (b) computed yield variability for Iloilo.

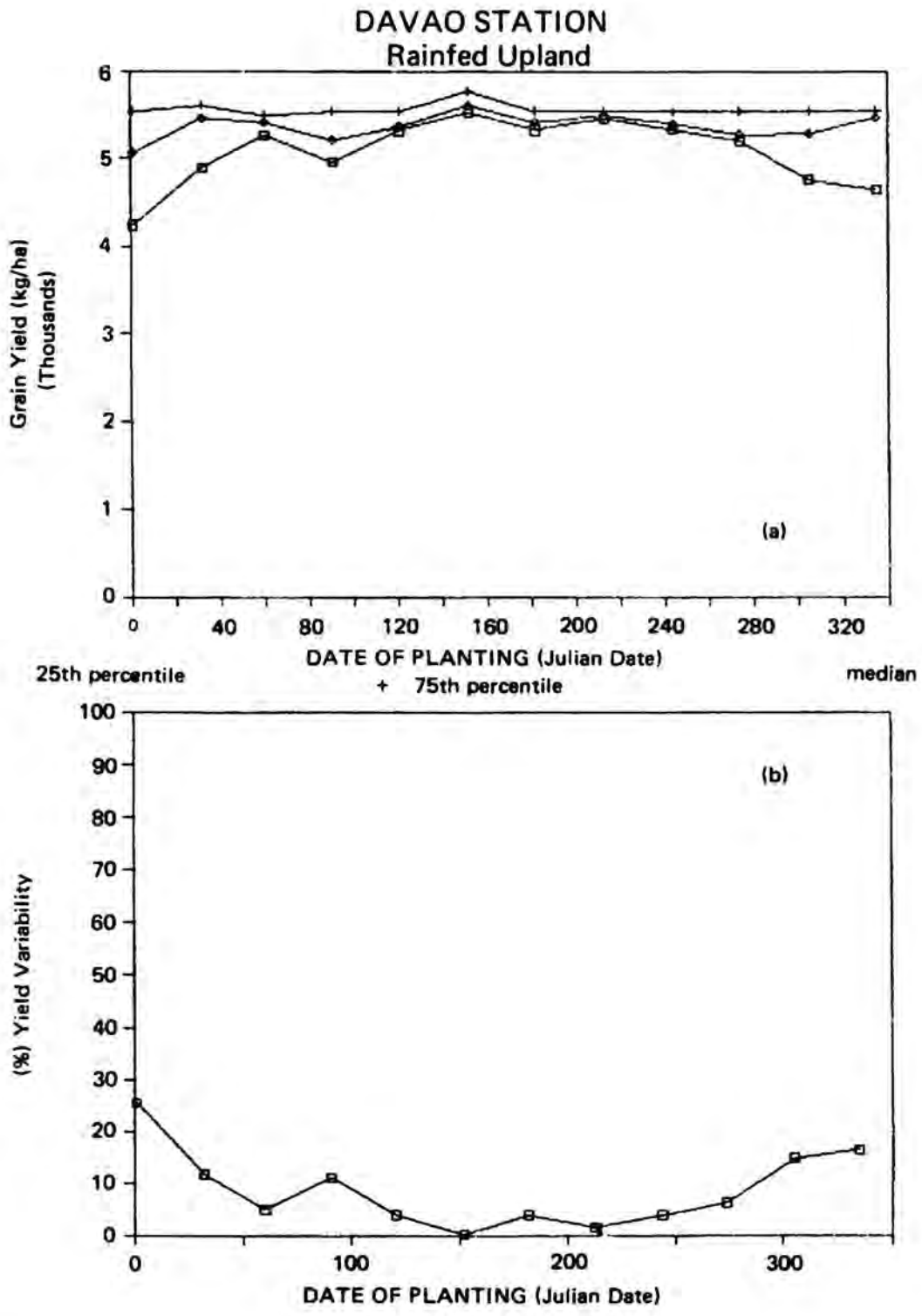


Figure 5. Simulated (a) median and quartile yields of rainfed rice; and (b) computed yield variability for Davao.

is simulated by treating the weeds as another crop that competes with the rice crop in water, nutrients, and sunlight. The crop-weed competition model can then be used to determine the frequency and timing of weeding during rice crop growing period.

## Concluding Remarks

Crop simulation has been shown in several practical applications to be a potentially useful tool in crop production research particularly in the quantitative evaluation of crop performance under different management situation and environmental conditions. The crop models that have been developed based on the present scientific knowledge and understanding of the ecophysiology of the crop can be used to identify and study the factors that influence growth and development, and thus define the knowledge or information gaps which can be addressed in future research.

Dynamic crop simulation models allow a more objective estimation of potential yield and evaluation of weather-related variability in crop production which are difficult to conduct otherwise. The use of crop simulation models in conjunction with other conceptual models provides for a cost-effective and efficient approach in agricultural research particularly on crop production.

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# A CUBIC EQUATION OF STATE FOR PHASE EQUILIBRIUM CALCULATIONS

Claro T. Llaguno and Errol A.C. Sanchez
*Chemical Thermodynamics Research Laboratory*
*Institute of Chemistry*
*University of the Philippines*
*Diliman, Quezon City*

**ABSTRACT**

A cubic equation of state for phase equilibrium calculations is proposed and tested for a number of pure compounds over the temperature range 0 5 $T_c$ < T $_H$ $T_c$ of interest in phase quilibria. Satisfactory numerical results were obtained for molecules of various shapes and dipole moments through the introduction of simple acentric factor-dependent dimensionless scaling factors. Deviations of calculated compressibility factors from results of well known correlations indicated superiority of the present equation over the Soave-Redlich-Kwong and showed performance comparable to the Peng-Robinson equation. Pure component fugacity coefficients based on the calculated compressibility factors were in good agreement with the widely accepted Soave-Redlich Kwong results, thus establishing utility of the equation in reproducing pure substance saturation conditons. The extension of the relation to mixtures with appropriate combining rules is the subject of subsequent studies.

## Introduction

The importance of the predictive capacity offered by multi-component vapor-liquid equilibrium (VLE) calculations cannot be over emphasized in the chemical system among the co-existing vapor and liquid phases at given temperature and pressure conditons allows the process engineer to make necessary adjustments in the system to meet whatever requirements the process entails. An approach to the solution of such phase equilibrium problems utilizes information on the volumetric behavior of the system in the form of an analytical relation. This method of calculation has the advantage of avoiding activity coefficients and their associated standard states, which in some cases are hypothetical. Cubic equations of state applicable to mixtures and satisfactory at both gas and liquid densities are best suited for this purpose.

One of the best known cubic equations was the Redlich-Kwong (1949), the earliest successful applications of which to VLE were given by Wilson (1964) and by Zudkevitch and Joffe (1970). In both these works, it was necessary to

improve on the original equation by assuming temperature dependent parameters established by equalizing fugacities along the vapor-pressure curve. Soave (1972) further generalized this technique of applying the Redlich-Kwong (RK): a more general temperature dependent parameter replaced altogether the $T^{-0.5}$ dependence in the attractive pressure term of the original equation. The modified form obtained, popularly known as the Soave-Redlich-Kwong (SRK) equation, has gained wide acceptance within the hydrocarbon industry because of its capability for generating reasonably accurate equilibrium ratios in VLE calculations. In a further development, Peng and Robinson (1976) pointed out that the SRK, as well as the original RK equation, still suffers from the incapability of generating satisfactory liquid density values despite the general acceptability of the corresponding vapor results. A Peng-Robinson (PR) cubic equation was thereby forwarded, claimed to yield equilibrium ratios as good as the SRK and more accurate liquid densities for a plus.

This paper presents an equation comparable in utility with the Soave-Redlich-Kwong and Peng-Robinson equations for phase equilirium calculations. The equation was originally formulated to meet some deficiencies in many cubic equations in the spirit of the discussion given by Abott (1973) where characteristics and behavior of cubic equations were analyzed. The original formulation, which is omitted in the foregoing equation in VLE calculations; in fact, it was done without any prior knowledge of the existence of the SRK and PR equations. The equation was only really then intended to provide good estimates of the volumetric properties of gases. Later encounters with the SRK provided the motivation for investigating the possibility that the earlier formulated equation might show similar performance in predicting VLE.

This paper is the first of two parts and deals only with the application of the proposed equation in generating compressibility factors and fugacity coefficients for pure substances. By initially ensuring that the equation satisfactorily reproduces saturation conditions for pure substances, extension to mixtures as done in the second part will only then require the search for appropriate combining rules.

## The Proposed Cubic Equation and Some Thermodynamic Derivation

Generally, the best known cubic equations are of the Van der Waals type which expresses the pressure as the sum of contributions from repulsive and attractive forces. In most instances the repulsive part is represented by

$$P_{repulsive} = RT/(v-b)$$

where v is the molar volume and b a temperature independent measure of the excluded volume, roughly the size of hard spherical molecules. The expression for attractive pressure component is what differentiates one cubic equation from another but still, it can be generalized as a function of some molecular interaction parameter, a, and of the molar volume, i.e.,

$$P_{attractive} = -a / g(v) \tag{1b}$$

The function g(v) may be taken to be of the form

$$g(v) = v^2 + r*bv + s*b^2 \tag{2}$$

Table 1. Four well known cubic equations

| *Equation* | *r* | *s* | *b* | *a* |
|---|---|---|---|---|
| Van der Waals | 0 | 0 | $RT_c/8P_c$ | $27R^2T_c^2/64P_c$ |
| Redlich-Kwong | 1 | 0 | $0.08664RT_c/P_c$ | $0.42748R^2T_c^2/P_cT_r^{0.5}$ |
| Soave | 1 | 0 | $0.08664RT_c/P_c$ | $0.42748R^2T_c^2/P_c * \{1+m(1-T_r^{0.5})\}^2$ |
| Peng-Robinson | 2 | −1 | $0.0778RT_c/P_c$ | $0.45724R^2T_c^2/P_c * \{1+m(1-T_r^{0.5})\}^2$ |

where m = m(w) , a quadratic function.

Table 1 lists four well known cubic equations and their corresponding values for r and s. The parameters a and b, likewise shown in Table 1, are obtained by evaluating a given cubic equation at the critical point where the first and second derivatives of the pressure with respect to the volume vanish.

The choice of the temperature dependence for the attractive pressure term is an important factor to consider as it has been the focus of attention in the improvements introduced by Soave (1972) over the Redlich Kwong equation. This temperature dependence may be viewed as a dimensionless scaling factor, a function of the reduced temperature $T_r$, in the interaction parameter a. As evident from Table 1, the SRK and PR equations show the same temperature dependence in a. The quantity m in their scaling factor is a function of the acentric factor w defined by Pitzer and co-workers (1955) as a measure of the deviation of the intermolecular potential from that of simple spherical molecules of "normal" fluids. Such incorporation of the acentric factor leads in part to the superiority of the SRK and PR equations over the Redlich-Kwong since the molecular interaction parameter is in effect corrected for the non-central character of the force of interaction of molecules of varying shapes and dipole moments. Note that for normal fluids w = 0.

The equation of state presently proposed is as follows:

$$P = RT/(v-b) - a/(v + 2b/3)^2 \tag{3}$$

where $a = 27\ R^2T_c^2 / 64\ P_c T_r^{2/3}$ and $b = 3\ RT_c / 40\ P_c$, as evaluated at the critical point. The termpature dependence in the parameter a may to a first approximation be taken as sufficient for other temperatures as well, although as seen in later developments, significant improvements and interesting observations are achieved by introducing an additional acentric factor dependent dimensionless scaling factor. In terms of equation 2, $r = 4/3$ and $s = 4/9$.

To generate compressibility factors for the coexisting liquid and vapor phases from the proposed relation, equation 3 is written in the form

$$Z^3 + Z^2\ (B/+ - 1) + Z\ (A - 4B/3 - 8B^2/9) - AB - 4B^2(1 + B)/9 = 0$$

where Z = compressibility factor, $B = bP/RT = 3\ P_r / 40\ T_r$, $A = aP/R^2T^2 = 27\ P_r / 64\ T_r^{8/3}$, $P_r$ = reduced pressure. This is then solved for its largest and smallest positive root under the coexistence curve, the roots being equal respectively to the vapor and liquid phase compressibility factors.

The basic condition for vapor-liquid equilibria is the equality of the vapor and liquid phase fugacities, $f^v$ and $f^l$, of each component i when distributed between the two phases in equilibrium:

$$f_i^v = f$$

$$f_i^v = f_i^l$$

Because the fugacities of a component in a mixture are proportional to the composition according to the thermodynamic relation

$$f_i^v = \phi_i^v y_i P \qquad \& \qquad f_i^l = \phi_i^l\ x_i P \quad .$$

$\phi$ being the fugacity coefficient, the equlibrium ratio

$$K_i = y_i / x_i \tag{6}$$

is then expressible in terms of the fugacity coefficients, i.e.

$$K_i = \phi_i^v / \phi_i^l$$

With the utilization of an equation of state to provide fugacity coefficients. the solution of the phase equilibrium problem is simply reduced to one of a problem of solving simultaneously a system of equations consisting of equations 6 and mass balance equations.

The fugacity coefficents of a component in a mixture are obtained from an equation of state through the general thermodynamic relation

$$RT \ln \phi_i = \int_V^\infty \{ (\partial P/\partial n_i)_{T, V, n_j} - RT/V \} \quad dV \; - \; RT \ln Z_i \qquad (7)$$

applied to both phases. In this paper, only pure component-fugacity coefficients are of concern. Equation 7 for a pure substance is

$$RT \ln \phi = \int_V^\infty \{ P/n_i - RT/V \} \quad dV - RT \ln Z + RT (Z - 1) \quad ,$$

for which the proposed equation (3) yields

$$\ln \phi = - \ln (Z - B) - A \; / \; (Z + 2B/3) + Z - 1 \qquad (8)$$

Equation 8 should give the same value whether one substitutes compressibility factors for the vapor phase or for the liquid phase obtained from equation 6 using vapor pressure data for pure substances.

*Numerical Results*

Compressibility factors for 12 substances were calculated using literature experimental data for the reduced temperature range of interest in many phase equilibrium investigations, $0.56 < T_r < 1.0$. Calculations were carried for approximately 25-30 data points for each compound, totalling roughly to around 340 data points. The accuracy of some data points used were quite uncertain due to low local availability of more recent literature vapor pressure data employing Newton's root searching technique, convergence towards a solution was easily achieved with 2 to 3 iterations, except at near critical temperatures where not even 10 iterations were sufficient. Subsequent use of the calculated liquid and gas compressibility factors yielded fugacity coefficients for both phases.

Table 2 shows the average results for each of the 12 substances examined at the specified temperature ranges. The presently proposed equation is compared with the SRK and PR equations in its ability to reproduce highly accurate gas and liquid compressibility factors predicted by well known correlations. Of course, the best test of performance is to compare the calculated results with experimental values. Because of the local unavailability of experimental literature data, such an approach is virtually impossible.

Accurate prediction of gas phase compressibility factors is not as much a problem as those for the liquid phase. Pitzer's corresponding state correlation (1955) involving the acentric factor as a third parameter still quite sufficiently represents gas phase compressibility factors despite the numerous correlations that have appeared since its conception. In this correlation, the compressibility factor is expressed as a first order expansion in the acentric factor,

$$Z = Z^{(o)} + W Z^{(1)}$$

Table 2. (a) Comparison between the proposed equation, SRK, & PR equations with respect to compressibility factor $Z_l$ & $Z_v$ predictions, (b) Absolute differences: $\ln \phi^v - \ln \phi^l$

| *Substance* | $T_r$ *range* | *Vapor Pressure Data Reference* | *Compressibility Factors Liquid Phase*[a] | | | *Ave. Absolute % deviation Gas phase*[b] | | | $\ln \phi^v$ $\ln \phi^l$ |
|---|---|---|---|---|---|---|---|---|---|
| | | | *Present* | *SRK* | *PR* | *Present* | *SRK* | *PR* | *(range)* |
| nitrogen | 0.5638–0.9996 | Reid *et al.* (1986) | 9.9258 | 7.6458 | 8.4691 | 1.2839 | 1.7201 | 1.6760 | 0–0.8841 |
| ethane | 0.6143–0.9428 | Timmermans (1950) | 9.8218 | 7.0265 | 5.9532 | 0.9856 | 1.4996 | 1.0078 | 0.0001–0.3809 |
| $CCl_4$ | 0.5629–0.9998 | Timmermans Boublik (1984) | 11.9619 | 12.7256 | 4.7317 | 2.1711 | 2.2938 | 1.0149 | 0.0000–0.1449 |
| neopentane | 0.5921–0.9871 | Boublik, *et al.* (1984) | 11.0548 | 9.7665 | 6.0990 | 2.0889 | 2.0705 | 1.1447 | 0.0005–0.0682 |
| 2,3 dimethyl butane | 0.5657–0.9953 | Timmermans | 13.3971 | 13.8262 | 5.3566 | 2.7705 | 2.4442 | 1.9579 | 0.0000–0.1449 |
| benzene | 0.5641–0.9982 | Timmermans | 12.1505 | 13.8463 | 4.8634 | 2.5908 | 2.4326 | 1.1243 | 0.0021–0.0848 |
| toluene | 0.5629–0.9997 | Reid *et al.* | 17.0129 | 20.3132 | 7.0188 | 3.5402 | 3.2517 | 1.1611 | 0.0000–0.2040 |
| diethylether | 0.5636–0.9983 | Timmermans | 14.1813 | 22.7584 | 8.9466 | 3.5226 | 2.6938 | 1.5306 | 0.0000–0.2631 |
| n-heptane | 0.5609–0.9948 | Timmermans | 10.3694 | 17.4767 | 4.0523 | 3.8120 | 2.2964 | 1.4791 | 0.0016–0.5839 |
| nonane | 0.5771–0.9992 | Reid *et al.* | 19.8013 | 23.1770 | 10.0110 | 4.4040 | 3.5812 | 2.5003 | 0.0004–0.9978 |
| decane | 0.5879–0.9991 | Reid *et al.* | 21.2598 | 25.1848 | 11.0226 | 4.5583 | 3.3028 | 1.6231 | 0.0003–1.1430 |
| $CO_2$ | 0.5694–0.9995 | Reid *et al.* | 8.8411 | 15.0164 | 6.6919 | 2.7047 | 2.2526 | 1.0832 | 0.0009–0.1034 |

(a) compared against Rackett correlation (1970, 1972) predictions
(b) compared against Pitzer's correlation (1958) predictions

The expansion coefficients $Z^{(o)}$ and $Z^{(i)}$ are functions of $T_r$ and $P_r$ and are numerically tabulated at regularly spaced temparture intervals. The acentric factor at each temperature is readily evaluated for any substance from the vapor pressure data through the relation

$$\log P_r = (\log P_r)^{(o)} + w\,(\partial \log P_r / \partial w)_{T_r}$$

where $(\log P_r)^{(o)}$ and $(\partial \log P_r / \partial w)_{T_r}$ are likewise expansion coefficients dependent only on the temperature and also tabulated numerically.

On the other hand, of the several liquid compresssibility factor correlations examined (Francis, 1957, 1959; Lyckman, *et. al.*, 1965; Rackett, 1970; Bhirud, 1978), the method of Rackett (1970) as modified by Spencer and Danner (1972) and as recommended by Reid and co-workers (1986) was found most satisfactory. The liquid compressibility factor takes the form

$$Z_l = (P_r / T_r)\, Z_{RA}\, [1 + (1 - T_r)^{2/7}]$$

where $Z_{RA}$ is a unique constant for each compound. This correlation also compared well with the more recent method of Hankinson and Thomson (1979) though slightly more convenient to use.

From Table 2, all three equations exhibit nearly the same performance in predicting gas phase values. On the other hand as consistent with Peng and Robinson's claim, their liquid compressibility factors are much more accurate than the Soave-Redlich-Kwong results. Note that the deviations reported here are relatively larger than those reported by Soave and Peng-Robinson for their equations since their predictions were compared in this paper against a certain correlation which naturally also deviates, though little, from experimental results. The liquid phase predictive capability of the presently proposed equation does not differ significantly from that of the SRK, although this is already a welcome result considering that the SRK includes the acentric factor as a third parameter.

As earlier mentioned, convergence problems in root searching appear at near critical temperatures for the proposed equation. It has been observed that the SRK and PR equations likewise suffer the same dilemma, although to a much lesser extent. Of course, other root searching techniques may be able to handle the situation. At any rate, unconverged results were not included in calculating the average percent deviations reported in Table 2.

The gas and liquid phase fugacity coefficients, determined from the compressibility factors predicted by the proposed equation, are seen to exhibit appreciable differences (Table 2). This clearly is a violation of the equilibrium condition and indicates further the necessity of introducing improvements in the original equation.

*An Improvement on the Originally Proposed Equation*

The unimpressive results obtained from the original equation does not come at all as a surprise because the constant quantities in the interaction parameter as evaluated at the critical point need not necessarily be the same for all substances and over all temperatures. Although a temperature dependence has already been initially taken and imposed, it is not quite safe to assume that such dependence is sufficient to correct for the difference in temperature between the critical point and any other state. Following Soave (1972), a dimensionless scaling factor $\alpha / T_r, w)$ may be introduced at temperatures other than the critical:

$$a(T) = a(T_c) * \alpha(T_r, w) \tag{9}$$

with $\alpha$, which is a function of the reduced temperature and the acentric factor, naturally satisfying the boundary condition $\alpha = 1$ at $T_r = 1$. The introduction of this scaling factor will in effect change the temperature dependence at temperature other than the critical and will simultaneously correct for the variation in the shape and polarity of molecules as reflected in the molecular interaction potential. The cubic compressibility equation (4) and the fugacity coefficient equation (8) becomes modified by multiplying A with $\alpha$.

The thermodynamic necessity of equal vapor and liquid phase fugacities at saturation conditions for pure substances provides the means of determining $\alpha$. Since for pure substances one may equivalently write equation 5 purely in terms of the fugacity coefficients, one obtains for $\alpha$.

$$\alpha = (64\, T_r^{8/3} / 27\, P_r) \quad \ln [(Z_l - B)/(Z_v - B)] + Z_l - Z_v$$
$$(Z_l - Z_v) / [(Z_v + 2B/3)(Z_l + 2B/3)] \tag{10}$$

An initial estimates of $\alpha = 1$ provides the same values for $Z_l$ and $Z_v$ as those reported in the previous section. Using these Z results, an improved value for $\alpha$ is obtained through equation 10. The calculation is repeated iteratively thereafter until a converged value for $\alpha$ is hit. The first iteration is usally sufficent.

Table 3 shows the average results for each of the substances examined. Clearly, significant improvement of the compressibility factor predictions are achieved (compare with Table 2). The Peng-Robinson predictions are just slightly superior over the present results. Convergence problems in root searching at near critical temperatures occurred but very seldom, just as with the SRK and PR equations. It is observed that large contributions to the average deviations obtained come from the near critical region despite the convergence. The fugacity coefficients show very good agreement with the SRK predicted values in Table 3. Incidentally, the difference between the predicted gas and liquid phase fugacity coefficients, $\ln \phi v - \ln \phi^1$, was of the order $10^{-16}$ thus satisfying the equilibrium condition. It is to be noted that all these results were achieved with $\alpha$ values

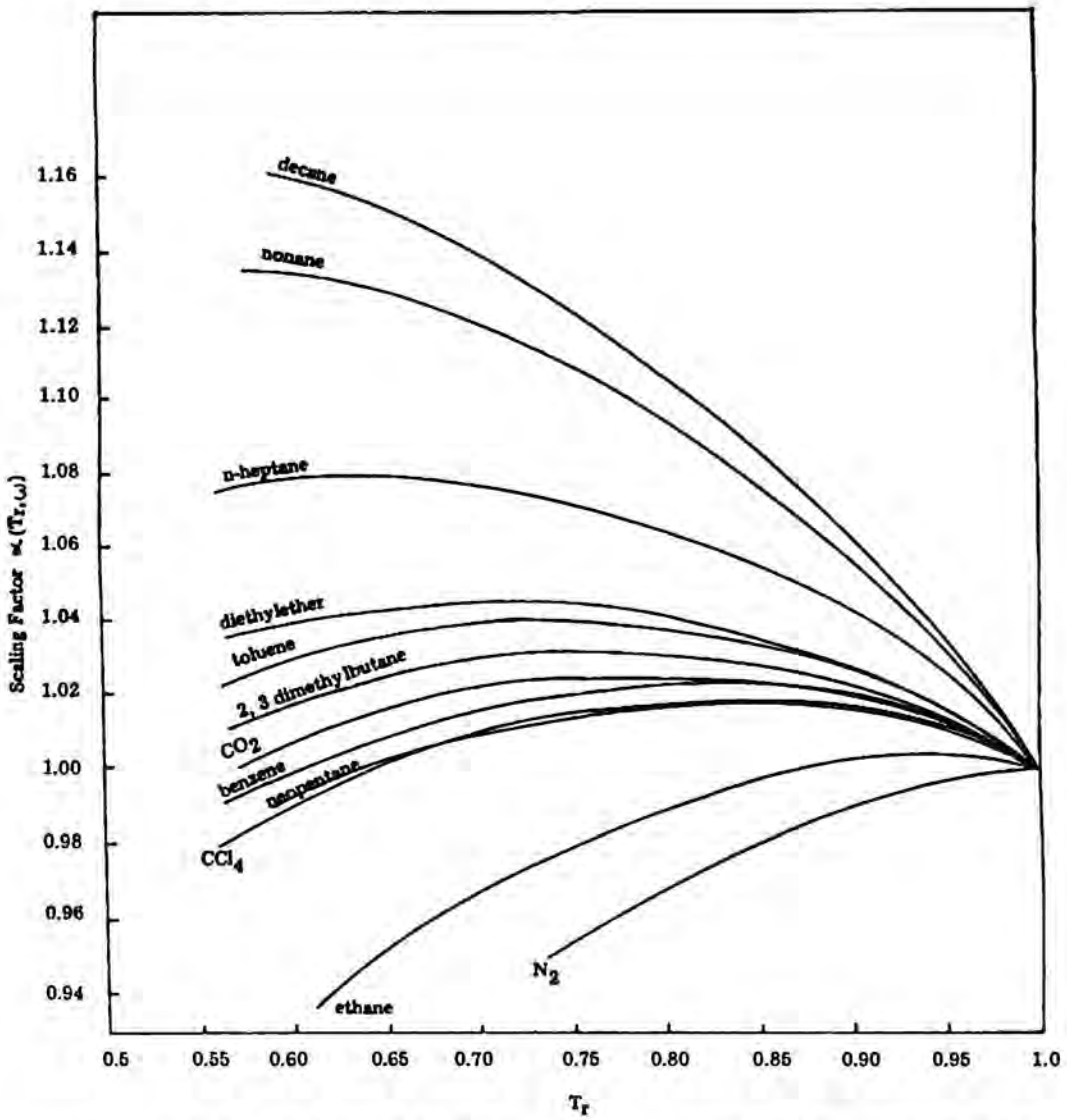
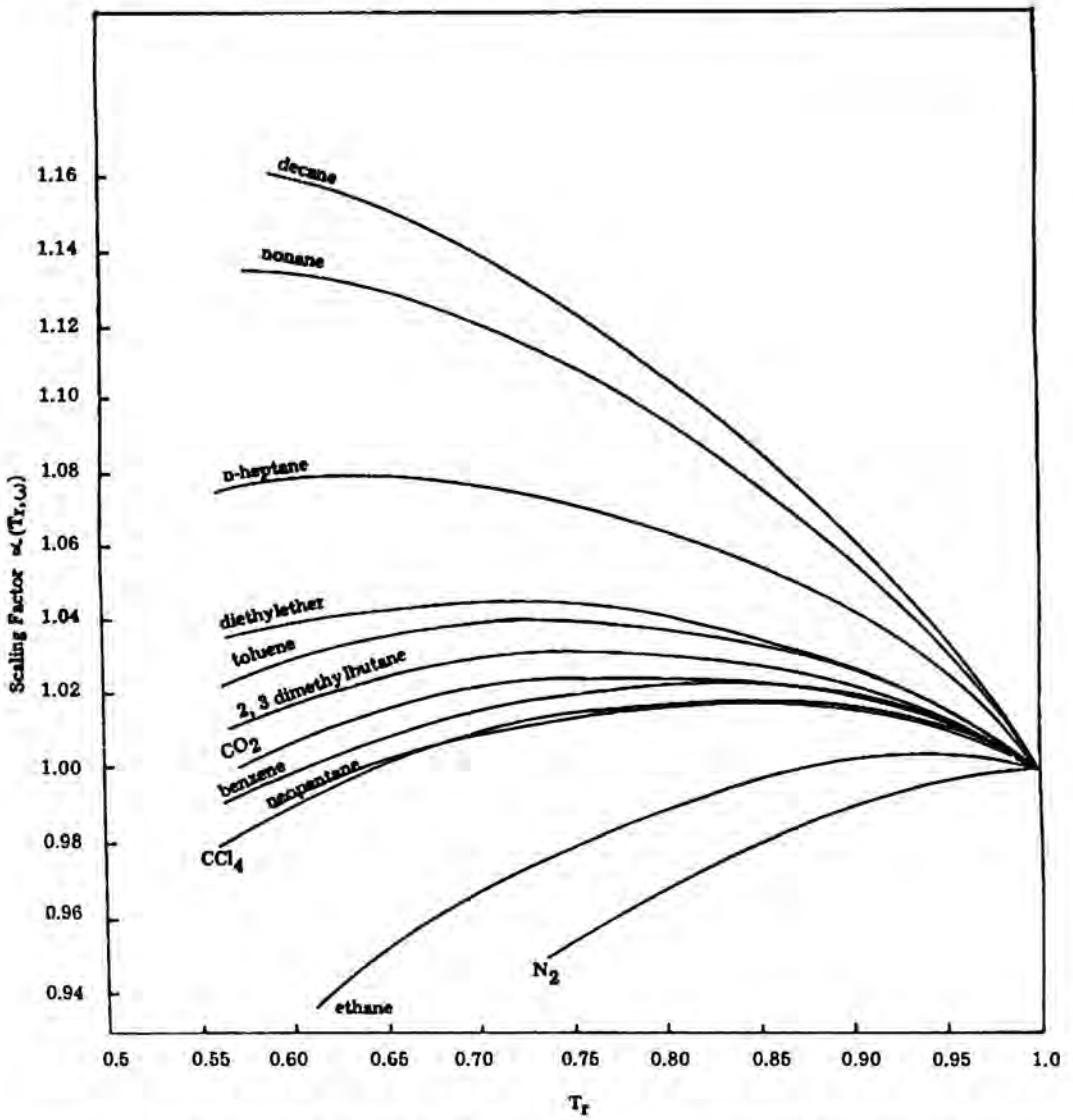



Figure 1: PLOT of SCALING FACTOR $\alpha(T_r, \omega)$ vs. REDUCED TEMPERATURE $T_r$

where $m(1-T_r)$ is the temperature varying slope of the $\alpha$ – w curve and $U(T_r)$ is a function only of the reduced temperature. The form (11) is consistent with the observations that the slope of the q–w curve decreases with the reduced temperature and that the slopes of the $T_r = 0.7$ and 0.905 plots were nearly the same factors of $1-T_r$. In addition, with this $1-T_r$ factor the disappearance of the acentric factor dependence at the critical point easily guarantees though not exclusively, the covergency of all $\alpha-T_r$ curves at $\alpha = 1$. The function $U(T_r)$ must now naturally satisfy the condition $U(1) = 1$. Further support for the form (11) is finally provided by plotting U (calculated from 11 using an m value as discussed in the succeeding paragraph) vs $T_r$ (Figure 3 includes only 3 substances). All points fell nearly on the same curve.

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where $m(1-T_r)$ is the temperature varying slope of the $\alpha - w$ curve and $U(T_r)$ is a function only of the reduced temperature. The form (11) is consistent with the observations that the slope of the $q-w$ curve decreases with the reduced temperature and that the slopes of the $T_r = 0.7$ and 0.905 plots were nearly the same factors of $1-T_r$. In addition, with this $1-T_r$ factor the disappearance of the acentric factor dependence at the critical point easily guarantees, though not exclusively, the covergency of all $\alpha-T_r$ curves at $\alpha=1$. The function $U(T_r)$ must now naturally satisfy the condition $U(1)=1$. Further support for the form (11) is finally provided by plotting U (calculated from 11 using an m value as discussed in the succeeding paragraph) vs $T_r$ (Figure 3 includes only 3 substances). All points fell nearly on the same curve.

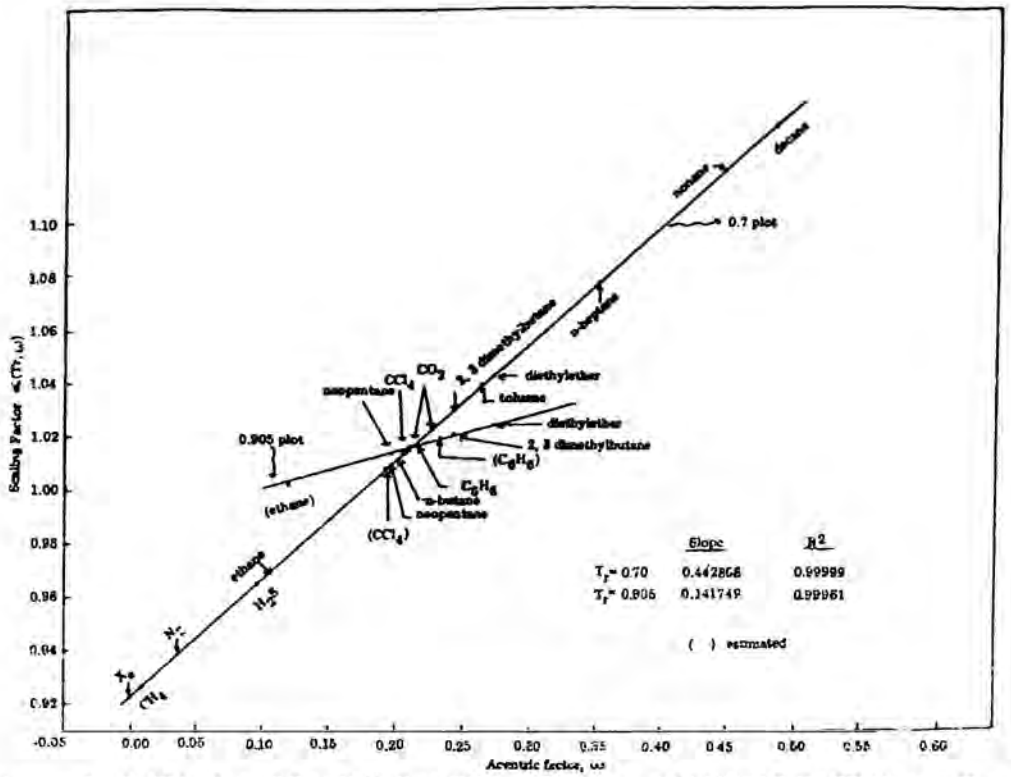


Figure 2: PLOT of the SCALING FACTOR $\alpha(T_r, \omega)$ vs. ACENTRIC FACTOR for $T_r = 0.7$ and 0.905

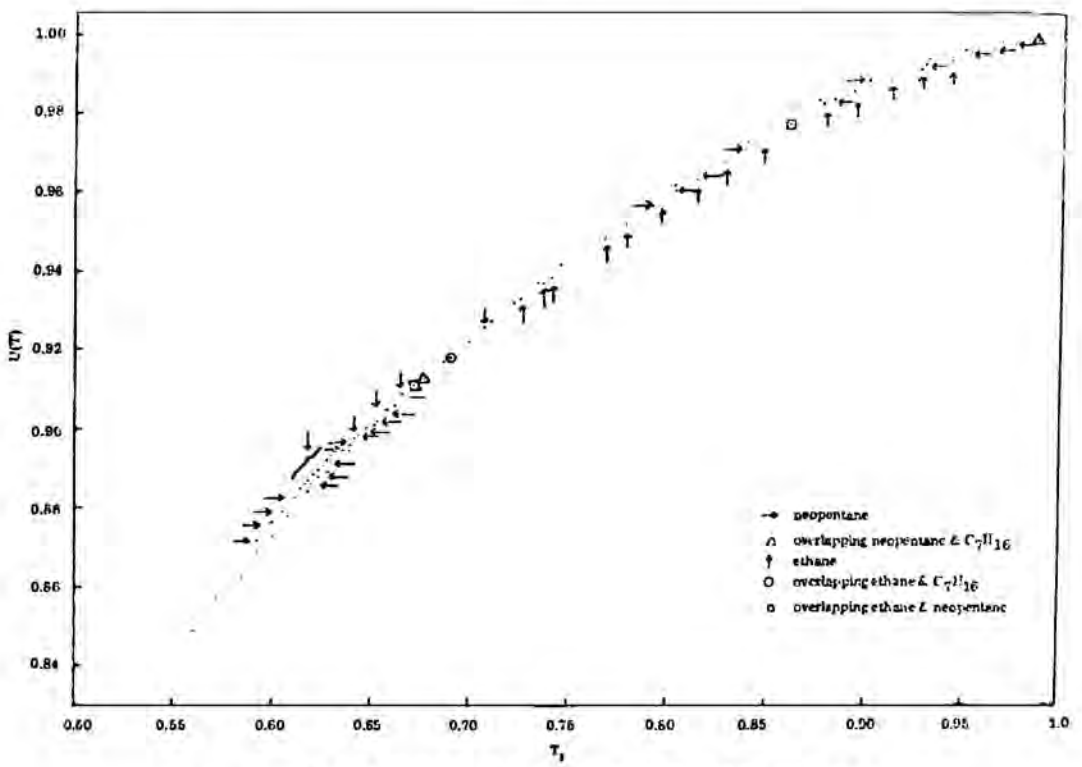


Figure 3: PLOT OF $U(T_r)$ vs. REDUCED TEMPERATURE $T_r$

The value of m in the acentric factor dependence is obtained from the slope of the $\alpha - w$ curve for $T_r = 0.7$ and is equal to 1.4921. This is in good agreement with that obtained from the 0.905 plot, equal to 1 4762. More substances were included in the regression of the $\alpha - w$ 0.7 plot so as to make the calculated m value more general and widely applicable to other substances as well that were not examined in this paper. Anyway, any limitations in the chosen m value can always be taken care of by $U(T_r)$. The choice of regressing data at $T_r = 0.7$ was really completely arbitrary and was perhaps only motivated by the desire to be consistent with Pitzer's original definition for w and by the fact that the existing data around that temperature was most abundant.

The function $U(T_r)$ was most satisfactorily and conveniently represented by the hyperbolic relation

$$U(T_r) = T_r / (h\,T_r + k) \qquad (12)$$

This may be rewritten in a linear form and then $T_r/U$ vs $T_r$ may be plotted so as to verify that equation 12 validly represents the remaining temperature dependence. For each data point, U was easily calculated since $U = \alpha - m\,w\,(1 - T_r)$. Whether one uses a constant value for w over all tempatures for a particular substance or a calculated value (as described in the previous section) at each temperature, the regression for $T_r/U$ vs $T_r$ yields nearly the same results. The regression line shown in Figure 4 gave a good correlation coefficient of 0.9992; the experimental plot (for 3 substances only in Figure 4) exhibits a slight curvature which is difficult to account for.

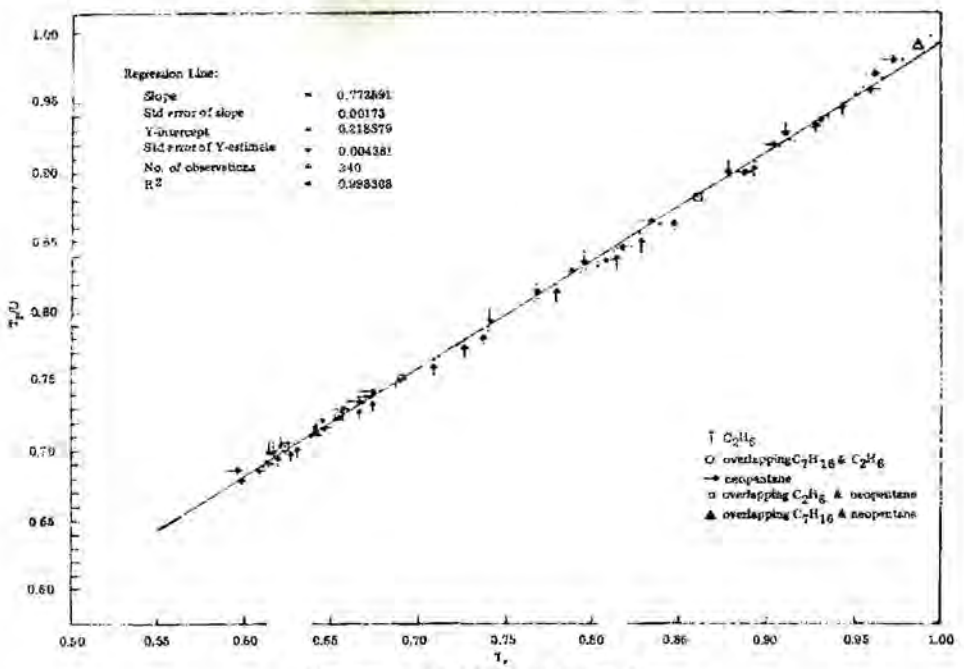


Figure 4: PLOT of $T_r/U$ vs. $T_r$

All in all then, the final form of the proposed equation is as follows.

$$P = RT/(v-b) - a(T)/(v + 2b/3)^2 \qquad (13)$$

where

$$a(T) = a(Tc) * \quad 1.4921\ w\ (1-T_r) + T_r/(0.7729\ T_r + 0.2189)$$

## Conclusion and Recommendations

The proposed equation 13 has been developed and shown to provide significant predictive capability in reproducing pure substance saturation conditions. The extension of the equation to mixtures should then be forthcoming. The values of quantities m, h, and k in equation 13 may further be improved and made more general by including more substances in the regression analysis. This involves further testing of the equation to probably at least 20 more substances. Finally, it is interesting to investigate whether $U(T_r)$ is indeed the limiting curve for the plots of $\alpha$ vs $T_r$. This can be established by testing normal fluids or compounds with acentric factors close to zero. Perhaps some theoretical investigations on the significance of this limiting curve may be pursued.

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# BIOLOGICAL SCIENCES

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# SCANNING ELECTRON MICROSCOPY OF THE INTEGUMENTAL SURFACES OF ADULT *GNATHOSTOMA DOLORESI* TUBANGUI, 1925, A PARASITE OF PIGS IN THE PHILIPPINES

Salcedo L. Eduardo
*College of Veterinary Medicine*
*University of the Philippines at Los Baños, College, Laguna*

**ABSTRACT**

The integumental surfaces of critically point dried adult *Gnathostoma doloresi* Tubangui, 1925 were studied by scanning electron microscopy.

The presence of structures whose occurrence and distribution appeared to be consistent in all specimens examined were revealed. The head bulb is armed with nine to ten rows of strongly curved and posteriorly pointed hoos arranged around the circumference of the lateral aspect of the bulb.

The body is covered all over with two kinds of spines, single pointed and multidigit. The latter cover the anterior one-third and the former the posterior two-thirds of the body. The multidigit spines consist of two forms and their extent of distribution appear to be consistent. Short broad spines with four to five digits of varying length cover a short area immediately after the neck. Long broad spines with three digits, in which the middle digit is distinctly longer than the lateral ones, cover the remaining anterior one-third. The three digit spines gradually diminish in size towards the posterior.

The pathology of the species as suggested by these structures is discussed. The species is compared with *Gnathostoma hispidium* with which it stands closest and which was also studied by SEM by other authors.

## Introduction

*Gnathostoma doloresi* is a nematode parasite occurring in the stomach of domestic and wild pigs. It was first reported in the Philippines by Tubangui (1925) and subsequently recorded in other countries such as India (Maplestone 1930), Japan (Miyazaki, 1950, 1957, 1960; Miyasaki *et al.*, 1951; Miyazaki *et al*, 1953; Morishita, 1951; Nishida, 1957), Singapore (Sandosham, 1953), Vietnam (Le-Van-Hoa *et al.*, 1965, 1967; Nguyen-Van-Ai, 1965) Taiwan (Chiu, 1959), USSR-Primorsk Region (Pigolkin, 1963), Thailand (Dissamaru *et al.*, 1966), New Guinea (Miyazaki, 1968; Talbot, 1969) and the British Solomon Islands (Talbol, 1969).

The first description (Tubangui, 1925) and subsequent redescriptions (Maplestone, 1930, Miyazaki, 1950, 1954; Ishii, 1956) were all based on light microscopy, including the brief description of seven species of the genus in a review given by Miyazaki (1960). It would be interesting therefore to examine this species under SEM; hence, this study.

## Materials and Methods

Ten specimens of *Gnathostoma doloresi* were processed for scanning electron microscopy following the procedure described by Eduardo (1981). Specimens were critically point-dried using carbon dioxide as drying medium and coated with gold. These were examined under a JEOL JSM-35C SEM unit at accelerating voltage of 25 kilovolts.

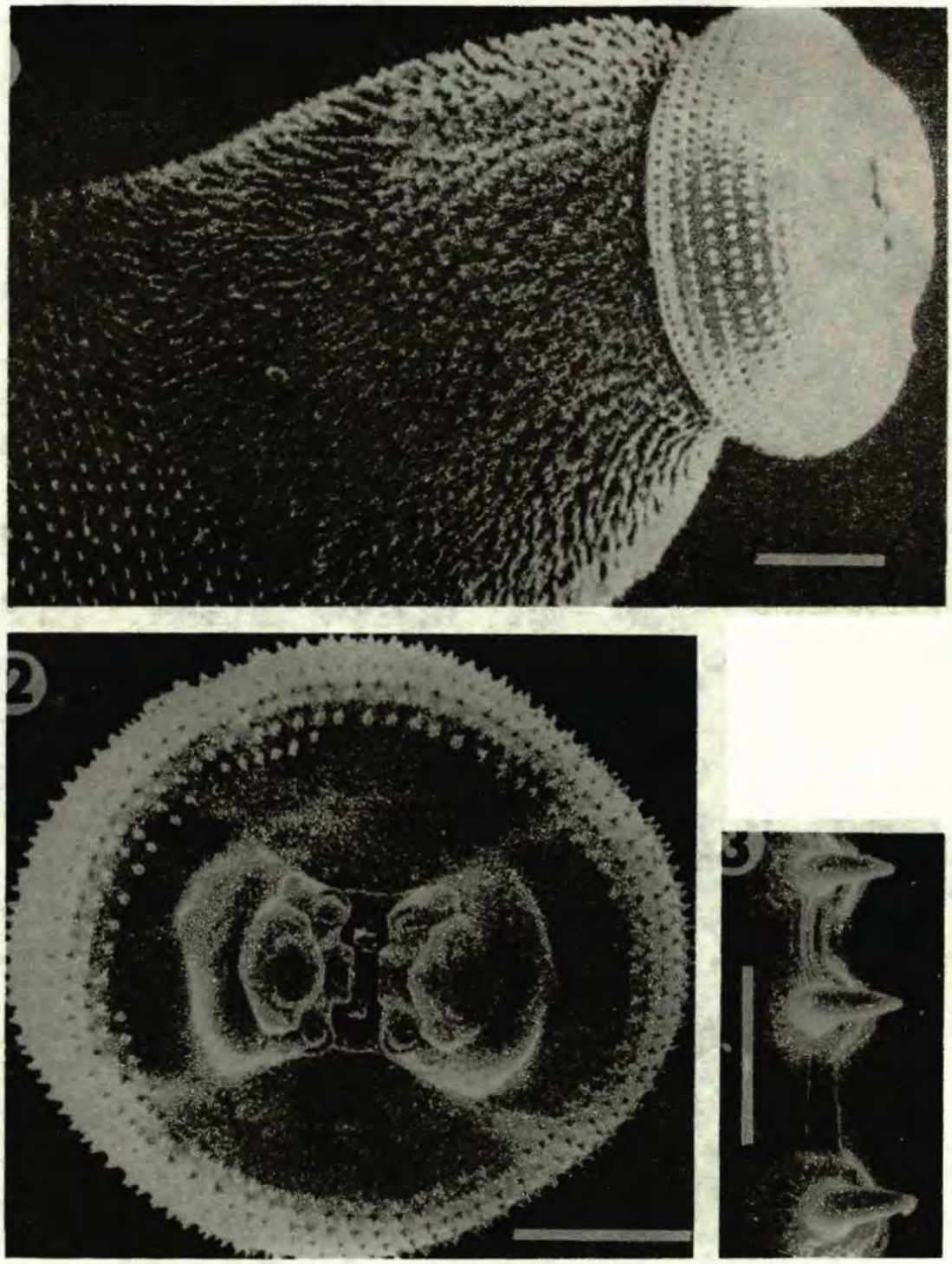
## Results and Discussion

As shown in Fig. 1, the head bulb is armed with hooks and the body is covered all over with spines. Although these structures have been observed in light microscopy, their exact details have not been clear. SEM does not only confirm their occurrence and distribution but also sheds light on their exact shape, size and surface details.

The hooks are arranged in nine to ten rows around the circumference of the head bulb (Fig. 2). In three specimens, however, the inner row of hooks appears incomplete, occupying only half of the circumference. Hooks appear strongly curved and posteriorly directed (Fig. 3). These structures and the head bulb, when inflated, enable the parasite to firmly attach itself on the gastric mocusa. Dislodgement of the worm would seem difficult. The sharp ends of the hooks could cause laceration of the mucosa as the parasite moves from one place to another. In a formalin-preserved stomach that harbored this species, holes on the mucosa were distinct, indicating the point of attachment. Attempt to count the number of holes and the number of parasite harbored showed that there were more of the former (about one-and-a-half more) than the latter, suggesting that some worms moved from one place to another.

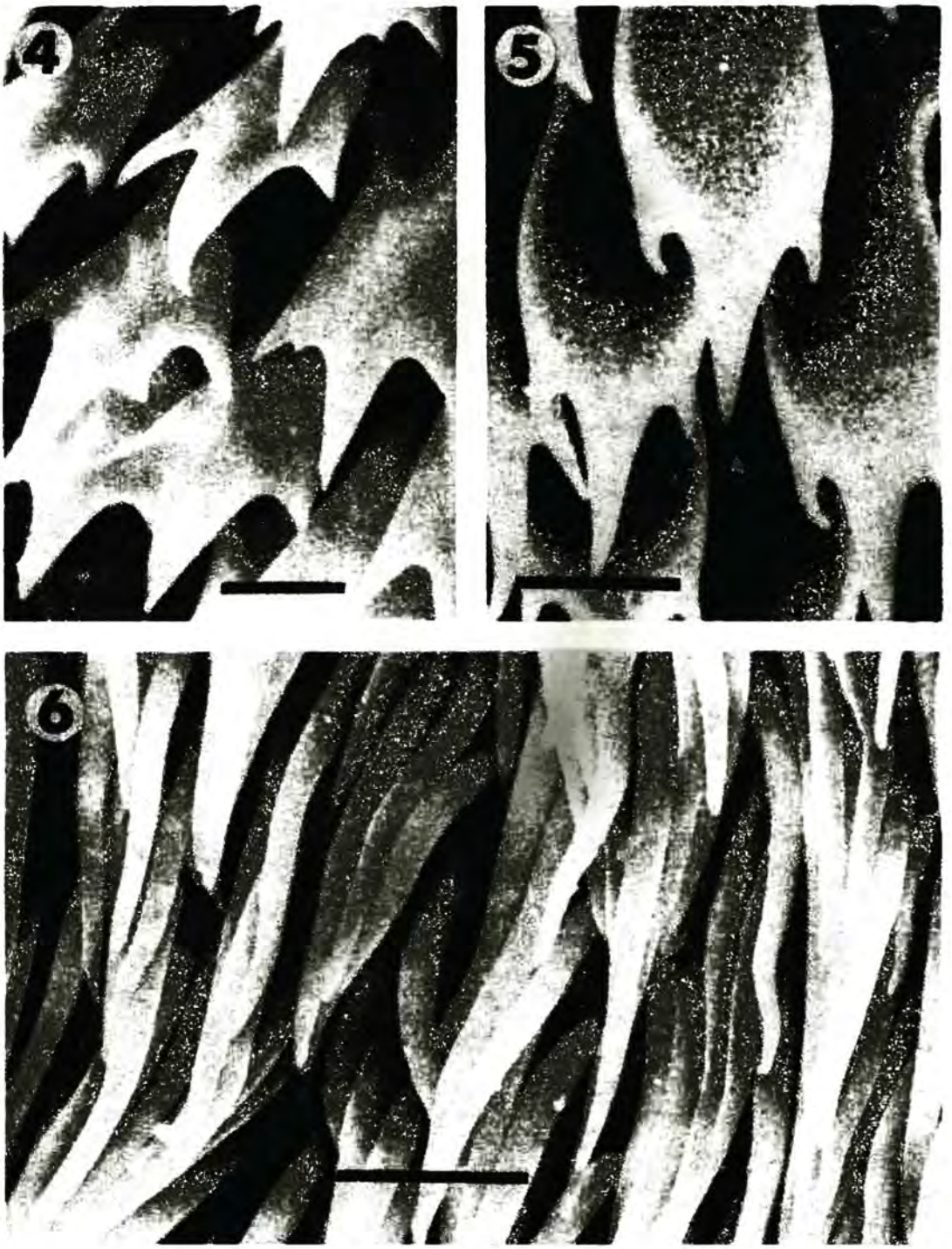
Figure 2 is an *en face* view of the anterior end and shows the mouth guarded by two lips, one on each lateral side. Each lip has a pair of dorsal and a pair of ventral papillae. One of the dorsal and one of the ventral papillae are median (nearer the mouth) and the other dorsal and ventral papillae are submedian (farther from the mouth). Thus, the submedian are within and in between the horizontal fields of the median papillae.

The spines covering the body of *Gnathostoma doloresi* consist of two kinds, single-pointed and multidigit. Save for the digits, the surfaces of these spines appear smooth. These digited spines could also contribute to laceration of the gastric mucosa. Their distribution appears to divide the body into two areas. The anterior third is covered with multidigit spines and the posterior two-thirds with the single-pointed spines. The multidigit spines can be further classified into two forms whose distribution appears to be consistent in all specimens examined. Spines which are short and broad and with four to five digits of varying length (Fig. 4) cover a short area immediately after the neck. They measure 37.7-53.3 microns (to the tip of the longest digit) and 24.4-30.0 microns in width. These are followed by broad and




*Gnathostoma doloresi*, SEM:

Figure 1. Antero-lateral view. Note hooks on head bulb and spines on body (scale bar = 200 microns); Fig. 2. *En face* view of anterior end. Note lateral lips, papillae and arrangement of hooks (scale bar = 200 microns); Fig. 3. Closer view of hooks on head bulb. Note that they are strong and posteriorly curved (scale bar = 20 microns).




*Gnathostoma doloresi*, SEM:

Figure 4. Spines on area immediately after the neck. Note four to five digits of spines (scale bar = 40 microns); Fig. 5. Spines with three digits covering remaining area of anterior one-third of the body. Note middle digit distinctly longer than the lateral ones (scale bar = 80 microns); Fig. 6. Single-pointed spines covering posterior two-thirds of the body (scale bar = 40 microns).

longer spines with three digits in which the middle digit is distinctly longer, about twice as long as the lateral digits (Fig. 5). The three digit spines cover the remaining anterior one-third of the body. Those in the more anterior part of this particular area measure 78.8-91.1 microns in length (to the tip of the middle digit) and 30.0-32.2 microns in width. Those on the more posterior part are smaller and measure 37.7-53.3 microns in length and 24.4-30.0 microns in width. The rest of the body (posterior two-thirds), as mentioned earlier, is covered with single-pointed slender spines (Fig. 6). They measure 37.7-46.6 microns in length and 2.8-3.3 microns in width.

Because the body of *Gnathostoma doloresi* is covered all over with cuticular spines, it stands closest to *Gnathostoma hispidium* and is frequently confused with it. SEM comparison on the distribution and characters of the body spines between *G. doloresi* as observed in this study and *G. hispidium* as observed by Kondo *et al.* (1984) and Koga *et al.* (1984) revealed some differences. The spines covering the area near the neck have four to five digits in *G. doloresi* while those in *G. hispidium* have five to ten digits. These are followed in the former species by spines with three digits in which the middle digit is distinctly longer than the lateral ones. In the latter species, there is a discrepancy in the observation of Kondo *et al.* (1984) and Koga *et al.* (1984). According to the former authors, this particular area is covered with a mixture of two-digit and three-digit spines. The digits of the two-digit spines are either equal in length or one is longer than the other. The digits of the three-digit spines are all almost equal in length. According the latter authors, however, this particular area is covered by spines with three digits and then replaced by two-digit spines. The three-digit spines are similar to that observed in *G. doloresi* in that the middle digit is much longer than the lateral ones. However, these spines, as observed by Koga *et al.* (1984) in *G. hispidium*, are longer and more slender than those observed in *G. doloresi*. Further, the middle digits are more than two twice as long as the lateral ones. Two-digit spines, as observed by Kondo *et al.* in their materials, were also observed by Koga *et al.* (1984) but all had digits almost equal in length.

The area covered by multidigit spines also differs in the two species. In *G. doloresi*, it is only the anterior one-third and in *G. hispidium* only the anterior one-fifth of the body. Consequently, the remaining area covered with single-pointed spines in the posterior two-thirds in the former species and the posterior four-fifths in the latter species.

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# ANTIMUTAGENS FROM *MOMORDICA CHARANTIA* LINN.

Amelia P. Guevara, Clara Y. Lim-Sylianco,
Fabian Dayrit, and Paul Finch

## ABSTRACT

A mixture of antimutagens, D, were isolated from the ethanol extract of the green fruits of *Momordica charantia* Linn., locally known as ampalaya, by solvent partitioning and repeated column chromatography. The major component of the mixture, D', was separated from the minor components by preparative high pressure liquid chromatography (HPLC). HPLC-pure D' was shown to be an intractable mixture of D'-1, as the major component, and D'-2, as the minor component.

The mixtures D and D' significantly reduced the number of micronucleated polychromatic erythrocytes induced by the well-known mutagen, Mitomycin C. At a dosage range of 12.5 ug – 50 ug per gram mouse, D' reduced the mutagenicity of Mitomycin C by 80%.

The structures of D' were elucidated by high-field proton nuclear magnetic resonance spectroscopy ($^{1}$N NMR), carbon-13 nuclear magnetic resonance spectroscopy ($^{13}$C NMR), Fourier transform infrared spectroscopy (FTIR), mass spectroscopy (MS), and chemical modification (acetylation, saponification, and methanolysis) followed by chromatographic and spectral analysis.

Spectral and chromatographic data indicated that D'-1 was 3-O-[6'-O-palmitoyl-β-D-glucosyl]-stigmasta-5,25(27)-dien while D'-2 was the stearyl derivative.

Structure-activity correlation studies suggested that the antimutagenic activity may reside in the peculiar lipid-like structure of the acylglucosylsterols which may allow them to be absorbed in the plasma membrane, thereby adversely affecting the membrane permeability towards Mitomycin C.

A computer search of the Biological Abstract and the Chemical Abstract revealed that no previous work has been published on the isolation, structure elucidation, and antimutagenic activity of these acylglucosylsterols. The structure elucidation of these novel compounds will be published in a forthcoming issue of *Phytochemistry* (in press).

## Introduction

Previous studies (Sylianco, 1986) showed that the expressions and/or decoctions of the leaves, fruits, and seeds of some 50 Philippine plants exhibited antimutagenic activity towards some well known mutagens like Mitomycin C, tetracycline, and dimethylnitrosoamine. This particular study on *Momordica charantia* Linn., locally known as ampalaya, was the first attempt to isolate, purify, and elucidate the structure of the active antimutagenic components from Philippine plants.

Antimutagenicity is a relatively new area of research, particularly the search for antimutagens of plant origin. The literature shows that a relatively few researchers have reported the isolation and characterization of some substances from plants found to inhibit the mutagenicity of various mutagens. These substances were found to be structurally diverse. Among the antimutagens found from plants were cinnamaldehyde from cinnamon bark oil (Ishii, 1984; Ohta, 1983), coumarin from lavender, woodruff, and sweet clover and unbeliferone from umbelliferae plants (Ohta, 1983), protoanemonin from Ranunculus and Anemone plants (Minakata, 1982), emodin from Rhubarb (Kushi, 1980), and epigallo-catechin-gallate from green tea leaves (Kada, 1985). Extracts of some common vegetables like cucumber, celery, lettuce, brocoli, spinach, cabbage, and parsely have also been shown to exhibit antimutagenic activity (Inoue, 1981; Morita, 1982; Shimoi, 1985). The active components of these vegetables await future research work.

The importance of antimutagens can not be overemphasized at this point. There are at present many mutagens in the environment and even in the common food we eat, many of which are thought to be related to cancer, genotoxicity, and aging. This has created worldwide interest in the study of antimutagenic agents and their modes of action in an effort to minimize, if not eliminate, such mutagens. Studies on antimutagens are important not only for the elucidation of the genetic mechanisms of mutagesis but also for the prevention of cancer (Ohta, 1983).

## Experimental

### *Isolation and Purification*

Fresh green fruits of *Momordica charantia* were homogenized in distilled ethanol at room temperature. The filtered extract was concentrated under reduced pressure at 40°C and subsequently partitioned between water, dichloromethane, petrol, aqueous methanol, and carbon tetrachloride using the method of Kupchan (Kupchan, 1978) as shown in Scheme I. The petrol and carbon tetrachloride extracts, which were shown by the bioassay tests to be antimutagenic, were subjected to repeated and sequential flash column chromatography using vacuum elution and pressure elution until thin layer chromatography (TLC)-pure fractions were obtained. Mixtures of hexane-ethylacetate of varying polarities were used as solvent. The elution was monitored by analytical TLC on precoated silica gel plates.

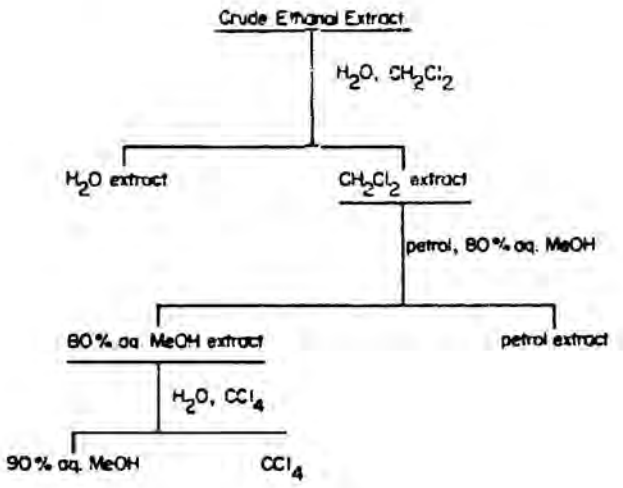
The TLC-pure fractions were finally subjected to preparative HPLC to isolate the intractable mixture D'.

### *Bioassay: The Micronucleus Test*

The micronucleus test (Schmid, 1978) was used to monitor the antimutagenic activity of the various fractions during the isolation process.

Seven- to nine-week old Swiss mice were used as test animals. Mitomycin C was used as the mutagenic control. A uniform dosage of 3 $\mu$g per gram mouse was

FRACTIONATION SCHEME




injected intraperitoneally to the test animals. Two treatments were given – 30 and 6 hours – before the mice were sacrificed.

The test extracts were dissolved in 50% dimethylsulfoxide (DMSO) and administered twice orally at varying dosages to the test animals using a feeding gavage. Three to five mice were used for each test dosage.

Immediately after sacrificing the mice, the bone marrow from the femura bones were suspended in fetal calf serum. The suspensions were centrifuged at 1000 rpm for 5 minutes and the cells in the sediment were smeared on glass slides. Three sides were smeared for each mouse. The air-dried slides were stained successively with May Grunwald and Giemsa stains. The slides were scored under a high power microscope by counting the number of micronucleated polychromatic erythrocytes (PCE) per 1000 PCE.

The data on the number of micronucleated PCE per 1000 PCE were processed on an IMB PC using the "Microstat" software, specifically the program on the analysis of variance. The calculated F ratios were compared with the critical F values found in statistical tables at 5% and 1% probability levels. The treatment effects were considered statistically significant when the calculated ratios were higher than the corresponding critical F ratios.

*Structure Elucidation*

The structure of the antimutagens were elucidated using high-field (400MHz) proton and carbon-13 NMR, Fourier transform infrared spectroscopy, and high and low resolution mass spectroscopy. Confirmation of the structure was obtained from chemical modification of the structure was obtained from chemical modification studies (acetylation, saponification, and methanolysis) followed by spectral and chromatographic analysis of the chemically modified products.

*Structure-Activity Correlation Studies*

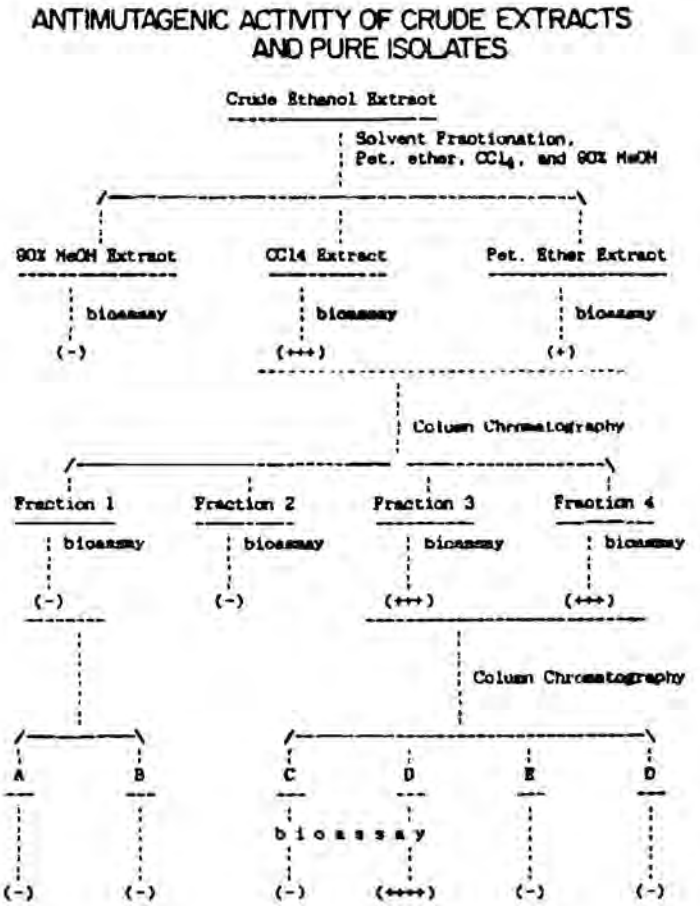
The structure-activity correlation studies were done by testing the antimutagenic activities of the native D* and the chemically modified products. The micronucleus test was used to monitor the antimutagenic activities.

## Results and Discussion

*Antimutagenic Activity*

Scheme 2 summarizes the results of the bioassay tracing the antimutagenic activity during the separation scheme.

Bioassay of the crude extracts showed that the activity was in the carbon tetrachloride and petroleum ether extracts (Figure 1). Further fractionation of these active extracts were done until TLC-pure isolaes, A, B, C, D, E, and F, were obtained. Bioassay data graphically presented in Figure 2 showed that only D was antimutagenic.



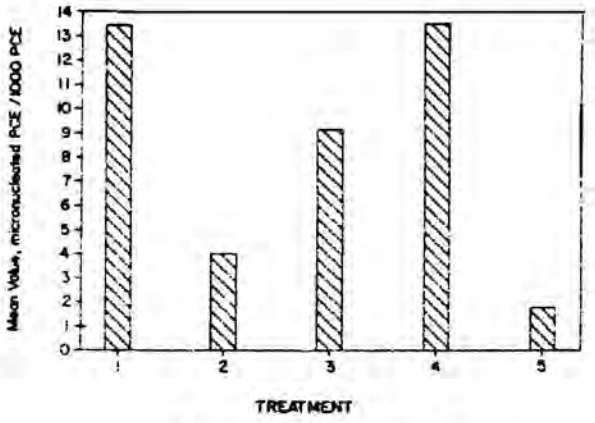



Figure 1. BIOASSAY OF CRUDE FRACTIONS.

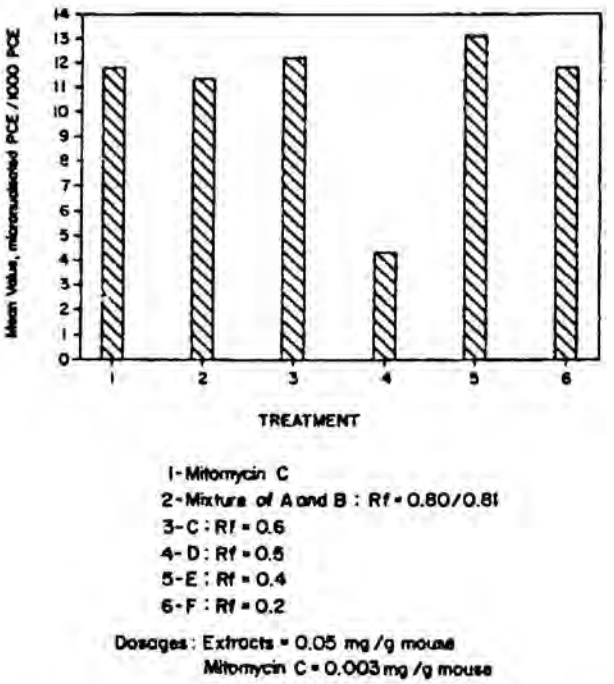



Figure 2. BIOASSAY OF PURE ISOLATES.

TLC-pure D was further purified by preparative HPLC to separate the major component, D'. Data shown in Figure 3 confirmed the antimutagenic activity of D' at a dosage range of 12.5 $\mu g - 50\ \mu g$ D' per gram mouse. At this dosage range, the mutagenicity of Mitomycin C was reduced by 80%. These data indicated that the antimutagenic principle of *Momordica charantia* is D'.

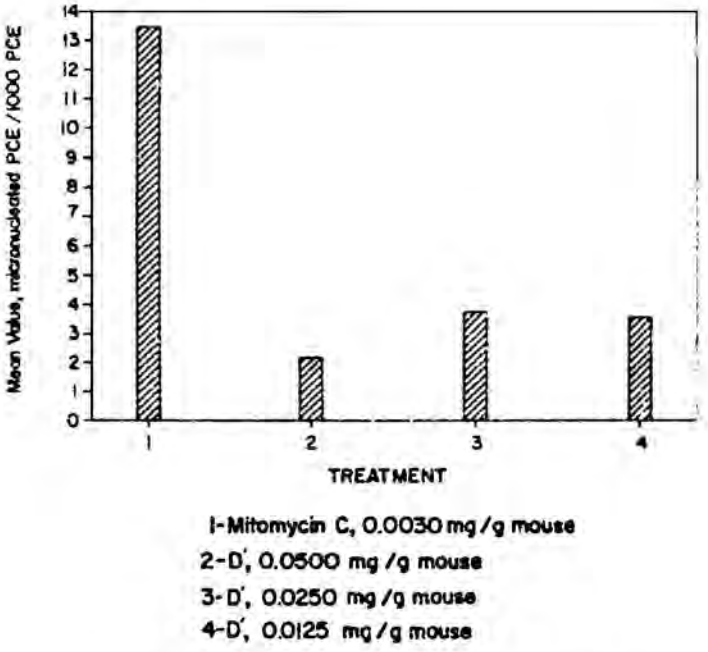



Figure 3. BIOASSAY OF HPLC-PURE D'.

*Structure Elucidation*

The $^1H$ NMR spectra of the native and acetylated (Figure 4) D' indicated D' was an acylglucosylsterol. The $^1H$ NMR characteristic signals attributed to the sterol moiety were consistent with published data for stigmasta-5,25(27)-dien-3$\beta$-ol (Sucrow, 1965; Akihisa, 1986; Garg, 1984). The identify of the sterol moiety was further confirmed by the electron impact mass spectrum of the free sterol, D'-S, obtained from the methanolysis reaction of D'. The spectrum showed a $[M^+]$ at m/z 412 corresponding to $C_{29}H_{48}O$. The mass fragmentation pattern (m/z 397, 394, 314, 299, 273, 255) was also consistent with the structure of stigmata-5,25(27-dien-3$\beta$-ol (Garg, 1984; Itoh, 1980). Final confirmation of the identity of the sterol moiety was obtained from the $^{13}C$ NMR spectum of D'. Comparison with available data from literature (Table 1) confirmed the presence of $\Delta^5$ – nucleus and a double bond at the 25(27) positions.

The very intense broad $^1H$ NMR singlet at 1.26 ppm (ascribed to a long methylene chain) and the triplet at 2.36 ppm (ascribed to methylene attached to a carbonyl group), the $^{13}C$ NMR signal at 174.6 ppm (ascribed to carbonyl carbon) and the intense signal at 29 ppm (ascribed to many methylene carbons)

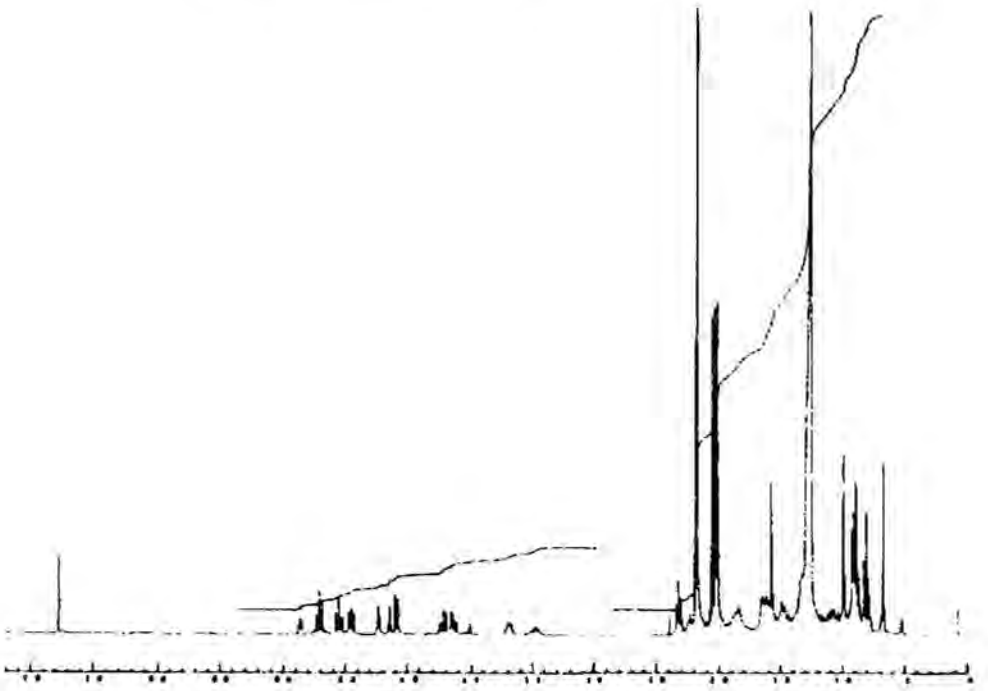


Figure 4. $^1$H NMR SPECTRUM OF ACETYLATED D' IN $CDCl^3$.

Table 1. $^{13}$C NMR Chemical Shifts

| | | *Sitosterol* | | *Stigmastadiene-3 β-OAc* | |
|---|---|---|---|---|---|
| *Carbon* | *D'* | *$\Delta^5$-3β-OH* | *$\Delta^5$-3β-OAc* | *Y $\Delta^{7,25(27)}$* | *$\Delta^{8,25(27)}$* |
| C-1 | 37.3 | 37.3 | 37.0 | 36.8 | 34.8 |
| 2 | 29.3 | 31.8 | 27.8 | 27.5 | 27.2 |
| 3 | 74.0 | 71.8 | 74.0 | 73.4 | 78.8 |
| 4 | 38.9 | 42.3 | 38.1 | 33.8 | 36.0 |
| 5 | 140.3 | 140.8 | 139.1 | 40.1 | 47.1 |
| 6 | 122.2 | 121.7 | 122.6 | 29.5 | 20.7 |
| 7 | 32.0 | 32.0 | 31.9 | 117.3 | 28.1 |
| 8 | 31.9 | 32.0 | 31.9 | 139.5 | 133.3 |
| 9 | 50.2 | 50.2 | 50.0 | 49.3 | 134.8 |
| 10 | 36.7 | 36.6 | 36.6 | 34.2 | 36.2 |
| 11 | 21.1 | 21.3 | 21.0 | 21.4 | 21.8 |
| 12 | 39.8 | 39.8 | 39.7 | 39.5 | 25.4 |
| 13 | 42.8 | 42.3 | 42.3 | 43.3 | 44.5 |
| 14 | 56.8 | 58.9 | 56.7 | 55.0 | 49.8 |
| 15 | 24.3 | 24.4 | 24.3 | 23.0 | 31.0 |
| 16 | 28.2 | 28.9 | 28.2 | 27.9 | 30.7 |
| 17 | 56.1 | 56.1 | 56.0 | 56.0 | 50.4 |
| 18 | 11.8 | 12.2 | 11.9 | 12.1 | 15.7 |
| 19 | 19.4 | 19.4 | 19.3 | 13.0 | 18.8 |
| 20 | 36.3 | 40.4 | 36.3 | 36.0 | 36.2 |

Table 1. $^{13}C$ NMR Chemicals Shifts (Continued)

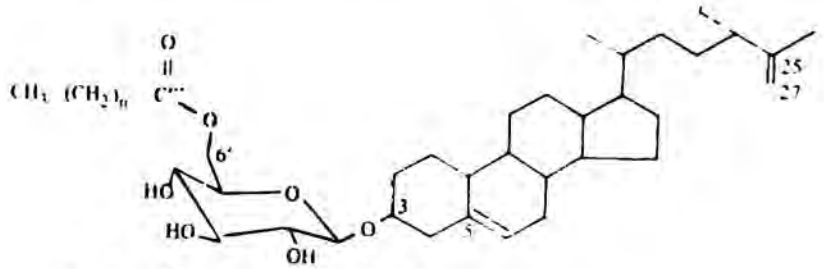
| | | *Sitosterol* | | *Stigmastadiene-3 βOAc* | |
|---|---|---|---|---|---|
| *Carbon* | *D'* | *$\Delta^5$-3β-OH* | *$\Delta^5$-3β-OAc* | *$\Delta^{7,25(27)}$* | *$\Delta^{8,25(27)}$* |
| 21 | 18.7 | 21.1 | 18.8 | 18.8 | 18.6 |
| 22 | 33.7 | 33.8 | 33.9 | 33.6 | 33.9 |
| 23 | 29.2 | 29.4 | 26.4 | 29.5 | 29.7 |
| 24 | 49.5 | 51.3 | 46.1 | 49.5 | 49.5 |
| 25 | 147.5 | 32.0 | 29.0 | 147.4 | 147.6 |
| 26 | 17.8 | 21.3 | 19.0 | 17.7 | 17.8 |
| 27 | 111.4 | 19.0 | 19.0 | 111.4 | 111.4 |
| 28 | 26.5 | 25.5 | 23.0 | 26.5 | 26.6 |
| 29 | 11.9 | 12.1 | 12.3 | 11.8 | 12.0 |
| 1' | 101.3 | | | | |
| 2' | 73.9 | | | | |
| 3' | 76.3 | | | | |
| 4' | 70.5 | | | | |
| 5' | 76.3 | | | | |
| 6' | 63.4 | | | | |
| 1" | 174.6 | | | | |
| 2" | 32.0 | | | | |
| 3"-14" | 29.7/29.3 | | | | |
| 14" | 22.7 | | | | |
| 15" | 14.1 | | | | |

were indicative of the presence of a long chain fatty acid. The gas liquid chromatogram-mass spectra of the fatty acid methyl esters obtained from the saponification reaction (followed by methylation) of D' confirmed the presence of palmitate and stearate at a ratio of 2.3: 1.

The $^1H$ NMR of the acetylated D' attributed to the sugar moiety indicated the presence of a β-D-glucose moiety. Confirmation of the presence of D-glucose was obtained from the gas liquid chromatogram of the trimethylsilylated glucose obtained from the trimethylsilylation of the unknown sugar obtained from the methanolysis reaction of D' .

A comparison of the glucosidic $^1H$ NMR and $^{13}C$ NMR signals of native D' and the glucosylsterol obtained from the saponification reaction of D' showed that only the signals due to the H'-6 and C'-6, respectively, were significantly shifted uplifted. These data indicated that the fatty acids were ester linked to the glucosyl moiety at the hydroxyl group at the 6' postion. The $^1H$ NMR spectrum of the acetylated glucosylsterol indicated that only one molecule of fatty acid is ester linked per molecule of glucosylsterol.

In the light of these data, it is suggested that the antimutagens present in *Momordica charantia*, which is the HPLC-pure D', is an intractable mixture of acylglucosylsterols. The major component of the mixture is 3-O-[6'-O-palmitoyl-β-D-glucosyl]-stigmasta-5,25(27)-diene (D'-1) and the minor component is the stearyl derivative (D'-2).



D'-1 : n = 14 (palmitate)
D'-2 : n = 16 (stearate)

*Structure-Activity Correlation*

A comparison of the bioassay data for the native D' and the chemically modified products obtained from the acetylation, methanolysis, and saponification reactions are graphically shown in Figure 5.

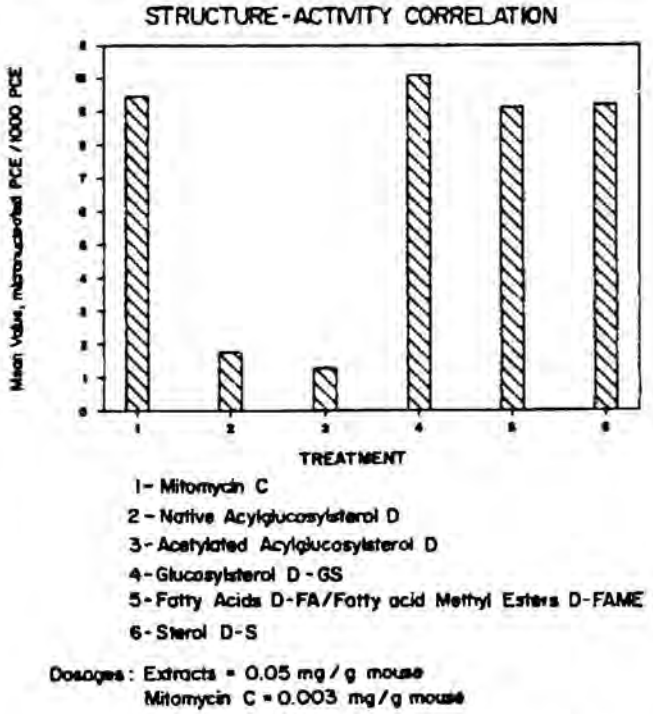



Figure 5. STRUCTURE-ACTIVITY CORRELATION

The acetylated D' also exhibited antimutagenic activity which suggested that the hydroxyl groups of the glucosyl moiety were not necessary for antimutagenic activity. It is, however, possible that the acetylated compound may be deacetylated *in vivo* to give back the active native antimutagen. On the other hand, the hydrophobic nature of the fatty acid and sterol moieties may be necessary for the activity.

The lack of antimutagenic activity of the free fatty acids, the free sterol and the glucosylsterol suggested that the activity may reside in the native arrangement of the three components – the fatty acid, the glucosyl moiety, and the sterol

moiety. It is, thus, possible that the antimutagenic activity may reside in the peculiar lipid-like nature of D'. As a lipid-like compound, ingestion of D' may result in the absorption in the membrane lipid bilayer, which could adversely affect the membrane permeability towards Mitomycin C and disrupt the cellular activity of the latter.

## Acknowledgment

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# CELLULOSE BIODEGRADATION STUDIES: APPLICATION OF rDNA AND PROTOPLAST FUSION TECHNIQUES

Saturnina C. Halos
*Natural Sciences Research Institute*
*University of the Philippines*
*Diliman, Quezon City, Philippines*

## Introduction

Cellulose biodegradation refers to the breakdown of cellulose to its component glucose units through the action of enzymes. This process has attracted scientific attention because of the complexity of the enzymes involved and the environmental, as well as economic, significance of the process.

Cellulose, the major structural polysaccharide of plants, is a hydrophilic linear glucose polymer with the anhydroglucose units bonded by B-1.4, glucosidic linkage (Ghose and Mishra, 1984). The number of glucose units may vary from 15 (α-cellulose) to more than 10,000 (α-cellulose) per molecule. The polymer has both crystalline and amorphous regions, the former referring to the portion more resistant to chemical/biochemical attack and the latter, to the portion of the cellulose chain that is prone to easy hydrolysis (Muhlenthaler, 1967).

Cellulose biodegradation is mediated by several enzyme systems. The more studied are the extracellular cellulase systems in fungi that have three components: endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) and B-glucosidases (EC 3.2.1.21) (Coughlan and Ljungdahl, 1988). The endoglucanases are found to be inactive against crystalline cellulose when acting alone, but hydrolyze amorphous cellulose and soluble derivatives of carboxymethyl cellulose (CM-cellulose). Endoglucanases are also referred to as CM-cellulases. Their attack on amorphous cellulose is characterized by random cleavage of B-glycosidic linkages. By contrast, cellobiohydrolases degrade amorphous cellulose by consecutive removal of cellobiose units from the non-reducing end of the substrate. Endoglucanases and cellobiohydrolases act together to degrade crystalline cellulose and the B-glucosidades complete the hydrolytic process by converting the resultant cellobiose to glucose or removing glucose from the non-reducing end of short cellooligosaccharides. Exoglucohydrolases (EC 3.2.1.74) from *Penicillium funiculosum* and *Talaromyces emersonii* catalyze the removal of glucose residues from the non-reducing end of cellodextrins but do not interact synergistically with endoglucanases in the hydrolysis of cellulose. Oxidative enzymes such as cellobiose oxidase (EC 1.199.18) and cellobiose: quinone oxidoreductase (EC 1.3.5.1) have been found to participate in cellulose degradation. Most, if not all, of the extracellular hydrolytic enzymes of

fungi are glycoproteins. Of the known aerobic cellulolytic bacteria, only three, *Clostridum thermocellum, Thermonospora sp.*, and *Microbispora bispora* possess culture filtrates active against crystalline cellulose. In anaerobic bacteria, an active cellulase complex referred to as cellulosome (Lamed *et al*, 1983) is bound to the cell surface. The bound enzymes are believed to be the more active cellulases in bacteria. However, one can see a more complex structure. The cellulosomes comprise 35 polypeptides ranging from 45kDA to about 200 kDA (Lamed, *et al*, 1983 and Hon-Nam, *et al*, 1986).

This paper describes our research work done at the Natural Sciences Research Institute (NSRI) at the U.P. Diliman campus and at the National Institute of Biotechnology and Applied Microbiology (BIOTECH) at the U.P. Los Baños campus. Research done at the NSRI mainly involves the use of rDNA techniques in studying cellulose biodegradation whereas at the BIOTECH, our work involved the use of protoplast fusion in improving cellulose biodegradation in fungi.

## I. *The use of recombinant DNA in cellulose degradation studies*

Recombinant DNA techniques refer to a set of procedures that obtains a piece of DNA from a donor species and inserts this piece to a self-replicating DNA molecule referred to as a vector that has been constructed to easily enter and multiply in a host cell.

At present, a number of laboratories worldwide have employed the recombinant DNA techniques in studying the cellulases of fungi and bacteria. This is based on the underlying concept of molecular biology that the structure determines the function. Primarily, people wanted to examine the structure of the cellulase genes to explain such phenomena as the proper and functional aggregation of a multicomponent complex or the varied activity of the enzyme depending on the source of substrate. In our studies, we wanted to do three things:

One was to clone the cellulase genes from a depressed mutant of *Cellulomonas fimi* and compare it with the genes from the wild type strain. The results would tell us if the genes themselves contain sequences involved in glucose repression and where these sequences would be located.

Two, we wanted to place the cellulase genes in a multicopy plasmid, place it back into the *Cellulomonas* cell to produce a high cellulase producer.

Three, we wanted to identify the exact gene products of the genes we isolated.

We started our work in May, 1986 and with the funds obtained from the U.S. Agency for International Development, we cloned and obtained restriction maps of two cellulase genes, did partial sequencing, identified our constraint in placing the cellulase genes back in the Cellulomonas and are still in the process of identifying the exact gene products of our two genes.

Our procedures involved construction of a *Cellulomonas* DNA library, molecular cloning and subcloning, restriction mapping, Southern blot hybridization, transformation experiments, and enzyme assays. Subcloning, restriction mapping, Southern blot hybridization and partial DNA sequencing were done by one of our

project personnel, Rose Caday, at the laboratory of Professor Seymour Fogel at the University of California, Berkeley, whereas the rest of the work was done at our NSRI laboratory. Details of our procedures were presented in three papers being prepared for publication.

We obtained a DNA library in *Escherichia coli* C600 composed of 3,000 clones using the shotgun approach. Of these, seven clones gave positive reaction when screened for cellulase activity by the Congo red assay. The presence of cellulase activity was further demonstrated by colorimetric activity (Table 1). However, only four clones were further assayed using flourogenic substances and these exhibited substantially increased activities as indicated by bright flourescence under uv light compared with the *E. coli* host. The culture filtrate of the four recombinant clones *E. coli* C602, C609, C610 and C612 were positive for B-glucosidase, cellobiohydrolase and xylanase activities (Table 2) whereas the cell-free extracts were positive only for B-glucosidase (Figure 1). The cell-free extracts of the *E. coli* host also showed high B-glucosidase activity but none of the other enzymes whereas the culture filtrate showed very weak B-glucosidase activity. The donor, *Cellulomonas* strain exhibited high activities for all three enzymes in both its cell-free extracts as well as its culture filtrate. These results indicate that the B-glucosidase, the cellobiohydrolase and the xylanase excreted by the *E. coli* host were of *Cellulomonas* origin. This may also mean that the host produced a lot more B-glucosidase, that some were excreted and the rest were kept inside the cell. This also indicates that both the *Cellulomonas* and the *E. coli* B-glucosidase genes were expressed.

The DNA inserts of recombinant clones, C602 and C610 were subcloned into *E. coli* HB101 for higher plasmid recovery. The recombinant plasmids were extracted and labelled with P32. These were hybridized with the digested *Cellulomonas* genomic DNA. Both inserts hybridized. These were also restricted with a total of 23 restriction enzymes. These two DNA inserts showed strictly different restriction maps indicating different DNA sequences (Figure 2). These genes may

Table 1. Colorimetric assay of cell lysates

| *Source of Enzymes* | *CMCase* *10* | *FPase* *10* | *B-clucosidase* *10* |
|---|---|---|---|
| E. coli C602 | 7.50 | 1.16 | 3.35 |
| E. coli C603 | 28.06 | 3.24 | 6.04 |
| E. coli C606 | 5.00 | 2.08 | 9.73 |
| E. coli C608 | 41.11 | 4.86 | 14.76 |
| E. coli C609 | 39.44 | 5.79 | 14.76 |
| E. coli C610 | 50.56 | 11.57 | 16.10 |
| E. coli C612 | 2.78 | 0.92 | 6.04 |
| E. coli C600 | 7.78 | 4.40 | 5.37 |

From: Halos, S.C. *et al.* (manuscript in preparation).

Table 2. Cellulase activity of culture filtrate from transformed and control cells

| *Source of Enzymes* | *Substrates* | | | |
|---|---|---|---|---|
| | *MUG* | *MUCb* | *MUxy* | *CMC** |
| E. coli C602 | ++ | ++ | ++ | + |
| E. coli C603 | nt | nt | nt | + |
| E. coli C606 | nt | nt | nt | + |
| E. coli C608 | nt | nt | nt | + |
| E. coli C609 | + | + | + | + |
| E. coli C610 | + | + | + | + |
| E. coli C612 | + | + | + | + |
| C. coli C600 | +/– | +/– | +/– | + |
| E. coli HB 294 | +/– | +/– | +/– | + |
| C. fimi | +++ | +++ | +++ | + |

| | |
|---|---|
| +++ | Strong fluorescence |
| ++ | Medium fluorescence |
| + | Weak fluorescence |
| +/– | Very weak fluorescence/clearing |
| *t | Presence of halo |
| nt | not rested |
| MUG | 4-methylumbelliferyl B-D glucopyranoside (B-glucosidase) |
| MUCb | 4-methylumbelliferyl B-D cellobioyranoside (Cellobiohydrolase) |
| MUXy | 4-methylumbelliferyl B-D xylopyranoside (Xylanase) |

From: Halos, S.C. *et al.* (manuscript in preparation)

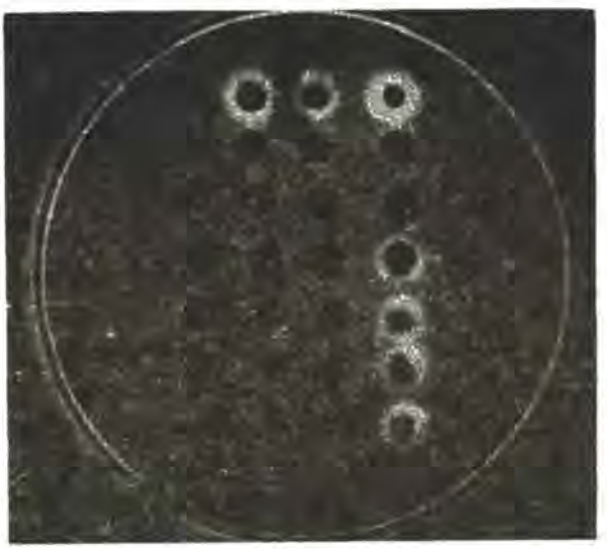

Figure 1. Fluorescence of crude extracts using 4 methylumbeliferyl B-D glycosides. Column 1, MUG; Column 2, MUCb; Column 3, MUXy; Column 4 Mixed substrates, no enzyme. Row 1, C602; Row 2, C609; Row 3, C610; Row 4, C612, Row 5, HB294; Row 6, C600, Row 7, *Cellulomonas fimi.* (Halos, C.S., J. Claudio, D. Sanchez, Manuscript in preparation).

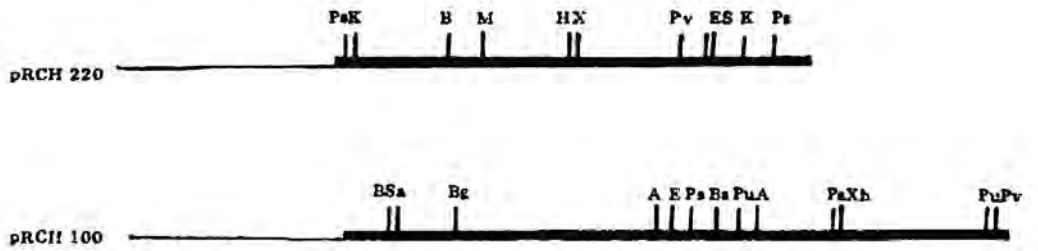


Figure 2. Restriction maps of cloned DNA fragments from depressed mutant of *Cellulomonas fimi* expressing cellulase activity. Ps, PstI; K, KpnI; B, BamHI; M, MluI; H, HindIII; X, XbaI; Pv, PvuI; E, EcoRI; S, SmaI; Sa, SalI; Bg, BglI; A, AvaI; Bs, BstxI; Pu, PvuII; Xh, XhoI. Pu sites were not clearly established from restriction map. (Halos, S.C. & R.A. Caday, manuscript in preparation).

represent the two separately migrating cellobiohydrolases that we have extracted from *Cellulomonas*. However, these restriction maps differed from restriction maps obtained for cellulase genes of various microorganisms, including the wild type of *Cellulomonas fimi* (Figures 3-7). It is possible that we have cloned other cellulase genes and that there could be at least four cellulase genes in *Cellulomonas fimi*. There were ten different DNA fragments expressing cellulolytic activities cloned for *C. thermocellum* and six in *Microbispora bispora*.

The cloned genes, including those obtained from other microorganisms and inserted in pBR322 or its derivatives, were used to transform the *Cellulomonas fimi* mutant. Although we were able to obtain transformation, the cells apparently could not maintain the plasmid. We have observed, however, that *Cellulomonas* has a plasmid which we can use to construct a cloning vector in the future.

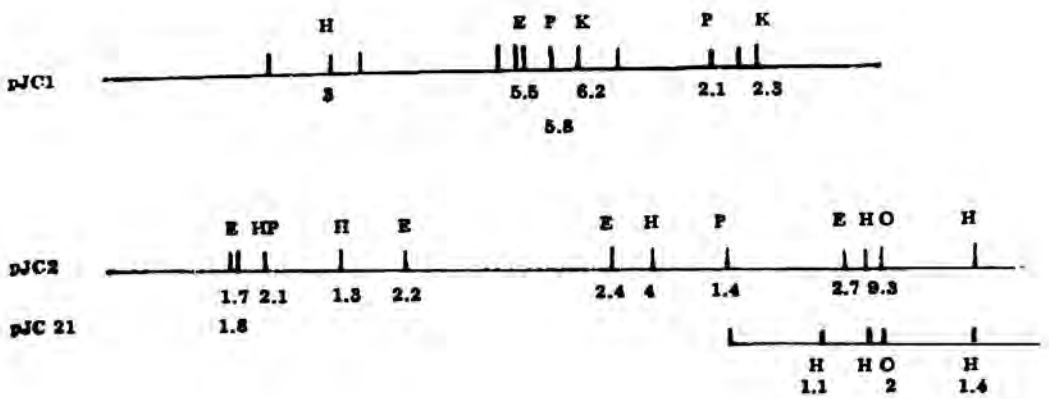


Figure 3. Restriction maps of cellulase genes from *Cellulomonas* sp. H, HindIII; E, EcoRI; K, KpnI, P, PvuII; B, BamHI. (Lohwongwatana, unpublished)

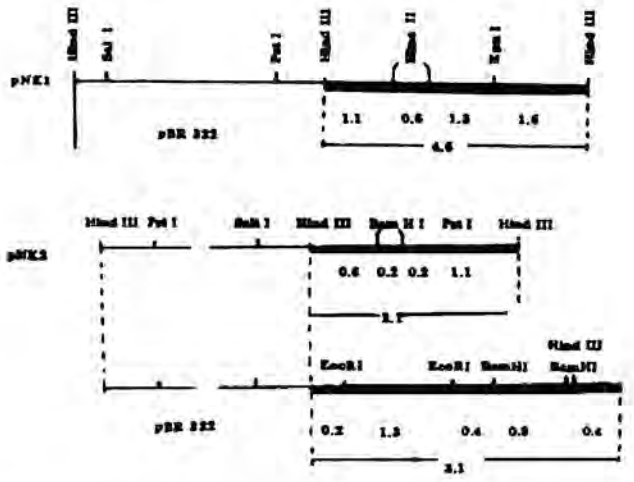

Figure 4. Restriction maps of cloned DNA fragments from *Bacillus* sp. expressing cellulase activity. (Horikoshi and Fukumori, 1988)

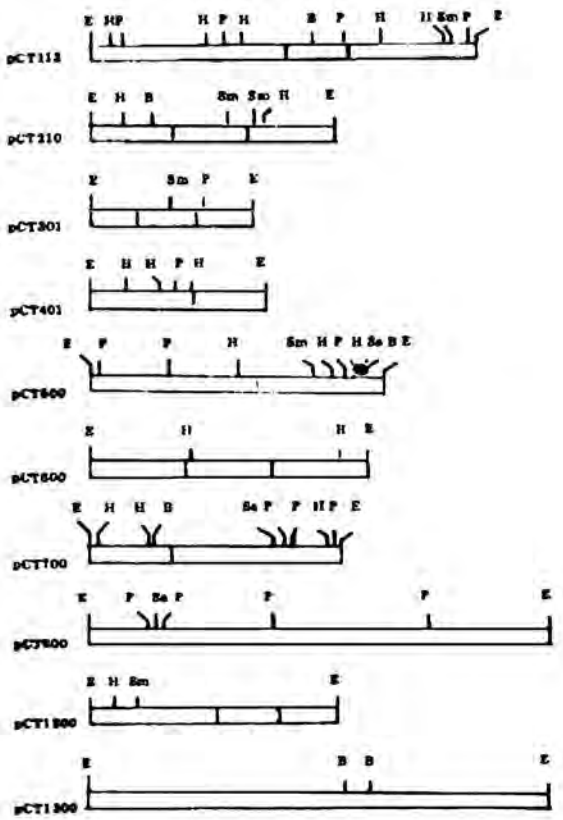

Figure 5. Restriction map of DNA from *Clostridum thermocellum* encoding CMC or MUCb-hydrolyzing activity. B, BamHI; E, EcoRI; H, HindIII; P, PstI; Sa, SalI, Sm, SmaI. pCT800 and pCT1300 contain 6 and 9 HindIII sites, respectively. (Beguin *et al.*, 1988)

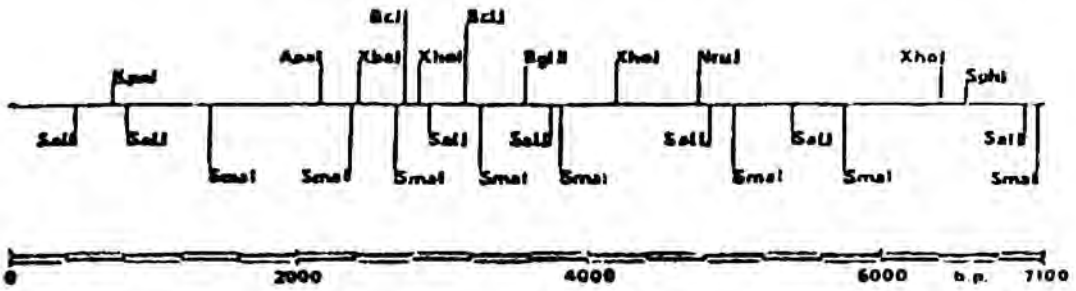


Figure 6. Restriction map of cloned DNA fragment from *Streptomyces* expressing cellulase activity. (Coppolecchia *et al.*, 1987)

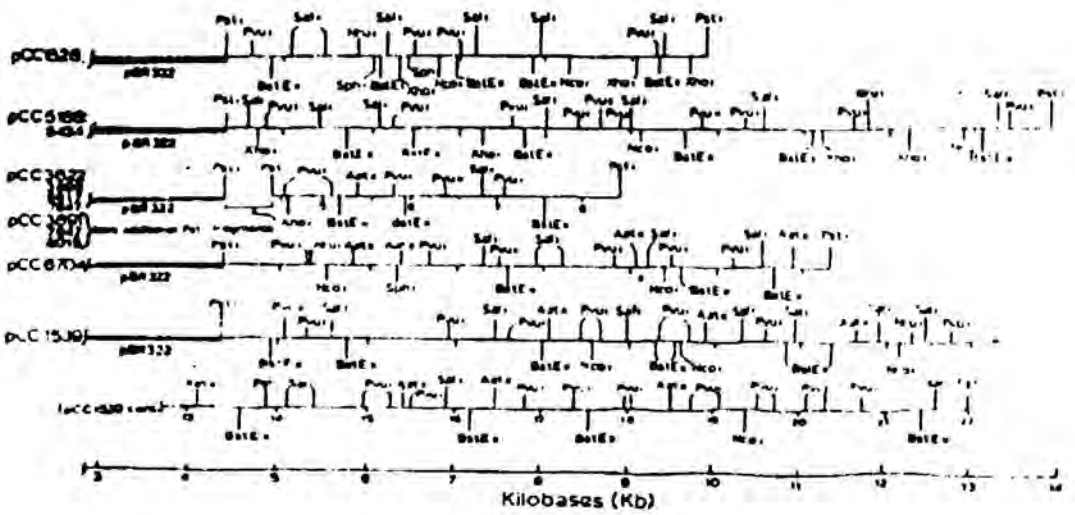


Figure 7. Restriction maps of cloned *Microbispora bispora* DNA fragments expressing active CMCase in *E. coli*. (Yablonsky *et al.*, 1988)

## II. *The use of protoplast fusion in cellulose biodegradation studies.*

Protoplast fusion refers to the creation of one cell out of two cells using an agent that removes the cell wall of bacteria, plants or fungi and an agent that makes these membrane-bound cells stick together and adjoin their membranes. With animal cells, only the use of a sticking agent is required. Protoplast fusion allows for the production of hybrids between totally unrelated species provided that viable fusants are obtained. In our studies, we are exploring the use of protoplast fusion in producing new fungal hybrids with improved cellulolytic activity.

Of the various fungal species producing cellulases, the *Trichoderma* and the *Penicillium* species are the ones currently used in the limited commercial production of cellulases. The advantage of using fungal cellulases lies in their relative stability to extraction procedures. However, the search for better sources of cellulases continues since no strain that can be used economically to produce the enzymes has yet been developed or isolated.

In our earliest project of screening for high cellulase producers, we identified, one isolate of *Penicillium funiculosum* Thom No. 171 that was as good as our reference strain, *Trichoderma reesei* RutC30, a strain developed for high cellulolytic activity by researchers of Rutgers University in New Jersey. In addition, *P. funiculosum* No. 171 produced higher levels of B-glucosidase activity than T. Reesei Rut C300 (Cruz, W.T., 1986). This prompted us to explore the possibility of using protoplast fusion to develop intergeneric hybrids of *Trichoderma X Penicillium* that would produce a hybrid cellulase complex incorporating the higher cellulase activities of both parents.

Our study involved establishing the procedures for protoplast isolation and renegeration for *P. funiculosum*, and *T. reesei* RutC30 using resources available to us, identification of suitable selection markers, characterization of fusants and assaying for their cellulolytic activities. Details of procedures and results are presented in two papers submitted for publication.

Of six enzyme preparations tested for protoplast production, a more efficient one was the combination of Novozyme 234 (Novo Industries, Inc.) and Zymolyaze 20T which released $3.6 \times 10^7$ for *T. reesei* and $1.4 \times 10^7$ for *P. funiculosum* per mg mycelia (Table 3 and Figure 8). This enzymes combination released protoplast in direct proportion to the amount of mycelia when exposed to the same concentration of enzyme (2.5 mg/ml Novozyme 234 and 5 mg/ml Zymolyase 20T) typical of an enzymatic reaction. *T. reesei* was more susceptible to these enzymes in that protoplasts were first produced from *T. reesei* 60 minutes following treatment of mycelium compared with *P. funiculosum* that started releasing

Table 3. Comparison of lytic enzyme preparations for the release of Protoplasts from *Trichoderma reesei RUT C-30* and Penicillum funiculosum *Thom* MG 171

| *Enzyme System* | *Protoplast yield Trichoderma reesei RUT C-30* | *Penicillum funiculosum Thom MG 171* |
|---|---|---|
| Celluclost | – | – – |
| Cellulase (laboratory prep.) | $3.7 \times 10^6$ | $2 \times 10^6$ |
| Novozyme 234 | $2.9 \times 10^5$ | $1.7 \times 10^6$ |
| Zymolase 20T | $0.5 \times 10^6$ | $0.28 \times 10^6$ |
| Celluclast + Zymolace 2OT | $0.1 \times 10^6$ | $0.016 \times 10^6$ |
| Novozym 234 X zymolase 2OT | $3.6 \times 10^7$ | $1.4 \times 10^7$ |

From: Pham, L. & S.C. Halos, 1988. manuscript for publication.

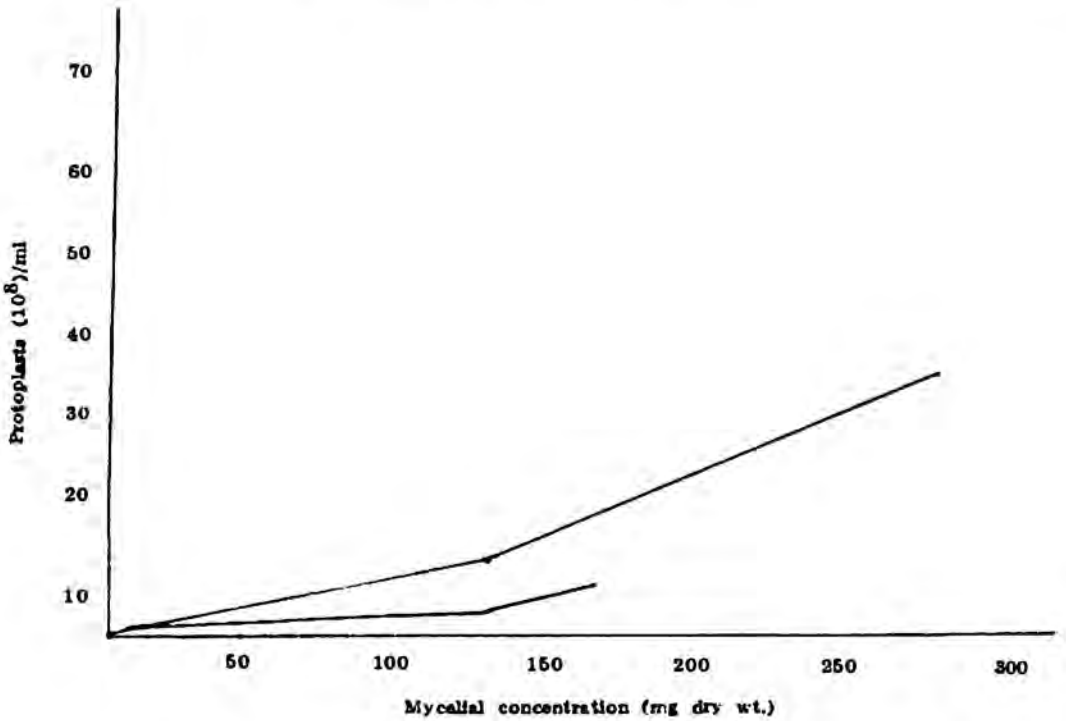


Figure 8. The effect of mycelial oncentration on protoplast formation of *Trichoderma reesei RUT C-30* and *Penicillium funiculosum Thom MG 171*. (Pham, L. & S.C. Halos, 1988. manuscript for publication).

protoplast 90 minutes after treatment. Furthermore, the highest yield of protoplasts was obtained with *Trichoderma* 2 hours after enzyme exposure, whereas with *P. funiculosum* it was 4 hours after. Also, *T. reesei* regenerated more at 92% than *P. funiculosum* which gave a 31.17% regeneration frequency. Regeneration occurred 3 hours after transfer to Winge medium.

Since we had no intention of altering the genetic makeup of our parental strains, we sought for innate properties of these strains to use as selection markers. We screened their resistance to different metal ions (Cu, Na, Co, and Hg), fungicides (Captan and Benlate) and antibiotic (nystatin) at different concentrations. We were able to identify complementing markers $Co^R$ and $Hg^S$ for *T. reesei* and $Co^S$ and $Hg^R$ for *P. funiculosum* (Table 4) as the primary selection markers (Figure 9). Fusants were then selected as $Co^R$ and $Hg^R$ colonies (Figure 10).

Fusants derived were viable and exhibited different morphologies (Table 5) which combine the properties of both parents. Fusants did not exhibit uniform characteristics which could be due to observations that mycelial fungi are multinucleated. Fusants might have represented different combinations of the 3 nuclei that are often found in one mycelial cell.

Table 4. The effect of Metal Ions on Protoplast Regeneration of *Trichoderma reesei* RUT C-30 and *Penicillium funiculosum* Thom MG 171

| *Metal Ions* | *Trichoderma reesei RUT C-30* | *Penicillium funicolusom Thom 171* |
|---|---|---|
| *Cu (10 ppm)* | + | + |
| *Na (10 ppm)* | + | |
| Cu (10 ppm) | + | + |
| Na (10 ppm) | + | + |
| Co (10 ppm) | + | – |
| Hg (1 ppm) | – | + |

+ = regenerated
– = did not regenerate

From: Pham, I. & S.C. Halos, 1988. manuscript for publication)

Protoplast from *Penicillium funiculosum.*

Regenerating protoplast from *P. funiculosum.*

Figure 9. Regeneration of *Penicillium funiculosum* protoplast. (Pham, L. & S.C. Halos, 1988. manuscript for publication).

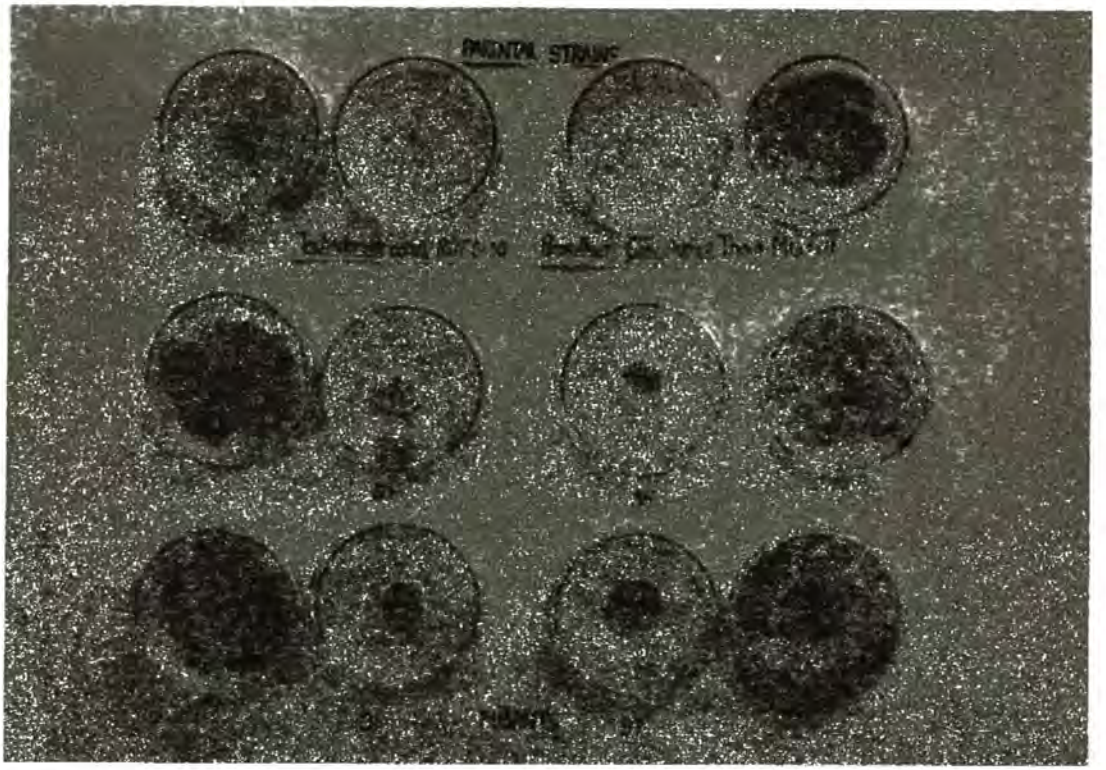

Figure 10. Parental Strains and Fusants derived from *Trichoderma reesei* RutC30 and *Penicillium funiculosum* Thom MG 171. (Pham, L. & S.C. Halos, 1988. manuscript for publication).
extreme left – without $Co^{+}$, $Hg^{++}$
center – with $Co^{+}$, $Hg^{++}$
extreme right – without $Co^{+}$, $Hg^{++}$

Table 5. Cultural characteristics of fusant strains following protoplast fusion compared tn parental strains

| *FUSANT NU.* | *COLONY COLOR* | *COLONY TEXTURE* | *SPORU-LATION* | *PIGMENTATION* |
|---|---|---|---|---|
| 14 | white | cottony | none | red |
| 20 | graying green | cottony | fourth day | dark brown |
| 24 | light blue to moss green | velvetty | second day | sulfur yellow |
| 34 | olive green | cottony | fourth day | red to brick red |
| 35 | olive green | cottony | fourth day | red to brick clay |
| 36 | blue to moss green | velvetty | second day | sulfur yellow |
| 37 | blue to moss green | velvetty | second day | sulfur yellow |

| *FUSANT NU.* | *COLONY COLOR* | *COLONY TEXTURE* | *SPORU-LATION* | *PIGMENTATION* |
|---|---|---|---|---|
| 46 | moss green | velvetty | – | dark brown |
| 47 | yellow green | velvetty | – | dark brown |
| 62 | olive green | velvetty | – | dark red |
| 66 | moss green | irregular | – | dark red |
| 71 | moss green | velvetty | – | brown |
| 74 | moss green | velvetty | – | brown |
| 76 | olive green | velvetty | – | dark brown |
| 77 | moss green | velvetty | – | dark brown |
| PARENTAL STRAINS | | | | |
| *T. reesei* RUT C-30 | yellow green to olive green | cottony | fourth day | sulfur yellow |
| *P. funiculum* Thom MG-171 | moss agreen | velvetty | fourthday | red |

From: Pham, L. & S.C. Halos, 1988. manuscript for publication.

Table 6. Enzyme activities of fusants and parental strains

| *FUSANT NO.* | *PROTEIN Mg/ml* | *CMCASE IU/ml/min* | *FPASE IU/m/min* | *B-GLUCOSIDASE IU/10 min.* |
|---|---|---|---|---|
| 14 | 0.38 | .157 | .007 | .101 |
| 20 | 0.68 | .055 | .051 | .037 |
| 24 | 1.09 | .565 | .154 | .126 |
| 34 | 1.52 | 1.52 | 1.31 | 7.79 |
| 35 | 1.55 | 4.65 | 1.19 | 4.267 |
| 36 | 0.95 | 3.90 | 0.697 | 1.877 |
| 37 | 1.93 | 3.90 | 1.04 | 1.319 |
| 46 | 1.14 | 5.46 | .36 | – |
| 47 | .553 | 2.17 | .36 | – |
| 66 | 1.25 | 5.46 | .332 | – |
| 67 | 1.38 | 5.05 | .408 | – |
| 71 | 1.59 | 4.83 | .344 | – |
| 74 | 1.86 | 5.23 | .398 | – |
| 76 | 1.25 | 4.42 | .352 | – |
| 77 | 1.45 | 5.62 | .359 | – |
| 78 | 1.92 | 3.56 | .334 | – |
| PARENTAL STRAINS | | | | |
| *T. reesei* RUT C-30 | 2.38 | 7.15 | 2.49 | .178 |
| *P. funiculosum* TThom MG-171 | 1.25 | 4.55 | 1.40 | 9.89 |

From: Pham, L. & S.C. Halos, 1988. manuscript for publication.

The cellulolytic activity of various fusants were in general lower than their parental strains (Table 6) Pham and Halos, 1988). However, preliminary results with the second generation or the progeny of two fusants indicate that these have higher CM-cellulase and FP-cellulase activities than the parental strains (Tables 7-8) (de los Reyes, M. 1988).

Table 7. Average CMcase activity (IU/ml), glucose production (mg/ml) and specific activity (IU/mg) in rice straw of the cellulase enzyme from the fusants and parental strains

| *Isolate Name/Number* | *CMcase act. (IU/ml)* | *Spec. act. (IU/mg)* | *Glucose yield (mg/ml)* |
|---|---|---|---|
| *Trichoderma reesei Rut C30* | *0.0759*$^{B}$ | 0.4258$^{B}$ | 0.1366 |
| *P. funiculosum MG 171* | 0.0557$^{B}$ | 0.6724$^{B}$ | 0.1002 |
| B18 | 0.3454$^{A}$ | 0.8665$^{A}$ | 0.6217 |
| B12 | 0.2857$^{A}$ | 0.7704$^{A}$ | 0.5143 |
| B13 | 0.3639$^{A}$ | 0.3808$^{A}$ | 0.6551 |
| B14 | 0.3605$^{A}$ | 0.7454$^{A}$ | 0.6490 |

From: de los Reyes, C.C., 1988

Table 8. Average FPase activity (IU/ml), glucose production (mg/ml) and specific activity (IU/mg) in rice straw of the cellulase enzyme from the fusants and parental strains

| *Isolate Name/Number* | *FPase act. (IU/ml)* | *Spec. act. (IU/mg)* | *Glucose yield (mg/ml)* |
|---|---|---|---|
| *Trichoderma reesei Rut C30* | 0.0073$^{B}$ | 0.0431$^{A}$ | 0.0787 |
| *P. funiculosum MG 171* | ND'$^{B}$ | ND'$^{A}$ | ND' |
| B18 | 0.0793$^{A}$ | 0.1581$^{A}$ | 0.8562 |
| B12 | 0.0260$^{A}$ | 0.0714$^{A}$ | 0.2809 |
| B13 | 0.0733$^{A}$ | 0.0767$^{A}$ | 0.7912 |
| B14 | 0.0963$^{A}$ | 0.1995$^{A}$ | 1.0402 |

[1] ND, undetectable

'values within a column having similar letters indicate that they are not significantly different according to DMRT result

From: de los Reyes, C.C., 1988

## Conclusion

We are just starting to explore the use of the techniques of the new genetics in cellulose biodegradation. In the use of protoplast fusion in improving the cellulose biodegradation ability of fungal strains, it appears that there is more promise

among the second generation fusants of *T. reesei* Rut C30 and *P. funiculosom* No. 171, whereby four second generation fusants exhibited higher cellulolytic activities than their parental strains. In using rDNA techniques, we are confirming results we obtained with other procedures on the presence of at least two genes for cellulases. These genes exhibit different sequences and their protein products exhibited different mobilities upon electrophoresis. Originally, we proposed to place one of these cloned genes back into the *Cellulomonas* cell; however, the *Cellulomonas* cannot retain the pBR322 plasmid. Hence, we are currently studying the *Cellulomonas* plasmid as a possible vector for the cloned cellulase gene.

## Acknowledgments

I wish to thank R.A. Caday, J.O. Claudio, D.R. Sanchez, L.J. Pham and T.C. Ilagan for their technical assistance. This work was supported by USAID Grant Number 492-5542-G-SS-6007-00 and BIOTECH.

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*Trans. Nat. Acad. Sci. & Tech. (Phils.)*
*1989.11:129-134*

# SUBLETHAL EFFECTS OF CADMIUM ON OVARIAN MORPHOGENESIS IN *TILAPIA NILOTICA*

Annabelle A. Herrera
and
Corazon Monica Baylosis
*Institute of Biology*
*College of Science*
*University of the Philippines*
*Diliman, Quezon City*

Onset of ovarian differentiation is at least one week delayed in Cd-treated juvenile Tilapia. Ultrastructurally, oogonia, primary oocytes and follicle cells show damaged membranes and cell organelles. While normally vitellogenesis starts at day fifty-three, most of the treated cells do not have yolk vesicles by this time. Ultrastructural features of steroidogenesis in theca cells are not evident by day forty-five. The ovary of treated fish appears smaller and contains less gametogenic cells than that of untreated fish.

All these observations indicate reproductive failure due to cadmium toxicity in *T. nilotica.*

## Introduction

Cadmium is largely accumulated in the kidney, pancrease, liver, spleen and intestines in mammals. Accumulated metal causes disorders, such as degeneration or malfunction of these organs. (Eisler and Gardner, 1973).

In teleosts, sites of major accumulation vary with species (Tokumaru *et al,* 1980). However, reports dealing with histological responses to cadmium are restricted to very short-term experiments on mature fish (Gardner & Yevich, 1970; Schweiger, 1957).

The purpose of this work is to analyze the ultrastructural effects of sublethal dose of cadmium on the histogenesis of the ovary of *Tilapia nilotica* from the embryo to maturity.

## Materials and Methods

*Tilapia nilotica* four-day posthatch larvae were exposed to 0.5 ppm cadmium chloride for eight weeks. Fish were harvested after four- and eight-week treatments and ovaries were processed for electron microscopy.

Specimens were fixed in 2.5% glutaraldehyde, washed in phosphate buffer (pH 7.2), postfixed in $OSO_4$, dehydrated in acetone and propylene oxide and embedded in araldite resin. Ultrathin sections were stained with uranyl acetate and lead citrate.

## Results and Discussion

By the second week of treatment, primordial germ cells reach the gonad blastema. Treated and untreated fish have the same ridge appearance and the PGCs have similar ultrastructural features (Herrera, 1987).

While the elongated primordium of the ovarian cavity appears on the sixteenth day in untreated fish, there is about one week delay in the treated larvae. By the fourth week, several pathological changes are observed (Figs. 1 and 2) in the germ cells and somatic tissues. (Herrera, 1988 a, b).

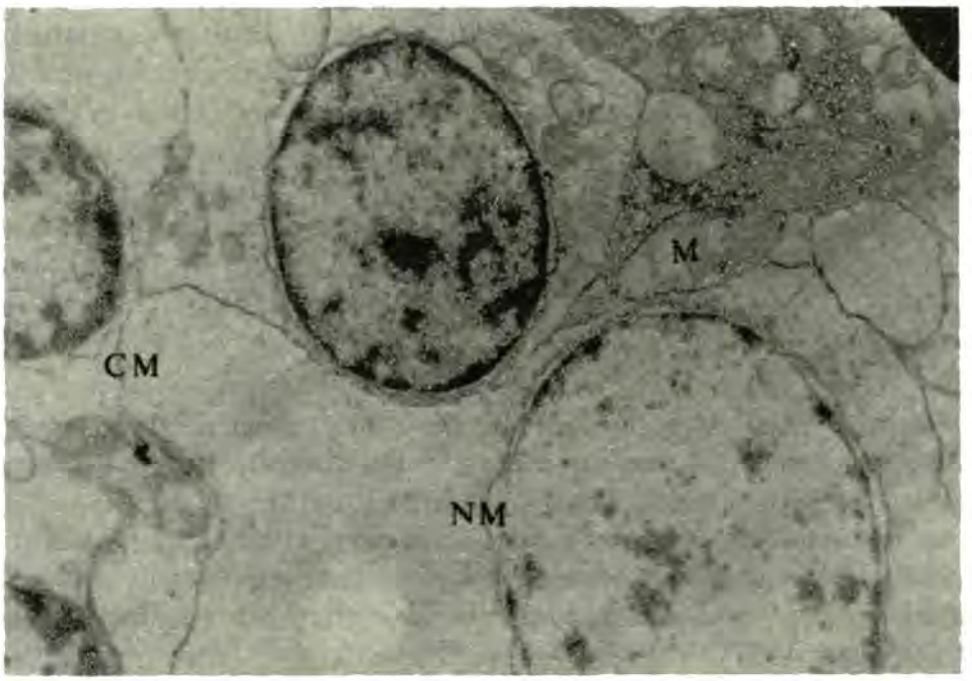


Figure 1. Swollen oocytes with dilated nuclear membrane and enlarged mitochondria and ER. 7,000x.

By seven to eight weeks, ultrastructural changes observed in the oocytes are dilated mitochondria, enlarged ER and formation of vacuoles and electron dense bodies. (Fig. 3)

Somatic cells closely show damage (Fig. 4) which probably explains delayed vitellogensis.

In the gonads, the deleterious effects in the gametogenic cells are reportedly due to injurious effects of Cd on the vasculature and on steroid synthesis (Sanga-

lang and O' Halloran, 1972). This was observed in the capillaries of the ovary (Figs. 5, 6).

This same condition of blood vessel destruction had been observed in the testis (Herrera, 1988c). This lead to aspermatogenesis, orchitis and epididymitis (White *et al.*, 1978).

Templeton and Cherian (1985) have offered an explanation to Cd-induced injury. Free Cd adversely affects numerous enzyme systems leading to pathological observations.

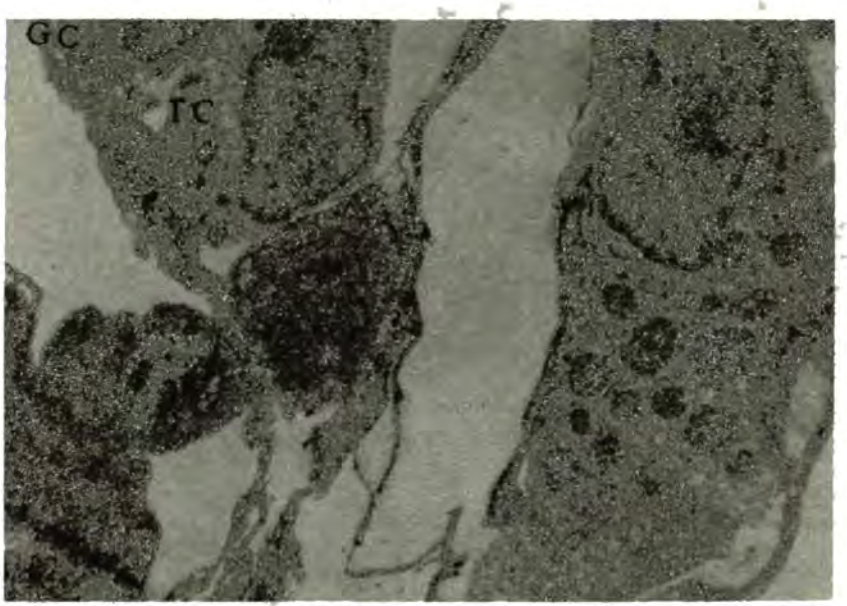


Figure 2. Granulosa cells (GS) and theca cells (TC) scattered in the ovarian cavity. 7,000x.

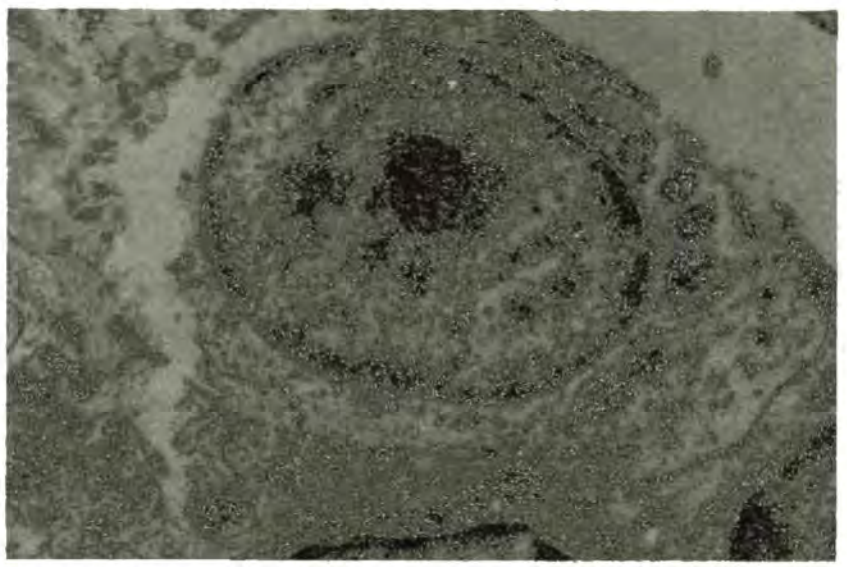

Figure 3. An egg cell with discontinuous cell membrane (CM), ruptured nuclear membrane (NM) and disorganized cell organelles. 5,000x.

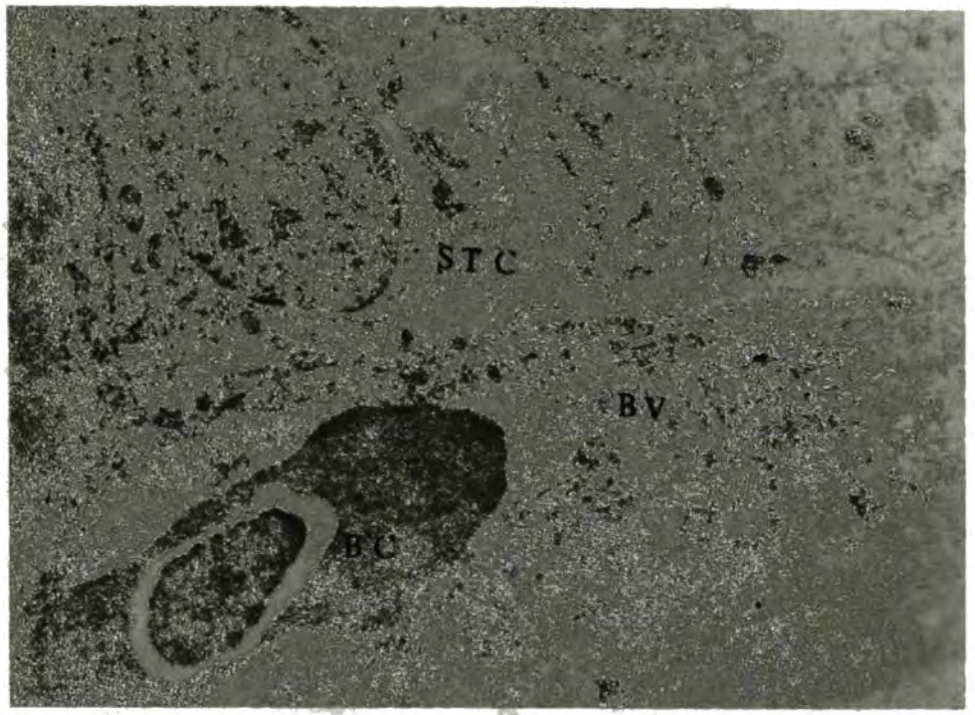


Figure 4. An atretic steroidogenic theca cell beside a destroyed blood vessel (BV). A blood cell (BC) with dilated nuclear membrane. 3,000x.

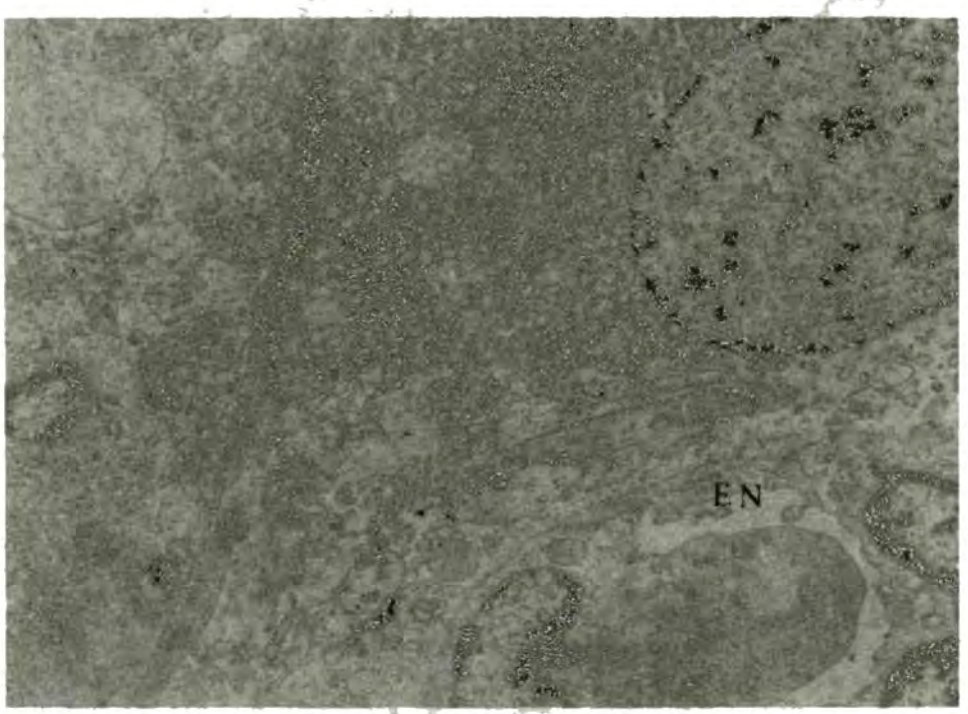


Figure 5. An intact capillary with healthy endothelial cells (EN). 7,000x.

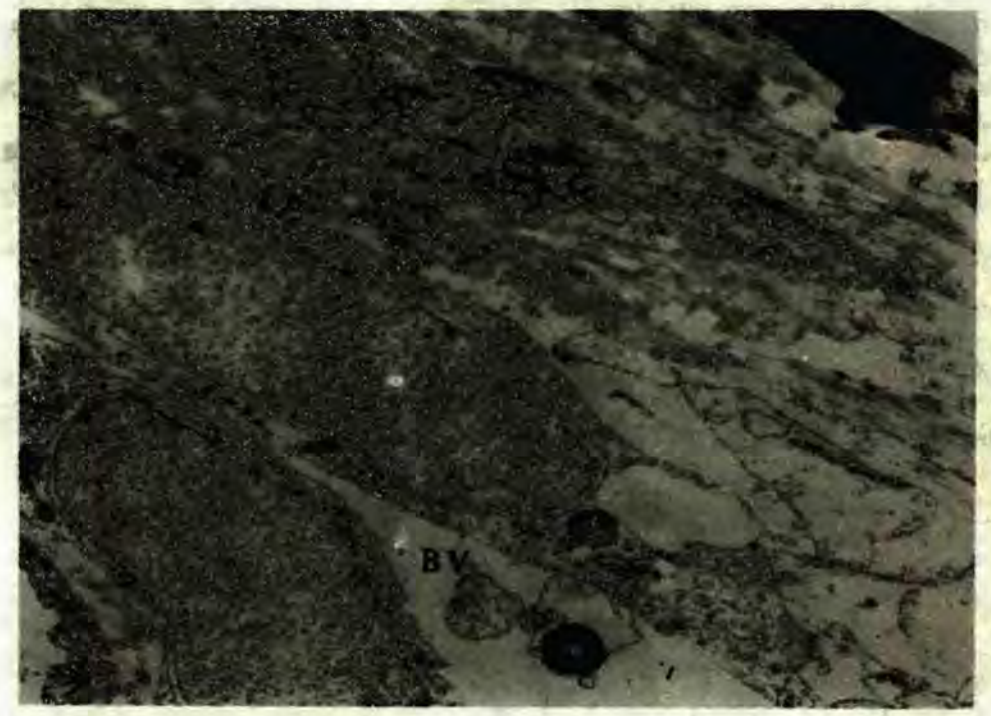


Figure 6. Damaged vascular and other somatic tissues of the ovary. 5,000x.

## Summary

Ovarian histogenesis is adversely affected by long-term exposure of fry to sublethal cadmium from the larval to the maturation stage. Onset of differentiation and vitellogensis are delayed. Gametogenic cells and somatic tissues show damage starting on the fourth week. The treated ovary is smaller and has less gametogenic cells than the normal. All these pathological changes indicate a high probability of reproductive failure.

## Acknowledgment

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# DETERMINATIONS OF TOXICITY ON SOME CORAL REEF CRABS OF LA UNION, PHILIPPINES

Glorina N. Pocsidio
*Institute of Biology, College of Science*
*University of the Philippines*
*Diliman, Quezon City*

**ABSTRACT**

The A.O.A.C. mouse assay for paralytic shellfish toxins on coral reefs crabs collected mainly from the area of Lingsat, San Fernando, La Union confirmed the toxicity of two species believed to have caused two of three known human deaths in the northern coastal province as well as added two species to the list of dangerous Philippine marine crabs which now include: *Atergatis floridus, Zozymus aeneus, Lophozozymus pictor, Demania toxica, Demania alcalai, Atergatis integrrimus, Carpilius maculatus, Calappa calappa, Dromidiopsis* sp., *Etisus splendidus, Etisus rhynchophorus, Ozius* sp., and *Pilumnus vespertilio*, the last two being the new additions. Lethal potencies of the coral reef crabs ranged from nondetectable toxicity to 209.8 MU/g and a total toxin content of 5475.8 MU which is more than sufficient to intoxicate a person. Because of the marked individual variability in toxicity levels even within and among the toxic species, it is advisable for the public to avoid coral reef crabs altogether as food.

## Introduction

During the past five or six decades or so, only very few cases of crab poisoning occurring in the northern coastal province of La Union are known. This is mainly because the fishermen in the area are quite aware that some crabs, especially those caught in the coral reefs, are deadly. At present with fast growing populations in the countryside and increasing numbers of people migrating from other towns to inhabit the sea coasts, there is need to warn the public about these crabs, especially since some people might be forced to glean on the rock beds for food.

Some time in 1935, a 57-year-old from a neighboring barrio had joined his fishermen friends in barrio Lingsat for torch fishing of sea cucumbers. As he walked apart from the group to warm himself by fire on the shore, he stepped on a crab which he then picked up to broil. Later, his companions saw him lying on the sand, dying with his mouth frothy. He had eaten what was described as a "hairy crab". This was the first fatality recounted by those who had heard from the witnesses.

On February 10, 1987, a fisherman aged about 59 and a woman in-law aged 54 from barrio Lingsat were rushed at about midnight to the Ilocos Regional Hospital in San Fernando, La Union. The man complained of spasms on the left side of

his body followed by his feeling of dizziness and numbness of both extremities. The woman complained of dizziness which started a few hours after eating supper.

Ten minutes after admission, the man was connected to a respirator and his secretions were sucked. Medications such as prostigmine and atropine sulfate were to no avail. Against medical advice, the man was discharged the following afternoon and died. The woman given the same medication but without being connected to the respirator recovered and was discharged three days later. Both had eaten a crab which was earlier discarded by other fishermen as unwanted catch of their nets and actually recognized by the man as poisonous. The family members could only explain the attempt to eat it as a case of suicide.

A third fatality was another fisherman from another barrio. He was enticed to eat a large reddish crab caught in his net. Fortunately, he had not shared his meal and thereby saved his housemates from a certain death.

Based on the descriptions of the crabs, the first fatality could have been due to *Pilumnus vespertilio.* The second case was due to *Atergatis floridus,* positively identified by members of the family of the victim from photos of various crabs. A barriomate of the third victim had pointed to *Etisus dentatus.*

Other countries have reported that several species of coral reef crabs could be toxic not only three or four. In this report the toxicity of only a few representatives of different species are presented.

## MATERIALS AND METHODS

Specimens were collected from September to December in 1987 and 1988. Except for two specimens of *Zozymus aeneus* which were collected from the coral reef in Paraoir, Balaoan, La Union, the crabs were all from Lingsat, San Fernando, La Union. Many were gathered by a tedious process of turning over and breaking the rocks at daytime during the low tide, while some were caught at night by torch fishing as the crabs characteristically come out of the rock crevices and forage for food at night. A few were caught in the nets of fishermen who donated them for the study.

The crabs were immediately frozen and either transported as such to the U.P.-NSRI laboratory in Diliman, Quezon City for the bioassay or used for the toxicity experiments in the collecting station. Most of the specimens were identified by Miss Marivene Manuel of the Carcinology Section of the National Museum in Manila.

The coral reef crabs collected were: Family Calappidae, *Calappa gallus;* Family Dromiidae, *Dromidiopsis dormia;* Family Grapsidae, *Plagusia depressa tuberculata;* Family Majidae, *Composcia retusa, Cyclocoeloma tuberculata, Tiarinia depressa,* and *Schizophrys aspera;* Family Parthenopidae, *Daldorfia horrida;* Family Zanthidae, *Actaeodes tomentosus, Atergatis floridus, Atergatis subdentatus, Carpilius convexus, Carpilius maculatus, Eriphia sebana, Etisus dentatus, Euxanthus melissa, Lachnopodus subacutus, Leptodius exaratus, Leptodius gracilis, Lophozozymus* sp., *Ozius* sp., *Pilumnus* sp., *Pilumnus vespertilio,* and *Zozymus*

*aeneus*. However, not all could be assayed as some crabs were too small or only one or two were available to provide homogenates for the assay and sample for species identification. The dates of collection of the crabs that were assayed are shown in Table 1.

Table 1. Lethal potencies of some coral reef crabs of La Union, Philippines

| *Species* | *Date of Collection** | *Weight of Crab, g* | *Sex* | *Lethal potency, MU/g* | *Total Toxin-Content, MU* |
|---|---|---|---|---|---|
| Family Calappidae | | | | | |
| *Calappa gallus* | 11-27-88 | 6.7 | M | – | – |
| Family Grapsidae | | | | | |
| *Plagusia depressa tuberculata* | 10-29-88 | 10.2 | F | – | – |
| Family Majidae | | | | | |
| *Composcia retusa* | *10-27-88* | *23.4* | M | – | – |
| Family Parthenopidae | | | | | |
| *Daldorfia horrida* | *9-15-87* | 84.5 | M | – | – |
| | | 102.2 | F | – | – |
| | | 141.1 | M | – | – |
| Family Xanthidae | | | | | |
| *Actaeodes tomentosus* | 9-16-87 | 20.3(9) | F+M | –** | –** |
| | 9-18-87 | 17.7(5) | F+M | –** | –** |
| | | 26.5(7) | F+M | –** | –** |
| | 10-27-88 | 23.7(5) | F | – | – |
| | | 23.3(7) | M | – | – |
| | | 38.8*5) | M | – | – |
| *Atergatis floridus* | 9-10-88 | 19.4 | M | –** | –** |
| | | 22.0(3) | M | 1.8 | 28.8 |
| | 10-27-88 | 16.0 | M | 1.8 | 28.8 |
| | 10-27-88 | 11.0 | F | – | – |
| | | 14.0 | F | 1.7 | 23.8 |
| | 10-29-88 | 19.0 | F | 2.0 | 38.0 |
| | | 20.0 | M | 2.0 | 40.0 |
| | 11-15-88 | 21.0 | F | 1.4 | 29.4 |
| | 12-2-88 | 17.3 | F | <1.5 | <26.0 |
| | | 13.3 | M | – | – |
| | 12-18-88 | 26.1 | M | 209.8 | 5745.8 |
| | 12-21-88 | 31.0 | M | 2.5 | 77.5 |
| | | 24.1 | F | 10.0 | 241.0 |
| *Atergatis subdentatus* | 9-30-88 | 236.7 | M | – | – |
| *Carpilius convexus* | 10-27-88 | 45.0 | M | – | – |
| | 10-29-88 | 14.3 | M | – | – |
| | | 32.3 | M | – | – |

| *Species* | *Date of Collection** | *Weight of Crab, g* | *Sex* | *Lethal potency, MU/g* | *Total Toxin-Content, MU* |
|---|---|---|---|---|---|
| | 12-21-88 | 12.8 | M | – | – |
| | | 12.3 | M | – | – |
| *Carpilius maculatus* | 10-29-88 | 30.0 | M | – | – |
| | | 15.4 | M | – | – |
| *Eriphia sebana* | 9-10-88 | 43.9 | M | – | – |
| | | 57.5 | F | – | – |
| | | 72.6 | M | – | – |
| | | 33.8 | F | – | – |
| | | 58.2 | F | – | – |
| | | 41.4 | F | – | – |
| | | 52.8 | M | – | – |
| | | 43.4 | F | – | – |
| | | 46.5 | F | – | – |
| | | 18.5 | F | – | – |
| *Lachnopodus subacutus* | 9-16-87 | 7.2(3) | F+M | – | – |
| *Leptodius exaratus* | 11-15-88 | 9.6 | M | – | – |
| *Lophozozymus* sp. | 9-16-87 | 15.0 | M | – | – |
| | | 23.3 | F | – | – |
| *Ozius* sp. | 9-12-88 | 12.6 | M | < 1.8 | < 22.7 |
| *Pilumnus* sp. | 11-15-88 | 19.0 | F | – | – |
| | | 21.0(3) | M | – | – |
| *Pilumnus vespertilio* | 9-10-88 | 12.7 | F | < 0.7 | < 8.9 |
| | | 9.8 | M | – | – |
| *Zozymus aeneus* | 12-15-88 | 18.9 | F | 2.6 | 49.1 |
| | 12-15-88 | 106.7 | F | 45.3 | 4833.5 |
| | | 74.5 | F | 15.8 | 1177.1 |

*All specimens collected from Lingsat, San Fernando, La Union except for *Zozymus aeneus* from Paraoir, Balaoan, La Union.

**Death of all three mice two to five hours after injection.

– Nondetectable toxicity

The toxicity of the different crabs were determined by the A.O.A.C. assay method for paralytic shellfish poisons as used by experimenters, particularly in Japan (Horwitz, 1965; Yasumoto, Raj, and Bagnis, 1984). Usually, crabs weighing at least 10g were tested individually. Those weighing less than 10g were combined for the test. The procedure was the same as that used to screen for paralytic shellfish poisons in molluscs (Pocsidio, 1987) with a slight modification as follows. After obtaining the median death time, the number of mouse units/ml was determined and the toxicity of the sample calculated as

$$\text{Toxicity (MU/g)} = \text{(MU/ml X dilution factor) X 2}$$

A death time > 60 min for survivors was considered as equivalent to < 0.875 MU/ml as recommended in the standard assay procedure.

## RESULTS AND DISCUSSION

The results of the mouse lethality tests are summarized in Table 1.

Crabs with dark or black pincers or those with equal sized chelipeds have always been classified by fishermen in La Union as the harmful ones. Remarkably, these crabs which have their "fingers" black or brown "as if marked as such by Nature" have been described in literature as those avoided by Amboinese natives in the Moluccas (Rumphius (1705) cited by Holthuis, 1968). These black-fingered species are common in Family Zanthidae. The observation regarding their unfitness for consumption is reasonably accurate since so far all xanthid species, except *Dromidiopsis* sp. of Family Dromiidae and *Calappa calappa* of Family Calappidae, have been found to be toxic. Moreover, equal sized chelipeds are not rare among coral reef crabs.

So far, the following xanthids have been demonstrated to be toxic, the latter four reported recently to possess marginal toxicities as in *Dromidiopsis* sp. and *Calappa calappa: Atergatis floridus, Zozymus aeneus, Lophozozymus pictor, Demania toxica, Demania alcalai, Atergatis* intege *integerrimus, Carpilius maculatus, Etisus splendidus,* and *Etisus rhynchophorus* (Alcala and Halstead, 1970; Carumbana, Alcala and Ortega, 1976; Koyama *et al., 1983; Yasumura et al,* 1986). Two more xanthids, *Pilumnus vespertilio* and Ozius sp., may be added to this list as evidenced from the results of the present assay.

The toxicity of *Atergatis floridus* and *Pilumnus vespertilio* which are claimed to have caused two of the human fatalities mentioned above is confirmed by the mouse lethality experiments.

Despite the black fingers and equal sized chelipeds, there was the entire range of toxicity from nondetectable toxicity to a lethal potency of 209.8 MU/g. The highest total toxin content in a crab was in one specimen of *Atergatis floridus* with 5475.8 MU. Ingestion of paralytic shellfish toxins above 3000 MU is assumed to intoxicate man (Yasumoto, Raj, and Bagnis, 1984).

Variability in the responses of the test animals to the crab extracts was likewise observed in the present experiments. A person may be more vulnerable than another, so one who treasures life on this earth should probably totally ignore the coral reef crabs as food.

It should alarm people also that a possible change in toxicity levels within a locality through time can take place. For example, a screening of toxic crabs by Hashimoto *et al.* (1969) on 72 species in the Ryukyu and Amami Islands produced only three species, namely *Atergatis floridus, Zozymus aeneus,* and *Platypodia granulosa,* but in 1983 from the same region and among the species assayed in 1969, seven additional species were found toxic (Yasumoto *et al.*, 1983).

A calcareous red alga, *Jania* sp., ingested by crabs was found to be a source of paralytic shellfish toxins (Kotaki *et al., 1983).* Poisonous crabs may also contain tetrodotoxin and palytoxin as well (Yasumura *et al.*, 1986; Yasumoto *et al.*, 1985). For the crabs found toxic in this study further investigation will have to be conducted, specially the particular toxins, origin of toxins, and the factors for their occurrences.

## Summary

Mouse lethality tests conducted on extracts of some coral reef crabs of Lingsat, San Fernando, La Union and Paraoir, Balaoan, La Union confirmed the toxicities of *Atergatis floridus* and *Pilumnus vespertilio* which were believed to have caused human deaths.

The study adds to the list of dangerous Philippine marine crabs two xanthid crabs, *Pilumnus vespertilio* and *Ozius* sp. It also adds evidence to the highly individual variability in the lethal potencies of the coral reef crabs.

A general caution to the public is to beware of coral reef crabs and to the content with the Portunid crabs of traditional delicacies.

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# SOCIAL SCIENCES

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# THE ERA OF THE KRIS: MORO RAIDS IN SORSOGON AND KABIKOLAN AND THEIR IMPACT ON PHILIPPINE HISTORY, 1571-1896

Luis C. Dery
*U.P. Baguio*

## Introduction

The Spanish era in the Philippines could also be called the era of Spanish-Moro wars. These wars were not just a history of both of the combatants. Downplayed in the writings by various historians was the role of the inhabitants in the places of conflicts, the psychological and physical impact of these conflicts on their lives and, more significant, the effect of these conflicts, on the development and history of the Philippines.

Spanish writers like Montero y Vidal and Barrantes highlighted the destructiveness of the Moro raids and the heroism of the Spaniards. On the other hand, Muslim writers like Saleeby and Majul emphasized the destructiveness of the Spaniards against the Moro homelands and the heroism of the Moros in warding off Spanish attempts to conquer them. This paper shall show other aspects of the conflict which Spanish and Muslim historians neglected to emphasize.

For more than two centuries, the Moro raiders left many lasting mementoes of their activities in Luzon and the Visayas. In Kabikolan, more than halfway into the 20th century, mothers still invoked the dreaded raiders' name, their saying *"hala, iya-on na an mga Moros* (now there, the Moros are coming) being sufficient to send their recalcitrant children scurrying home. In Sorsogon, remains of many *baluartes* and *intramuroses* (fortified enclosures) where people sought refuge and protection when the raiders came still dot the coasts. The various churches are also mute witnesses to the fire and fury that raged around them. Their thick walls were so made to serve as ramparts for those inside to fight the raiders. Nowhere in the Philippines than in Sorsogon and Kabikolan could one find so many churches, facing the coasts, with very tall *simburios* (churchtowers) built by the people to serve as lookouts for the feared raiders from the south. Local places even got their names because of the raiders. One is the town of Gubat, Sorsogon so named by the inhabitants because when some Spaniards came around asking for the name of the place, the people shouted *"gubat, gubat"* (raid, raid) for the Moro raiders happened to be around. Even the local dialects were enriched by the raiders. Natives of Sorsogon would describe a brash, troublesome, and undisciplined person as *"may pagka-Moros"* (like a Moro).

Various factors prompted the Moro raids. European inroads in the Far East saw the Dutch and the Spaniards, in particular, competing for further expansion of their colonial holdings. By the 16th century, Moro leaders were discomfitted to see their homelands circumscribed by the Dutch, Portuguese, and British in the south and by the Spaniards in the north. The expansionist policies of these European powers, notably the Spaniards, impressed in the minds of the Moro leaders the matter of their survival in their own lands. Mindanao and, later, Sulu sought to stem this dangerous trend by undertaking internal consolidation and offensive operations against the Spaniards.

*The Era of the Kris*

The long, mournful sounds of the *budyong* (conch shell), the fierce pealings of church-bells, and the series of smoke signals coming from burning patches of cogon heralded the presence of the Moro raiders. Their practice of attacking at dawn, when the inhabitants were in deep slumber, accentuated the terror and the chaos that accompanied their attacks. They came at the onset of the *vendavales* (southwest monsoons) and left when the *amihan* (northern monsoons) set in. The *vendavales* aided their sea crafts in travelling from their southern lairs to their northern destinations in the Visayas and Luzon and the *amihan* in propelling them back to their bases.

The first major attacks occurred in 1590-91 when fifty *caracoas*[1] of Mindanaoans and Joloans devastated Cebu, Negros, and Panay. Their depredations so terrified the inhabitants that many of them abandoned their coastal settlements and retired into the security of the hinterlands.[2]

They came again in 1599-1600. In 1599, fifty *pancos* of some 3,000 Mindanaoans, Joloans, and Camucones attacked Panay and Negros. They left loaded with booty and some 800 captives. Datus Sali and Silongan, the raiders' leaders, returned in 1600 with about 8,000 men in seventy *caracoas* and devastated Panay. In 1602, forty-eight *caracoas* of Moros raided Cebu, Tayabas, and Batangas. In Mindoro, they sacked its capital town, barely missing the incoming Governor General of the

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[1]Bartholomew Leonardo Argensola, *The Discovery and Conquest of the Molucco and Philippine Islands* (London: 1708), p. 17, distinguished the *caracoa* and the joanga. *The caracoa* was a sea craft which uses oars, is open and bigger, steered by two rudders, one ahead and the second astern. The Ternatans called the *caracoa* as *joanga*, except that the *joanga* differ "in having two half-moons of wood, painted or guilt, rising above the keel at the head and poop. About 100 men row in each of them, to the sound of a tabor and a bell. They carry 20 soldiers and six musketiers. The rest are employed about four or five brass guns. Both the men that row and the soldiers are armed with *campilanes*. . . and shields, and abudance of *calabays* and *sagus*, being along canes burnt in the fire to harden them, which they throw without tacking, as the Moors do their darts."

[2]Vicente Barrantes, *Guerras Piraticas de Filipinas contra Mindanaos y Joloanos* (Madrid: Imp. de Miguel G. Hernandez, 1878), p. 9.

Philippines, Don Pedro de Acuna, who was aboard a small ship accompanied only by two *champans.*[3]

In 1616, Governor General Juan de Silva led an ill-fated expedition to Malacca. In so doing, he depleted the Spanish forces in the Islands. The Mindanaoans and Joloans exploited this weakened state of the country and raided many places, reaching as far as Batangas. In this year, too, occurred the first major raid against Sorsogon. Sixty *caracoas* of raiders surprised the Spanish garrison in Bagatao Island on October 18, 1616, destroying a galleon and two *pataches* being built there, killing 200, and capturing another 100 inhabitants plus 30 Spaniards. More than one million pesos worth of guns, artillery, property, and supplies were taken or destroyed by the raiders.[4]

The Joloans again raided the Bagatao shipyard in Sorsogon in 1627. They carried off much booty besides throwing to the sea some 1,000 *fanegas* of rice which they could not carry. From Bagatao, the Joloan raiders proceeded to Romblon and Leyte. They carried away more than 300 captives.[5]

In 1634, eighteen *caracoas* of 1,500 Mindanaoans and Joloans attacked Leyte, Cebu, Sorsogon, Albay, Camarines, and Tayabas. In Tayabas, they got the Alcalde Mayor of the province and almost got the Archbishop of Manila, Fr. Miguel Garcia Serrano, who was then conducting his diocesan visits.

The following year, Datu Ache raided the Gulf of Sorsogon, destroying again the rebuilt Bagatao shipyard, including many nearby towns. The two years' booty of Datu Ache's raids was reputedly immense: 2,000 ounces of gold, an equal amount in silver, numerous firearms, and 1,500 inhabitants captured, and 30 Spaniards killed (five of them friars).[6]

Samar province was plundered in 1636. In 1646, Sorsogon Gulf was devastated, resulting in the destruction and death of the villages of Ibalon and Duma-

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[3]Fr. Jose Torrubia, Orden de S. Francisco, *Dissertacion historico-politica y en mucha parte geografica de las Islas Philippinas, extension del Mahometismo en ellas, grandes estragos que han hecho los Mindanaos, Joloes, Camucones y Confederados de esta Secta en nuestros Pueblos Christianos, 1753.* (m.s.), pp. 13, 21-23.

[4]*Ibid.*, pp. 30-31; "Informatory Memorial addressed to the King. Juan Grau y Monfalcon, Madrid, 1637," Emma Blair and James Robertson, *The Philippine Islands, 1493-1898* (Cleveland, Ohio: The Arthur H. Clark Co., 1903-1909), Vol. 27, p. 195 (henceforth to be cited as BR); "Relation by Capt. Sebastian de Pineda, May 26, 1619," BR, Vol. 18, p. 182.

[5]"Relation of 1627-28. Unsigned, July 1628," BR, Vol. 22, pp. 203-210; "Historia de la Orden de San Agustin, Juan de Medina," BR, Vol. 24, p. 139.

[6]Jose Montero y Vidal, *Historia general de Filipinas desde el descubriemento de dichas islas hasta nuestros dias* (Madrid: Est. Tip. de la Viuda e Hijos de Tello, 1894), Tomo I, p. 190; Diego Diaz de la Carrera, *Sucesos felices que por mar y tierra ha dado N.S. as las armas Espanolas en las islas Filipinas contra los Olandes, por fin del ano de 1636 y principal del de 1637* (Madrid: 1639), ms.; "Events in Filipinas. Fr. Juan Lopez, Cavite, July 23, 1637," BR, Vol. 27, pp. 314-5; "Corcuera to Felipe IV. Manila, August 20, 1637," BR, Vol. 27, p. 350; "Moro raids repulsed. Manila, 1755," BR, Vol. 48, p. 50.

naog. In 1662-1663, many Visayan provinces, especially Leyte and Samar,[7] suffered a similar fate.

In the succeeding years the unrelenting fury of the Moro raiders was shown. Scattered records mentioned continuous raids. A 1695 account noted that Bacon and its visita, Sorsogon, had only churches and habitations made of nipa and bamboo because they were routinely destroyed by the Camucones and Mindanaoans (por quemarlas muy ordinario el enemigo Camucon y Mindanao).[8] According to local historians like Mariano Goyena del Prado, the Moros came to Albay in 1730 and "in two years captured a total of 200 Christians." Many towns in Sorsogon Gulf were raided in 1737, 1740, 1746, 1749, and 1781.[9] Likewise, the natives of Ligao, Albay in their 1772 petition to build *baluartes* along the town's coast, mentioned a village that was destroyed by a Moro raid in 1736.[10]

The 1740 raid in Sorsogon Gulf must have been so severe that in 1742 the Spanish transferred the capital of *Provincia de Ibalon* from the town of Ibalon to the town of Albay Viejo. Located at the Gulf of Albay opposite the Gulf of Sorsogon, Albay Viejo is away from the routes of the Moro raiders.

The bloodiest Moro raids occurred during the decade of the 1750's. The immediate cause was the Spanish colonial government's decision to conquer once and for all the Sultan of Sulu. At a *Junta de Guerra* (Council of War) in October 1751, a war of fire and blood *(guerra a fuego y sangre)* was declared against all the Mindanaoans, Joloans, Tirones, and Camucones. An expedition under Col. Antonio Ramon de Abad invaded Jolo in 1752 but was "disgracefully beaten." The Joloans retaliated and "invaded the Philippines in their turn, and successfully desolated and laid waste the Spanish provinces for a period of three years."[11]

Beginning in late 1752, sixty-eight *joangas* of Moros desolated Kalibo, Aklan; thirty-eight razed Ilog, Negros; fifty-seven sacked Banton Island; twenty-five besieged Palompon, Leyte; seventeen pillaged Calapan, Mindoro; two thousand

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[7]Montero y Vidal, *op. cit.*, Tomo I, p. 322; Fr. Casimiro Diaz, *Conquistas de las Islas Filipinas* (Valladolid: Imp. Libreria, Heliografia y Tallar de Gravados de Luis N. Gaviria, 1890), pp. 637-9.

[8]"Entrada de la Serafica Religion de Nuestro P.S. Francisco en las Islas Filipinas, 1695," Wenceslao E. Retana, ed., *Archivo del Bibliofilo Filipino* (Madrid: Imp. de la Viuda de M. Minuesa de los Rios, 1895), Tomo I, p. 13.

[9]Mariano Goyena del Prado, *Ibalon: Ethnohistory of the Bikol Region*, tr. by Ma. Lilia F. Realubit (Legaspi City: AMS Press, 1981), p. 50.

[10]*Ereccion de Pueblos-Camarines Sur, 1781-1833*, Folio 300-308b. Consulta del Alcalde mayor de Camarines en que acompaña la presentacion del Gobllo y comun de prales del pueblo de Ligao solicitando se les conceda licencia para construir dos baluartes en los sitios nombrados Marigondon y Panganiran para defenderse de los Moros. Camarines Sur, 18 de Enero 1810.

[11]John Crawfurd, *History of the Indian Archipelago* (Edinburgh: Archibald Constable and Company, 1820), Vol. 2, pp. 471-472.

Maranaos besieged Iligan, Mindanao for two months, etc. Of the eighteen towns of Mindoro, only two (Nauhan and Calapan) escaped total destruction. No less than 160 *joangas* of Moros were reported to have made Mindoro as their base in raiding nearby islands.

The Iranuns and Maranaos came next in 1753 and repeated the same scenes of pillage and desolation. Various places in Mindanao, were raided such as Iligan, Initao, Caraga, Layauan, Tagoloan, Lubungan, and Iponan. In July 1753, the raiders destroyed Surigao, Higaquet, Pahuntungan, and the entire destrict of Butuan. In the Visayas, they attacked Camiguin, Romblon, Tablas, Banton, Simara, and Sibuyan. Ticao Island was sacked while Calapan, Mindoro and Calavite, Marinduque were destroyed. Perhaps the Moro killer of the *padre ministro* of Calavite expressed the fury of the Moros against the Spaniards and their native allies when he said: "*Español mato a mi padre, yo tambien mato Español*" (A Spaniard killed my father, I will also kill a Spaniard). The raiders attacked even faraway Masinloc and Sta. Cruz, Zambales.[12]

The same acts of pillage and plunder were repeated in 1754, also a sad year for Sorsogon. In March 1754, the Leyte towns of Hinondayan, Cabalian, Liloan, Sogod, Maasin, and Biliran were destroyed by the raiders. Two months later, most of the towns in Panay were raided. In June, the town of Bacon, Sorsogon was destroyed. In July, the *Santissima Trinidad* anchored at Ticao carrying the incoming Governor General, Manuel de Arandia. He dispatched a *caracoa* carrying the parcels of letters from Acapulco to be sent to Manila. Approaching the Gulf of Sorsogon, the messengers were overcome by the Moro raiders. Had Arandia landed with his men, the Moro raiders could not have asked for more – a Governor General for a captive.[13]

In August 1754, the towns along the shores of Sorsogon Gulf were attacked. Macalaya, Donsol, Sorsogon, and adjacent towns were despoiled.[14] The raids so terrified the people of Donsol that they abandoned their coastal site and transferred to an interior one which was a day's travel to the coast. In September, the raiders occupied the capital of Albay Province, Albay Viejo. It took the Spaniards and residents of nearby towns three days of fighting to retake Albay Viejo.[15]

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[12]Montero y Vidal, *op. cit.*, Tomo I, pp. 508-539; Juan de la Concepcion, *Historia General de Filipinas* (Madrid: Agustin de la Rosa y Balagtas, 1788), Tomo XIII, pp. 1-36. 190-224.

[13]Juan de la Concepcion, *ibid.*, Tomo XIII, p. 232; Goyena del Prado, *op. cit.*, pp. 115-6.

[14]*Ereccion de Pueblos-Albay, 1800-1858*, Tomo III, Folio 402b. Sr. Alcalde mayor, El Gobllo, Capitanes pasados, cabesas de barangay y demas vecinos principales de esta cabecera, reunidos. . . relativo al puerto de Putiao. Albay, 26 de Septiembre 1830.

[15]Juan de la Concepcion, *op. cit.*, Tomo XIII, pp. 19-191.

By 1755, the Moro raiders had entrenched themselves in nearby islands, like Marinduque, using them as their bases for raiding and seriously threatening the entire western coast of Luzon.[16]

The year 1756 continued to witness the persistent Moro raids. Communications of provincial colonial officials with Manila mentioned the continued Moro raids against the various provinces and islands in Luzon and the Visayas.[17]

In 1757, the fiercest of the Moro leaders, Datu Salicala, came and struck fear in the hearts of the Christianized peoples of Luzon and the Visayas. The ferocity of his raids which brought him as far as Manila Bay, was such that the mere mention of his name sufficed to send people scurrying to the interior. He left with more than a thousand captives.[18]

In 1759, the Fathers Provincial of the various religious orders reported to the Governor General the sad state of affairs in their respective jurisdictions. The Fray Capellan of Imus, Cavite, reported that the *contra-costa* of Mindoro was a major Moro base from which the raiders had inflicted immense damage on unsuspecting travellers passing through the island.

The Father Provincial of the Augustinians also reported the various Moro depredations against Panay, Capiz, Iloilo, Cebu, and Batangas. He mentioned that in 1758 the Moros raided the towns of Taal and Batangas, four towns in Capiz, and the entire province of Iloilo.

The Father Provincial of the Franciscans gave a similar gloomy picture for Tayabas and Kabikolan. The Tayabas towns of Mayaboc, Polillo, Gumaca, Mauban, Atimonan, Pagbilao, and Binangonan de Lampon suffered heavily from Moro raids. In Camarines, the towns of Ragay and Lupi were destroyed while the towns of Oas, Libon, Guinobatan, Libmanan, Calabanga, and Cagsaua were miserable because the raiders often destroyed their croplands. The Father Provincial estimated that in 1758 alone more than 280 persons in Kabikolan died fighting off the raiders and the population decreased due to captivity, deaths, or dispersions in the mountains.[19]

The Moro raiders came almost every year. Until the British occupied Manila in 1762, the Spanish colonial authorities were fully preoccupied with the Moro menace. A series of natural calamities also occurred on the eve of the British occu-

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[16]*Cedulario, 1756-1771,* Folio 66-67. Real orden del 1[e] de Septiembre de 1756 manifestando quedarse enterado el Rey del desembarco de los moros en Balayan, cometiendo destrosos y de haberse libertado de los mismos la Ysla de Marinduque. Madrid, 1° de Septiembre de 1756.

[17]*Cedulario, 1756-1771,* Folio 259-260. Real orden de 17 de Octubre de 1757 manifestando quedar enterado el Rey de la carta sobre las irrupciones de los Moros en provincias. Madrid, 17 de Octubre de 1757.

[18]Juan de la Concepcion, *op. cit.*, Tomo VII, p. 4.

[19]*Cedulario, 1758-1768,* Folio 89-239. Real cedula de 1° de Noviembre de 1758 Su Magestad previniendo al Gobernador de Filipinas lo que debe executar para contener los insultos y excesos que cometen los Moros Joloanos y otros confinatos en aquellas Islas.

pation dispiriting the country and its inhabitants. Thus, what the British found when they came was a prostrate country people which Fr. Juan de la Concepcion graphically described as *"un desmayado cuerpo, sin Espiritu y sin Sangre."*[20]

The British occupation left the Moros in full control of the seas. And even after the British left in 1764, it took many more years for the Spaniards to rehabilitate their war measures against the Moros. In the meantime, the latter raided with impunity areas as far as Palanan, Cagayan where they captured a Dominican friar and a Spaniard on January 7, 1771.[21] Two years earlier before, they raided various towns in Mindanao and the islands of Cebu, Camiguin, Panay, Sibuyan, Mindoro, Iloilo, Leyte, Negros, Samar, Albay, and Masbate. They reached up to Bataan where they razed the towns of Mariveles and Cabcaben. The raiders had become so confident that in the island of Inangpolongan, in the strait between Iloilo and Negros, they built a *tangcal* (a corral or enclosure) where they gathered all their captives in their raiding sorties before transporting them to the slave markets in Jolo or Borneo.[22]

In October 1772, Manito, Albay was raided. Two raids hit nearby Putiao town in 1772-1774 causing its inhabitants to abandon the town.[23] The Moros raided again various towns in Sorsogon, Albay, and Camarines in 1782, 1786, 1796, and 1799. The 1796 raid must have been heavy for the raiders wiped out the town of Himoragat, Camarines Sur.[24]

The raiders came again in big numbers in 1805. On May 28, they captured the goleta *San Vicente* loaded with the entire 1804 tributes collected from Albay Province (consisting of 2,332 pesos and 512 cavans of rice).[25] Elsewhere, the town

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[20] Juan de la Concepcion, *op. cit.*, Tomo VII, pp. 3-11.

[21] Montero y Vidal, *op. cit.*, Tomo I, p. 259; *Consultas-17th Century.* Consulta del Lugar Theniente Justicia Maior en que da cuenta se haverse avistado quatro imbarcaciones de Moros. Vigan, 6 de Julio 1760.

[22] Barrantes, *op. cit.*, pp. 52-56; Montero y Vidal, *op. cit.*, Tomo I, pp. 231-260.

[23] *Ereccion de Pueblos-Albay, 1856-1897*, Tomo VI, Folio 184-191b. Expediente en que participa haber resuelto que los Naturales residentes en el sitio nombrado Manito pasasen al monte Capuntucan por las rasones que expresa. Albay, Enero 27 de 1772; *Ereccion de Pueblos-Albay, 1800-1858*, Tomo III, Folio 402b. Sr. Alcalde mayor. . . . (see footnote 14).

[24] *Ereccion de Pueblos-Camarines Sur, 1781-1883*, Folio 99-104. Expediente creado a Consulta del Alcalde Mayor de Camarines del antiguo pueblo de Himoragat, de la formacion de un baluartillo para colocar un falconete y de la libertad de tributos por dos anos. Camarines, 1803.

[25] *Varias Provincias-Albay*, Tomo XI. (testimonio de) Don Slavador Jose de Ybarra para conducir 512 cavanes de arroz limpio al Real Almacenes de Manila abordo de goleta San Vicente, Cabecera de Albay, 19 de Mayo 1805; Testimonio de las diligencias practicadas por la goleta San Vicente apresada por los Moros en la ensenada de Botag cargada de Reales intereses el 29 de Mayo 1805; Carta del Governadorcillo de Bulan Don Juan Camposano (to) Alcalde Mayor de Albay Don Manuel Garay, 31 de Mayo 1805; Carta de Governadorcillo de Sorsogon Don Juan Silverio, 1º de Junio 1805; Declaracion del grumete del goleta San Vicente Don Juan Ynocencio, 3 de Junio 1805.

of Mambulao, Camarines was attacked and fifteen fishermen were captured by the raiders. During their stay, they effectively blocked travel and trade between Mauban, Tayabas and Sorsogon.[26]

In 1810, the parish priest of Bulan, Sorsogon reported that during the previous year the town was raided by the Moros.[27] In November 1810, the Bishop of Nueva Caceres reported that one of the grave problems of overseeing his diocess was the extreme dangers posed at sea by the Moros. An example he cited was the raid in October 1810 by some 170 *pancos* of Moros from Sorsogon and Catanduanes. Their raid in Libmanan, Camarines Sur was unforgettable for its inhabitants. Not only did they take 100 of them in captivity, the Bishop noted, but they disrobed all the women, made their *tapis* into sacks for the palay they got and forced the naked women to carry the palay to their *pancos* before leaving.[28]

The Moros made another big raid in 1818. As early as February, the *armadillas* of the provinces of Albay were alerted of the presence of 170 *pancos* of Moros in the vicinity of Indan and Polillo Island.[29] By July, the Moros had already inflicted serious damages. On July 2, near Rapu-Rapo Island, they captured two armed *paraos* of the towns of Tiui and Tabaco and the *falua* of Albay Province. On July 6, they sacked Sangay, Camarines Sur capturing 84 of its inhabitants, including the town parish priest and *governadorcillo*. On July 9, they captured the entire tributes of Catanduanes and sacked the towns of Talisay and Indan, Camarines Sur.

By August, the Spanish colonial authorities had fitted twenty vessels to fight the raiders. On August 7, fifty *pancos* of raiders again occupied Indan. The Albay *armadilla* could not give aid because of the presence of thirty-one *pancos* of raiders which landed at Magdalena, Masbate Island. Another group of raiders, numbering some 3,000, was sighted heading towards Palapag, Samar about the end of August 1818.

The heavy presence of the Moro raiders from Samar to Catanduanes and the frenetic preparations made by the Spaniards and the local inhabitants made a major

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[26]*Ereccion de Pueblos-Camarines Sur, 1799-1820*, Folio 79-87. Partes sobre el arribo de varios pancos de Moros en en los pueblos de Mambulao y Ragay de Camarines, 1805; *Ereccion de Pueblos-Albay, 1799-1864*, Tomo II, Folio 106-119. Oficio del Alcalde Mayor de Albay participando hallarse crusando sobre las costas de Sorsogon y Casiguran 40 pancos de Moros que han apresado un Pontin; Alcalde Mayor de Albay Don Domingo Navea (to) Gobernador General, Albay, 23 de Noviembre 1806.

[27]*Santa Visita*, Box 4-A-2, Folio 188-193. Santa Visita del pueblo de Bulan, 1810.

[28]*Gobierno Obispados Sufraganeos, 1697-1893*, Box 4-E-13, Folder 1741-1918. Obispo de Nueva Caceres (to) Sr. Don Antonio de Zulaybar, Arzobispo de Manila, Nueva Caceres, Noviembre 4 de 1810.

[29]*Ereccion de Pueblos-Albay, 1772-1836*, Tomo I. Partes del Alcalde Mayor de Albay sobre los incursiones de los Moros piratas en los pueblos de su provincia, robando y cautivando sus habitantes, y de los combates sostenidos por sus armadillas contra dichos piratas. Albay, 1818-1821.

battle inevitable. The ceaseless reconnoitering of the seas by the defenders finally paid off. A battle ensued between the Spanish forces and the Moro raiders near the Encenada de Pitogo, Tabogon Bay on the night of October 25. The battle resulted in the loss of fourteen *pancos* besides forcing some five hundred of the raiders to flee on land.

Darkness enabled the rest of the raiders to escape while the Spanish forces regrouped under the leadership of Don Pedro Estevan, ex-*governadorcillo* of Tabaco, Albay and overall commander of the Spanish *armadillas*. At dawn of October 26, they intercepted another forty *pancos* of raiders led by no less than "Prince Nune, the son of the Sultan of Mindanao." After thirteen hours of battle, the heat of the fight taking place from eight in the morning to four in the afternoon, Estevan's forces captured another nine *pancos*, sunk twelve, and freed thirty captives. Prince Nune, however, escaped capture when one of his leaders, Datu Gampon, returned to the battle scene and spirited him away. Two weeks after his October 25-26 encounters, more than sixty corpses of the raiders were washed out on the shores of Caramoan and another fifty-nine captives freed while some 1,000 raiders fled to the mountains of the said towns. Until late December 1818, remnants of the Moro fugitives continued to be captured or killed by the local inhabitants.[30]

The 1818 Battle of Tabogon Bay was very significant not only to the Bikolanos but to the local colonial authorities as well. Thereafter, the Moro raiders were no longer as intrepid and daring in conducting their attacks. Their defeat at Tabogon Bay impressed the raiders with the stiffened and organized resistance against their pernicious raids. Reports after this 1818 battle showed that the Moros were reduced to preying on fishermen or isolated travellers while hiding in the numerous isolated coves or islets; or capturing those who happened to cross their paths; or raiding isolated and sparsely-populated *visitas* or settlements.[31]

There were still sporadic raids – the last taking place in 1896, according to local accounts in Sorsogon – but for the Moros their era of raiding and destroying Luzon and the Visayas with impunity was forever gone. By the last half of the 19th century, it was the turn of the raiders to be progressively on the defensive as the onslaught of the Cross against the Crescent mounted.

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[30] *Ibid.*, see Folios 256-351.

[31] *Ereccion de Pueblos-Camarines Sur, 1831-1883*, Folio 168-193. Partes de Moros, Camarines Sur, Junio 5 – Septiembre 15 de 1834; *Ereccion de Pueblos-Camarines Sur, 1785-1837*. Informe sobre todos los acontecimientos de Moros en la Provincia de Camarines Sur durante el ano 1835; *Ereccion de Pueblos-Albay, 1841-1894*, Tomo IV, Folio 40. Partes de Moros. Albay, 1841; Folio 194 – Alcalde Mayor de Albay Jose Velarde (to) Gobernador Gral, Albay, 22 de Septiembre 1847; *Cartas, 1847-1860*. Gobernador General Narciso de Claveria (to) Sr. Secretario de Estado. . . y Gobernacion de Ultramar, Manila, 20 de Octubre 1847; *Piratas* – Bundle II. Albay, Ano de 1860. Comunicaciones sobre de aparicion de piratas en las aguas de dicha provincia.

*The Effects of the Moro Raids*

"This unending war with the Moros overburdened our Government and every governor general." the Spanish historian Vicente Barrantes thus summed up the effects of Moro resistance and counter-attacks against Spanish colonial expansionism. The Moro resistance had so affected the Spanish authorities that no less than Governor General Marquina commented that the wars with the Moros was an evil without remedy (*era un mal sin remedio*).[32] To the Spanish colonial authorities, the Moro raids were not only detrimental to their interests in the Philippines but they were also the root cause of the depopulation of many coastal areas and of their lack of commercial and agricultural growth, especially in the Visayas.[33] Governor General Basco y Vargas, in 1778, attributed the decadence of the Islands to the continuous Moro raids which disrupted peace and order, stopped inter-coastal trade and commerce, destroyed many towns and fields, and carried many inhabitants to captivity.[34]

One of the obvious effects of the raids was the substantial number of captives taken from the various coastal towns of Luzon and Visayas. In 1621, Fr. Hernando de los Rios Coronel mentioned the "more than ten thousand captives" taken during the past decades.[35] In 1634, Archbishop of Manila Fr. Miguel Garcia Serrano wrote the Spanish king that during the first thirty years of the 17th century more than 20,000 Christians were captured by the Moro raiders.[36] Vicente Barrantes cited a report by the Father Provincial of the Recollects that in Paragua (Palawan) alone no less than 10,000 were killed or taken captive during the years 1719 to 1751.[37]

The decades of the 1750's, during which time the heaviest raids were recorded, saw a greater population reduction of many coastal areas. Panay was reduced from 1,500 tributes in 1750 to only 500 in 1757; Romblon, from 1,370 to 995; Aklan, from 1,164 to 549; and Banga, from 1,020 to 754. Ibahay lost 229 tributes and Tibiao 200 tributes.[38] In Mindoro, from 1752 to 1766 more than 1,000 in-

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[32]Barrantes, *op. cit.*, p. 139.

[33]Ildefonso de Aragon, *Plan general de defensa de las Islas Filipinas con su plano hidrografico y topografico, ano de 1829* (ms.), pp. 189-190.

[34]*Spanish Manila* – Bundle V. Armadillas corsarias contra Moros. Bando del Sr. Basco de 22 de Agosto de 1778 sobre formar armadillas en las provincias para la persecucion de los Moros y privilegios que se conceden a los voluntarios que quieran hacer el corso. Real Palacio de Manila, 22 de Agosto 1778.

[35]"Memorial y relacion para su Magestad. Hernando de los Rios Coronel, Madrid, Fernando Correa, 1621," BR, Vol. 19, p. 265.

[36]Montero y Vidal, *op. cit.*, Tomo I, p. 165; Barrantes, *op. cit.*, p. 49.

[37]Barrantes, *ibid.*, p. 232.

[38]Montero y Vidal, *op. cit.*, Tomo I, pp. 541-2; Juan de la Concepcion, *op. cit.*, Tomo XIV, pp. 325-6.

habitants were killed and another 1,300 captured, four of them friars.[39] Fray Manuel Matos, Bishop of Nueva Caceres, wrote the Spanish king on June 29, 1758 that around 8,000 inhabitants of Kabikolan were captured by the Moro raiders in 1757.[40] The Spanish historian, Jose Montero y Vidal, estimated that an annual average of 500 persons were taken captive by the Moros from the various places that they raided.[41]

Undoubtedly, a substantial number of inhabitants were either taken or killed by the Moro raiders every year, although it is difficult to confirm the figures given by the friars. Even the Father Provincial of the Franciscans admitted in 1759, that it was difficult to determine the exact number of persons captured or killed in their jurisdictions in Tayabas, Camarines, and Albay. However, he was certain that the *padrones de almas* (population lists) in the various towns they administered showed many members missing or unaccounted for and that definitely they were either captured or killed, or had taken refuge in the mountains. The Franciscan Father Provincial was sure that the above were the reasons for the depopulated towns in their jurisdictions.[42] In other words, the number of captives taken by the raiders was not the sole cause for the depopulation of many coastal towns. The destruction, desolution, and terror that the raiders instilled in the hearts of the inhabitants drove many of them away from their coastal habitations into the interior. Consquently, this evacuation was reflected in the growth of settlements in the interior and in the reduced number of tributary populations manifested in the *padrones de almas* of the various coastal towns.

Abetting the Moro's successes was the Spanish colonial policy of prohibiting the inhabitants from carrying any form of arms which the latter could have used for self-protection. The prohibition was intended to control the vagabonds and *tulisanes* (bandits or robbers) who had become daring and destructive, too. However, this policy did not affect the vagabonds and *tulisanes* as much as the general inhabitants, especially those in the coastal areas, who were rendered helpless before the Moro raiders. Only after the destructive Moro raids of the 18th century was the ban to carry arms eased by the Spanish colonial authorities as they passed

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[39] *Cedulario, 1760-1768*, Folio 79b-83. Memorial de Fr. Juan de San Agustin, Procurador y Comisario de Provincia de San Nicolas de Augustinos Recoletos Descalzados. Manila, 11 de Noviembre 1766.

[40] Domingo E. Abella, *Bikol Annals* (Manila: n.p., 1954), p. 106.

[41] Montero y Vidal, *op. cit.*, Tomo I, p. 369.

[42] *Cedulario, 1758-1768*, Folio 105-107. Fray Francisco de Santa Rosa, Provincial de San Francisco (to) Fr. Miguel de Espeleta, Manila, Junio 15 de 1759.

on to the native population in 1799 the burden of conducting the wars against the Moro radiers.[43]

The raiders succeeded in surprising their preys in many of their attacks by using Christian renegades to guide and pinpoint to them rich areas which were least defended. A native of Bulusan, Sorsogon named Martin Sto. Domingo was one of the well-known renegades who aided the Balanguigui raiders. He was, in fact, an influential member of the group headed by *Panglima* Taupan, the chief of the Balanguiguis. Convicted to life imprisonment, he was later pardoned on June 30, 1859 and allowed to return to Bulusan after serving the Spaniards as an interpreter when the *Rajamuda* of Siocon was being investigated.[44] Besides using renegades, the raiders also used captured Spanish flags to camouflage their real identity or masquerade as fishermen or traders.[45]

Facilitating the raiders' successes against many coastal towns was their establishment of various bases and settlements in many islets which were located right at the backdoors of the Christianized towns. In the islands of Mindoro Burias, Samar, Leyte, Masbate, Polillo, and Paragua were many Moro settlements which served as bases and rendezvouz points for the raiders in attacking nearby islands or coastal areas.[46]

What truly terrified the coastal inhabitants was the Moro raiders' tactic of attacking at dawn when the people were in deep slumber, or their practice of burning the town, the church, and the croplands. One account described Moro raids thus:

> The villages which they had ravaged were pitiful to see, being either burned to the ground or abandoned or deserted; for the inhabitants who

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[43] *Spanish Manila* – Bundle VII. Mayo 16 de 1740. Bando. Armas Prohibidas. Bando de Don Gaspar de la Torre prohibiendo llevar armas ofensivas como son cuchillos, punales, almaradas, bayonetas, trabucos, pistolas de faltriguerra, flechas y otros semejantes. Governor Manuel de Arandia also issued a bando on March 18, 1755 and on September 27, 1756; Governor Basco y Vargas issued one, too, on February 27, 1783; Governor Basco y Vargas issued one, too, on February 27, 1783, Governor Marquina on March 5, 1789; and Governor Aguilar on January 31, 1799.

[44] *Mindanao y Sulu* (unclassified bundle). Julio de Tolosa, Secretario de Estado de Gobierno Superior Civil, Manila, Junio 15 de 1859 and Junio 21 1859; *Mindanao y Sulu, 1859-1861.* Julio de Tolosa, Secretario de Govierno, Manila, Septiembre 8 de 1859.

[45] *Piratas* – Bundle II. Piratas y Cautivos. Acuerdo del Oficio del Consul de Singapur de unos pancos piratas y aprehension en poder de los mismos de una bandera Espanola, 12 de Junio 1862; Albay, ano de 1860. Comunicacions sobre de aparicion de piratas en las aguas de dicha provincia, [May-October 1860].

[46] *Ereccion de Pueblos-Samar, 1769-1798*, Tomo I. Razon de lo todo lo acontecido a Don Juan Miguel del Castillo en el tiempo que estuvo cautivo entre los Moros. Manila, Febrero 17 de 1775; Expediente en que el alcalde Mayor [de Samar] pide licencia para perseguir a los Moros que and an infestando aquella provincia. Joseph Santos Sanchez Diaz, Catbalogan, Septiembre 5 de 1770.

> were able to escape from the hands of the enemy hid themselves in the thickets of the mountains/and even/ the gospel ministers were compelled to flee in this same way . . . . Even thus they were not always able to flee, for some, cut to pieces, fell into their hands; others were captured and ransomed at great cost, or died of ill-treatment in their captivity. Those barbarians did not spare the churches, but rather plundered them with an infernal fury; burned them, and trampled under foot the ornaments; broke the images and profaned the vessels; and impiously clothed themselves with the sacred vestments. The most unbearable thing of all was to see all those evils unchecked, or friends dishearted, the enemy unresisted, and the villages defenseless.[47]

From Manuel Matos' February 24, 1757 circular to the religious ministers of the towns of Lagonoy, Malinao, Tabaco, Albay Viejo, Sorsogon, Casiguran, Donsol, and Bulusan gives an idea of the state of affairs in the Bikol region, as well as of the impact of the Moro raids on the region. Fray Matos rebuked the towns' religious ministers for the neglected state of their parishes and the dispersed inhabitants of these towns, many of whom lived in the mountains without the benefit of the *"Santos Sacramentos."* In very strong words, Fray Matos told them that –

> The Indios have their synagogues, the Moros their mosques, the Gentiles their temples, the heretics their churches – all except the Christian Catholics of my Bishopric do not have theirs.

Except for their roofs, Fray Matos added, the churches in Kabikolan could be likened to a snake-pit (*madrigueras de culebras*) or to the hut of an *indio cimarron*, as most of them were poorly-built structures (*malformados camarines).*[48]

The Moro raids also caused the decline or death of many coastal villages especially in Sorsogon. The June 1754 raid against Bacon erased the town from the Bikol map for two decades. The town appeared again in the Nueva Caceres' *Estado General de Almas* (General Population List) in 1781.[49] Also, the August 1754 raids against Macalaya, Donsol, and Sorsogon drove the terrified inhabitants of Donsol from their coastal site to a place which was a day's travel to the coast.

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[47] "Fortunate successes in Filipinas and Terrenate, 1636-1637. Unsigned, Madrid, 1639," BR, Vol. 29, p. 116.

[48] *Gobierno de Obispados Sufraganeos*, Box 4-E-13, Folder 1741-1918. Fr. Manuel, Obispo (to) Sres. Curas de los partidos que al margen se expecifican. Nueva Caceres, Febrero 24 de 1757.

[49] *Cedulario, 1771-1829*, Folio 213. Plan de Tributos y almas segun sus estados y clases que al presente se numeran en las provincias, pueblos y misiones que en el Obispado de Camarines estan al cuidado y cargo de los Religiosos Descalzos de N.S.P.S. Francisco de la Provincia de S. Gregorio de estas Islas con expresion de sus nombres, y hedades. Fecho por el Ministro Provincial de dicha Provincia y ruego y en cargo del Illmo. Sr. Obispo Don Fr. Juan Antonio Gallego con arreglo a las Liquidaciones ultimas del año inmediato pasado de 1778.

The Donsol folks returned to their coastal site only in 1822. The same case was true of Albay Viejo town. Occupied for three days by the raiders in September 1754, many of its inhabitants refused to return even after the raiders had already been driven away.[50] The town of Manito, opposite Albay Viejo, was relocated by its inhabitants to a mountain site, away from its exposed location at the Gulf of Albay.[51]

Two successive raids against the town of Putiao in 1773-1774 led to its abandonment by its inhabitants. Putiao was resettled only in 1799 when some residents of Albay Viejo reestablished the townsite in a new place called Inang, which was further inland.[52] Moro raids forced the inhabitants of Bulan, Sorsogon to relocate their townsite half a league inland (one league is equal to three miles).[53] Pantao and Macabogos, early sites of the Spanish shipyards in Sorsogon and destroyed by the massive Moro raids in 1616, 1627, and 1635 remained abandoned until the 1820's when there were sustained efforts to rehabilitate them.[54] The local accounts of Gubat, Sorsogon mentioned that its inhabitants moved the townsite four times in different places in the interior due to frequent Moro raids.[55]

Other settlements in Sorsogon were less fortunate. Ibalon, Dumanaog, Yguey, Bontugan, Macalaya, Gate, Otabi, Busaingan, and Boton failed to recover their pre-Moro raids prominence when most of their inhabitants refused to return and rebuild them. Ibalon, the former capital of Albay Province, became a mere *sitio* of Casiguran by 1781. Some of these settlements, however, were repopulated during the late half of the 19th century. Among these were Yguey which became part of the new town of Magallanes and Busaingan which became the new town of Santa Magdalena.

Many other places adjacent to Sorsogon were deserted, if not depopulated for many years. As late as 1824, despite the Spanish authorities' effort to repopulate Burias and Masbate, the former remained largely deserted while Masbate had only few and poor inhabitants (*con muy corto de pobres habitantes*) because the said islands were frequently used by the Moro raiders and by the bandits from the southern Tagalog provinces as their hideouts.[56] The sea separating Burias from

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[50]Juan de la Concepcion, *op.* cit., Tomo XIII, pp. 190-191.

[51]*Ereccion de Pueblos-Albay, 1856-1897*, Tomo VI, Folio 184. Expediente en que participa haber resuelto que los Natureales residentes en el sitio nombrado Manito pasasen al monte Capuntucan por las rasones que expresa. Albay, Enero 27 de 1772.

[52]See footnote 14.

[53]*Memoria de Albay, 1844*, see entry for Bulan.

[54]*Memoria de la Provincia de Camarines Sur, 1826*, Folio 16.

[55]Isaias Estropigan, Jr., *Historical Background of Gubat* (n.d., n.p.).

[56]*Bandos y Circulares* – Bundle XII. Sobre la repoblacion de Burias y Masbate propuesta por Don Gregorio Cordero, 14 de Mayo 1824.

Sorsogon was just a half-day'navigation and the people of Sorsogon called Burias Pass a perennial lair of the Moros (*madriguera perenne de los piratas Moros*).[57]

As late as 1826, Fr. Francisco Aragoneses, friar-curate of Oas, Albay, observed that the western coasts of Kabikolan from Putiao, Albay to Pascao, Camarines Sur were deserted although their interior was full of various types of inhabitants, living freely and not paying tributes.[58] Mindoro was described as depopulated (*punto despoblado*).[59] Samar, several decades after the destructive raids of the 1750's, continued to be inhabited by terrified people. It was miserable and its *caja de comunidad* in 1819, according to its Alcalde Mayor, contained not even a single *cuarto*.[60]

Moro raids not only encouraged the inhabitants to settle in the interior, but they also forced the Spaniards to resettle people. Relocation or reconcentration of the inhabitants of isolated coastal towns or *visitas* was also the reason why a good number of towns or *visitas* vanished from the map of Bikol, especially in Sorsogon, during the 18th century. On February 13, 1735 the Spanish king approved a proposal by the Governor General to relocate the inhabitants of isolated *visitas* to prevent them from being helpless preys of the Moro raiders.[61] When Moro raids intensified during the 1750's, this measure was reiterated by Governor General Manuel de Arandia on October 18, 1755, who ordered that isolated towns or *visitas* being defenseless, all their inhabitants should be reconcentrated in their respective capital towns where they could expect adequate security. Thus, by March 1757 all the inhabitants of Mindoro's twenty-two towns, *visitas*, and *rancherias* were integrated into only seven towns.[62] The same thing happened in

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[57] *Ereccion de Pueblos-Albay, 1800-1858*, Tomo III, Folio 135-139. Año de 1828 a 1831. Expediente instruido a solicitar del Gobernadorcillo y Principales del pueblo de Donsol, la Provincia de Albay, en que piden permiso para trasladar aquel pueblo al sitio de la Barra en donde tiene una visita, asi mismo piden tambien que se les reserven de pagar el tributo por un año y que se les faciliten armas para fortificar los tres Baluartes.

[58] *Memoria de la Provincia de Camarines Sur, 1826*, Folio 23b.

[59] *Ereccion de Pueblos-Mindoro, 1784-1878*. Oficio del Sr. Comandante General de Marina de V.S. [Gobernador General] Manila, 23 de Noviembre 1829.

[60] *Ereccion de Pueblos-Samar, 1749-1848*. Tomo T. Sobre la intranquilidad de los habitantes de Samar por la multitud de los pancos de Moros que se presentan en las costas de la ensenada asi mismo sobre la falta de fondos de los naturales para construir faluas, tambien sobre tanorias, etc. Samar, 1819.

[61] *Cedulario, 1734-1739*, Folio 289-296. Real cedula de 13 de Febrero de 1735 aprobando la providencia que dio sobre la reduccion apoblado de los naturales que se hallaban dispersos.

[62] *Cedulario, 1756-1771*, Folio 187-189. Real orden de 12 de Marzo de 1757 aprobando las providencias que dio para la reunion de los pueblos y tributantes dispersos de la Ysla de Mindoro y ordenandole aplique las corresponddientes para la habilitacion de las Yglesias destruidas en ella, por los Moros, y quede cuenta de su importe y ejecucion, en la forma que se espresa. Buen Retiro, 12 de Marzo 1757.

Albay, Romblon, Burias, and Masbate where many of the isolated settlements were merged with their respective capital towns. By October 1755, the inhabitants of Burucan, Palanog, Baleno, and Burias were reconcentrated in Mobo, Masbate. All the inhabitants in Ticao Island were reconcentrated in the port of San Jacinto. The inhabitants of Macalaya were resettled in Sorsogon, Sorsogon; those of Ibalon in Casiguran; those of Matnog in Bulusan; and those of Marigondon and Pola in Donsol.[63]

The relocation order was immediately carried out in Albay Province so that before the end of 1755 the province had only ten towns.[64] Two years later, the province was further reduced to only eight towns.[65] Only after twenty years did the relocated inhabitants return to their former towns or *visitas*.[66]

The raiders' dominance of the seas literally ended interisland trade and travel during the 18th century. On November 12, 1779 the Governor General wrote the Spanish king that for the past ten years, the inhabitants of Samar and Leyte could not trade with Manila because of Moro infestation of the seas.[67] A similar situation was described by the Franciscan Father Provincial who wrote the Governor General on January 26, 1770 that the inhabitants in Tayabas, Camarines, Albay, and Sorsogon were very poor because they could not trade with other places due to Moro dominance of the seas (*los pueblos estan muy pobres por no haber traficar sus generos acausa de los Moros*).[68]

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[63]*Spanish Manila* – Bundle VII. Poblaciones. Bando circular de 18 de Octubre (1755) para que los Alcaldes Mayores de estas Islas ordenen y dispongan en las respectivas jurisdicciones sus pueblos, haciendose junten y establezcan sus naturales, no permitiendo rancherias ni casas dispersas.

[64]*Cedularios, 1755-1756.* Año de 1755. Expediente formado en virtud de Superior Providencia sobre que el Excellentissimo maior de este Superior Gobierno ponga testimonio del expediente sobre la baja del Real Situado en Mexico e egualmente Oficiales Reales certifiquen el caudal que componen el cuerpo de la Real Hacienda con distincion de ramos y rentas, gastos y consignaciones para el conocimiento del recidio anual.

[65]*See footnote 48.*

[66]*Bandos y Circulares* – Bundle XIV.Bandos. Julio 28 de 1775. Estadistica de todos los pueblos de estas Yslas con distincion de provincias y almas que administran cada una de los ordenes religiosos.

[67]*Cartas, 1778-1857.* El Gobernador de Philipinas da cuenta con testimonio de los motivos que han tenido los Naturales de las Provincias de Catbalonga, Leyte y Samar en las Visayais para no haber venido en diez años al trafico y comercio en la Capital, reducidos a los insultos de los Moros, y haberse determinado traer en 43 embarcaciones frutos y efectos mediante aque por la armadilla que esta en continuo corso, experimentado por este medio la abundancia y abaratur de frutos en esta republica. Manila, Noviembre 12 de 1779.

[68]*Cedulario, 1766-1778,* Folio 148-183. Real cedula de 31 de Julio de 1766 en que S.M. repite el encargo de que procure contener los insultos y exesos que cometen los Moros Mindanaos. Joloanos y otros confinantes a estas Islas.

In 1826, the Governor General commented that unless the raiders were contained, inter-coastal trade and commerce would cease.[69] As late as 1838, the Alcalde Mayor of Albay reported that half of the annual palay harvest of Catanduanes Island was taken away by the Moro raiders.[70]

The Moro raids likewise exacted very substantial damages against Spanish colonial finances. The destruction wrought by the raiders upon many coastal towns always left the inhabitants in dire circumstances making them unable to pay their tributes to the colonial government. Moreover, the longer the raids lasted, the greater the extent of destruction brought upon the besieged towns. Such was the case for the towns of Cuyo, Calamianes; Palompon, Leyte; and Gasan, Marinduque. Consequent to the prolonged attacked by the Moros, the three towns successfully petitioned the colonial government for a dispensation from paying their tributes. While the dispensation meant relief for the the inhabitants of these towns, it meant no income to the colonial coffers.[71]

The Moro raids drained the colonial treasury. As early as 1722, the colonial authorities in Manila noted the heavy financial exhaustion of the government treasury in maintaining or repairing churches and government institutions routinely burned and plundered by the Moro raiders. The central government enjoined the provincial officials to exert extra efforts (*asiste tarde y manana*) in the collection of tributes to ease the heavy financial drain.[72] On July 14, 1755 the Governor General anxiously petitioned Madrid for additional aid because the finances of the Philippines were exhausted by the continuous Moro raids, by the eruption of Taal volcano which destroyed many nearby towns, and by the other needs of the country which required equal urgent attention.[73]

The situation remained unchanged in the following years. On November 7, 1769 authorities reported to Madrid that from 1762 to 1769 Manila received from Mexico a total financial aid amounting to 489,662 pesos. Of this amount, 245,025 pesos was spent for the construction of two *fragatas* (light, fast-sailing warship)

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[69] *Cartas. 1825-1826.* Folio 206. Gobernador General de Filipinas (to) Sr. Secretario del Despacho de Marina. Comercio, y Gobernacion de Ultramar. Manila, 4 de Febrero 1826.

[70] *Varias Provincias-Albay.* Bundle VII. Provincia de Albay, 1838. Copia del expediente sequido sobre el convenido de los pueblos a dar de los fondos de arbitrios por Cabeceria 3 p. 5 res. y un cuartillo para ayuda de gastos de Faluas a fin de su buena organizacion. Jose Maria Peñaranda. Sorsogon, 10 de Marzo 1838.

[71] *Cedulario. 1756-1771.* Folio 110-112. Real orden de 4 de Septiembre de 1756 aprobando la dispensa de tributos concedida a los pueblos de Cuyu en Calamianes, Palompon en Leyte y Gasang en Marinduque por la defensa que hicieron contra la invasion de los Moros, y el arrendamiento de los tributos de la provincia de Tondo. Madrid, 4 de Septiembre de 1756.

[72] *Cedulario. 1706-1722.* Folio 100b-101. Decreto del Superior Gobierno sobre que asiste tarde y mañana los Oficiales mayores y menores. Manila, Agosto 14 de 1722.

[73] *Cedulario. 1756-1771.* Folio 124-126. Real orden del Septiembre de 1756 manifestando haberse recomendado al Virrey de Nueva España atienda a las urgencias de estas Yslas. Madrid, 6 de Septiembre 1756.

and three *galeras* for the Moro wars. The rest of the 489,662 pesos was spent in the war against the British, the repairs of Manila's fortifications, payment of salaries, etc.[74]

From 1778 to 1793, the colonial government spent another 1,519,209 pesos for the salaries, ships, and expeditions sent against the Moros. The amount "demonstrated the extraordinary cost of the incessant war against the Moros since the start of Spanish rule in the Philippines.[75] By 1826, the colonial government was annually spending 50,000 pesos in the war against the Moros, an excessive amount according to the Governor General's report in 1826 to Madrid.[76]

The enormous amounts expended by the Spanish colonial government did not include those spent by the parish priests and the inhabitants who, without much help from the colonial government, took it upon themselves to build the *castillos* (watchtowers), *baluartes* and *intramuroses* (fortified or *paraos* (small, fast-sailing vessels) in defending themselves against the Moros. They also excluded the value of destroyed towns, churches, properties, and croplands; the captured funds and goods (like the capture of the entire 1804 tributes collected from Albay Province and the entire 1818 tributes collected from the island of Catanduanes); and the broken families and orphaned children of those killed or taken captive by the Moros. Finally, there was also the substantial amount of supplies and services annually shouldered by the inhabitants (following the colonial government's decision in 1799 to pass on to them the burden of defense) in maintaining and provisioning the coastal naval forces ordered formed by the colonial authorities and which the people themselves constructed and manned through the *polos y servicios.*[77]

It is important to point out that the expenses mentioned did not include those shouldered by the inhabitants of Mindanao and Sulu and the destruction suffered whenever the Spanish colonial authorities retaliated against the Moros.

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[74]See footnote 68.

[75]Montero y Vidal, *op. cit.*, Tomo II, p. 369; Barrantes, *op. cit.*, pp. 154-155.

[76]*Cartas, 1825-1826.* Folio 206. Gobernador General de Filipinas (to) Sr. Secretario de Estado y del Despacho de Marina, Comercio, y Gobernacio de Ultramar. Manila, 4 de Febrero 1826.

[77]*Varias Provincias-Albay*, Tomo I. Expediente a Consulta del Alcalde Mayor de la Provincia de Albay sobre exencion de tributos de la tripulacion de las cuatro lanchas y seis faluas que componen la armadilla de otra provincia. Albay, Agosto 11 de 1828; *Ereccion de Pueblos-Albay, 1799-1864.* Tomo II. Juntas celebradas por los Gobernadorcillos y Principales de la Provincia de Albay. . . para perseguir a los Moros y contener sus hostilidades. Albay, 15 de Mayo 1799; *Spanish Manila*-Bundle V. 1778 Bandos. Armadillas corsarias contra Moros. Bando del Sr. Basco de 22 de Agosto de 1778 sobre formar armadillas en las provincias, para la persecucion de los Moros y privilegios que se conceden a los voluntarios que quieran hacer el corso.

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[77]*Varias Provincias-Albay*, Tomo I. Expediente a Consulta del Alcalde Mayor de la Provincia de Albay sobre exencion de tributos de la tripulacion de las cuatro lanchas y seis faluas que componen la armadilla de otra provincia. Albay, Agosto 11 de 1828; *Ereccion de Pueblos-Albay, 1799-1864*, Tomo II, Juntas celebradas por los Gobernadorcillos y Principales de la Provincia de Albay. . . para perseguir a los Moros y contener sus hostilidades. Albay, 15 de Mayo 1799; *Spanish Manila*-Bundle V. 1778 Bandos. Armadillas corsarias contra Moros. Bando del Sr. Basco de 22 de Agosto de 1778 sobre formar armadillas en las provincias, para la persecucion de los Moros y privilegios que se conceden a los voluntarios que quieran hacer el corso.

*Reasons for the Prolonged Moro Raids*

The war between the Cross and the Crescent spanned almost the entire period of Spanish rule in the Philippines. Why, it may be asked, did it take the militarily superior Castilians more than two centuries to finally neutralize the southern kin of the Tagalogs and the Visayas?

Part of the reason for this prolonged conflict lay on the Spaniards themselves. They were chronically plagued with scarcity of funds to maintain the war and pursue it to its logical end. Moreover, the Moros were not their only enemies. These conditions were aggravated by the Manila colony's utter dependence on financial aid from Mexico. The dependence was so critical that the sinking of the galleon carrying the aid was enough to spell disaster for the colony. The lack of funds thus prevented the colonial government from sustaining the farflung areas with arms and ammunition. The lack of arms and gunpowder pervaded throughout the era of the Moro raids and was constantly reiterated in almost all of the petitions by the coastal inhabitants to the colonial government.[78]

There was also the inescapable problem of graft and corruption among the colonial officials tasked to enforce the measures against the Moros. It is important to point out that many, if not most, of the Spaniards who came to the Philippines were guided by less patriotic motives. Many of the Alcaldes-mayores of Tayabas, Camarines, and Albay were repeatedly censured by the colonial government for neglect of their duties.

Montero y Vidal sarcastically commented that during the destructive raids of the 1750's the naval forces sent to pursue the Moro raiders were themselves engaged in smuggling while the Moros were destroying a nearby island.[79] Barrantes also noted that the alcaldes-mayores during the 18th century used for their business interests the *armadillas* designated to pursue the Moros and even sold the arms and artillery destined for defenses of the coastal areas.[80] In fact, the major reason for the 1799 decision to transfer the responsibility of defending the coastal towns from the alcaldes-mayores to the gobernadorcillo, with the *cura parroco* as guarantor, was in the irrefutable finding that the former did not use the arms and vessels for their destined purposes.[81]

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[78] *Ereccion de Pueblos-Albay, 1799-1864.* Tomo II. Juntas celebradas por los Gobernadorcillos y Principales de la Provincia de Albay.... Albay, 15 de Mayo 1799. See, for instance, the various petitions of the towns in Albay for firearms and gunpowder; *Ereccion de Pueblos – Camarines Sur, 1797-1852,* Folio 107-149. Defensa contra Moros: Nuevo plan de defensa contra Moros propuesto por el Alcalde Mayor de Camarines, 1819, see also the various petitions of the towns of Camarines for firearms and gunpowder.

[79] Montero y Vidal, *op. cit.*, Tomo I, p. 508.

[80] Barrantes, *op. cit.*, p. 177.

[81] *Cedulario, 1758-1768.* Real cedula de lo de Noviembre de 1758 S.M. preveniendo al Gobernador de Filipinas lo que debe executar para contener los insultos y exesos que cometen los Moros Jaloanos y otros confinantes que aquellas Yslas, see Folios 120-129.

Other factors further hindered the Spanish anti-Moro efforts. Jagor noted in 1860 that the smoke of the Spanish ships amply warned the Moro raiders to evade or avoid them and escape. Moreover, some Spanish squadron commanders did not really comply with orders to pursue the Moro raiders; they simply came "to show the distressed provinces that their outcry was not altogether unnoticed.[82] No less than Governor General Narcisco de Claveria complained to Madrid in 1846 about the lack of zeal and discipline among the men of the government's naval forces in performing their duties. At one time, he was outraged when he learned that his orders to the naval squadrons in Cavite and Cebu to pursue the Moro raiders were deliberately disobeyed. What the two squadrons did, according to the Governor General, was simply to cruise along (*los dejaron pasearse*) thereby permitting the Moros to escape.[83]

Notwithstanding the many defects that hindered the anti-Moro campaign, the Spanish objective to end the destructive Moro raids was realized in 1848 when Governor General Narcisco de Claveria destroyed the Balanguiguis' stronghold in Sulu and, in 1858, when Governor General Norzagaray garrisoned Balabac – an island right in the heart of Moroland.[84] Also, Spanish use of steamboats in the 1860's effectively neutralized Moro naval threat. Then, in 1864, the prohibition imposed on the Joloanos and Samals against building any big seacraft (as to do so would have meant their treatment as ordinary pirates and prisoners of war) deprived the southern natives the last means by which they could continue their defiance of the Spaniards.[85]

## Conclusions

The centuries of Moro raids deeply affected the inhabitants of Kabikolan and many parts of the Philippines. The Moro raiders came, as Cesar Adib Majul conclusively showed, in retaliation against Spanish efforts to subjugate their homelands. They raided Spanish-held territories to offset Spanish plans to conquer them.

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[82] Feodor Jagor, *Travels in the Philippines* (London: Chapman and Hall, 1875), p. 225, see footnote.

[83] *Cartas, 1846-1855*. Folio 160-167. Gobernador General Narciso de Claveria (to) Sr. Secretario de Estado del Despacho de Marina, Comercio y Gobernacion de Ultramar. Manila, 14 de Noviembre 1846.

[84] *Cartas, 1847-1848*. Gobernador General Narciso de Claveria (to) Sr. Secretario de Estado y del Despacho de la Gobernacion del Reyno. Zamboanga. 28 de Febrero 1848; *Cartas, 1858-1861*. Folio 176-189. Gobernador General (to) Sr. Ministro de Estado y Ultramar, 21 de Febrero 1858; Folio 377-392 – Gobernador Militar y Politico de Balabac (to) Gobernador General Norzagaray, 24 de Marzo 1858.

[85] *Piratas* – Bundle III, part 2. Consejo de Administracion. Pirateria, 1860 a 1869. Gobernador Politico-Militar de Mindanao (to) Sr. Presidente de Consejo de Administracion, Manila, 2 de Junio 1864.

The raids destroyed the bases or sources of support for Spanish expansionism. What was more significant, insofar as Sorsogon and the Bikol region were concerned, was the impact of their retaliatory raids. The effects were incalculable – in terms of numerous towns destroyed, thousands of persons killed or sold to slavery, and the immense expenses incurred by all those involved. The raids also altered the course of Philippine history by dividing the inhabitants into two camps – Christians and Moros – a division which continues to haunt the present generations. It could further be said that, in the light of the tremendous expenses shouldered by the Spanish colonial government and the immeasurable destructions of Spanish-held territories, the Moros of Mindanao and Sulu succeeded in making the Spaniards pay dearly for the efforts to conquer their homelands.

The psychological impact of the Moro raids on the sub-consciousness of the people must have been devastating, too. More than halfway into the 20th century, mothers in Kabikolan still invoked the dreaded memories of the fierce raiders to discipline their recalcitrant children. Documentary sources mentioned three important near-misses by the raiders. They almost captured an Archbishop of Manila and two incoming Governors-General. One wonders what could have been the impact on Spanish colonial rule in the Philippines had the raiders captured these symbols of Spanish power.

Hidden behind the popular view that Moro raids were destructive were, in fact, several significant contributions by the raiders. One was the raids led to the consolidation of the interior with the coastal areas of the provinces. It is true that Moro raids forced many coastal inhabitants to relocate their habitations deep into the interior. However, the dissipation of the raids also led the inhabitants who evacuated to the interior to return to their former coastal settlements. Not all of the evacuees, though, joined these back-to-the-coast movements. Those who remained consolidated the interior with the coastal areas of the provinces for they served as the core or pioneer settlers of the interior areas of the said provinces (as in Sorsogon). And this was the reason why visitas and *barrios* proliferated in the hinterlands.

The Moro raids also forced the Spanish colonial authorities to undertake various infrastructure projects, such as roads and bridges, as part of their defensive measures particularly to facilitate communication between towns in jointly combatting the Moro raiders. These roads and bridges also served as conduits among the inhabitants of the various towns of Sorsogon and Kabikolan in performing other essential human activities, especially trade.

The heroes of the Moro raids or Spanish-Moro wars, as colonial historians like Barrantes and Montero y Vidal as well as Saleeby and Majul would lead us to believe, were supposedly the Spaniards or the Moros themselves. Not so, as documentary sources would reveal. The Spaniards, for instance, got all the accolades because they were the colonial masters and they did not allow any native to command any military expedition against the Moros of Mindanao and Sulu. Definitely, the Spaniards and the Moros did not have a monopoly of courage and bravery.

In fact, records showed the Spaniards several times engaging in activities to enrich themselves rather than pursuing the raiders.

In Kabikolan, many natives outshone both the Spaniards and the Moros and performed almost superhuman feats in combatting the raiders. They were the unsung *gobernadorcillos* and *falueros* who led and manned the provincial naval forces (*armadillas*) to make the coastal areas safe against the raiders. Foremost of these forgotten local heroes was Don Pedro Estevan, *gobernadorcillo* of Tabaco, Albay whose exploits became legendary to the inhabitants of eastern Kabikolan. Because of his feats he was, perhaps, the only anti-Moro campaigner among the natives and the Spaniards who was awarded by the Spanish king a medal of valor (*medalla de las del premio al valor*).

Of greater import of the Kris' impact on the Bikol inhabitants was that it prevented them from pondering on the broader but crucial issue that they were the unwitting and helpless tools of the Spaniards in the latter's war of conquest against the Moros. Unrelenting Moro pressures prevented the Bikol inhabitants from entertaining thoughts about overthrowing Spanish rule in the region, as did their counterparts in the Tagalog or Ilocos provinces. Proof of this was the fact that the Bikolanos did not even initiate a revolt against Spanish rule throughout the Spanish era. Apparently, the fury against the Moro raids drove the inhabitants to side with their colonial masters. Of course, there were instances of native resistance against the Spaniards. However, these instances did not reach the stage of armed rebellion largely because colonial exactions and Moro pressures kept the inhabitants perennially preoccupied with how to cope with these twin adversities. To survive, even if they had to live with colonial impositions and Moro raids, strengthened the will to live of the Sorsoganos and the Bikolanos. Apparently, they stoically accepted the idea that, like the devastating typhoons that they suffered annually, they could not escape from these two forces – the Spaniards who ruled the land and the Moros who prowled the sea.

The dissipation of the Moro raids provided the setting for the revival and growth of the Sorsoganos and the Bikolanos during the 19th century, demonstrating their innate capacities to develop. But, as during the era of Spanish rule and Moro raids, they were not the ones who enjoyed the fruits of their labors. It was to be, as aptly described by Norman Owen, a period of "prosperity without progress."

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# EVOLVING, CRAFTING AND IMPLEMENTING THE PHILIPPINE NATIONAL DRUG POLICY AND THE GENERICS ACT OF 1988 – A MODEL IN SCIENCE AND TECHNOLOGY POLICY-MAKING

Quintin L. Kintanar, M.D., PhD. Academician
Alberto Romualdez, Jr. M.D.

## Introduction

The story of the landmark Philippine National Drug Policy (PNDP) and the ensuing Generics Act of 1988 (G.A. 1988) has all the elements of Science and Technology policy-making. It is therefore important that it be properly recorded and documented so that lessons applicable to other fields of Science and Technology can be drawn from it.

The Philippine National Drug Policy (PNDP) was declared by Presidential Executive fiat while the Generics Act of 1988 passed through the legislative mill.

This paper describes in detail the process of evolution of both the PNDP and G.A. 1988 so they can serve as models of Science and Technology policy-making in the Philippine contemporary environment.

## A. PHILIPPINE NATIONAL DRUG POLICY

### *IDEATION STAGE*

As with many good ideas, the birth of PNDP was the result of the right combination of factors and circumstances. Perhaps the most crucial of these was the installation of a new government and a new management team at the Department of Health (DOH) as a result of the peaceful EDSA Revolution of February, 1986. The second critical factor was the presence of sensitive development managers in the person of Sec. A.R.A. Bengzon, Undersec. Rhais Gamboa and others who were quick to recognize a need and grab the opportunity for a major policy initiative in the pharmaceutical field. The third factor was the strong felt need to provide good quality and affordable drugs to the people. In fact, in all of the President's regional consultation, one of the most frequent issues raised was that drugs and medicines are beyond the reach of the majority of the Filipinos. The fourth factor was the worldwide problem of inadequate access to essential drugs and irrational use of drugs which led to the launching of WHO's Essential Drug Action Program in 1981.

As Sec. Bengzon has described it, the National Drug Policy can be said to have been largely born out of serendipity. Just a few months in office and needing to provide drugs for the health care programs, he asked Undersecretary Rhais Gamboa to look into the existing drug policies. It turned out that there was no

long-term comprehensive drug policy. Drugs were procured as needed on an *ad hoc* basis. Procurement seemed largely based on previous practices carried over from one administration to another. Worse, there was lopsided favoring of a single group of companies close to the former President, giving credence to the rumor that grease money changed hands when drugs and medicines were procured by government.

It became obvious to the DOH management that graft and corruption and the lack of a long-term comprehensive drug policy could be solved with a new National Drug Policy. Sec. Bengzon can be best described as the architect of the New Philippine National Drug Policy as well as of the Generics Act of 1988.

## *THE EVOLUTIONARY PROCESS*

As soon as the imperative for a new National Drug Policy was accepted, preparatory work began with the creation of a special Task Force on Pharmaceuticals composed of:

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| Mrs. Milagros S. Castro | – | Chairman |
| Dr. Antonio Gonzaga | – | Member |
| Dr. Natividad de Castro | – | Member |
| Mrs. Lina Esquivel | – | Member |
| Mr. Federico Gonzales | – | Member |
| Dr. Antonio Perlas | – | Member |
| Dr. Estrella Paje-Villar | – | Member |

The Task Force was to gather as much primary and secondary information through research, interviews, consultations, solicited position papers seminar-workshops and conferences. Work started in mid-1986, initially to determine the scope of policy formulation. The actual conduct of research and consultation continued all the way up to April 1987. The research included local studies and publications on the Pharmaceutical System done in other countries or by the World Health Organization or other International Bodies. One such study was by the UN Asia-Pacific Development Institute (UNAPDI) – a commissioned study on the pharmaceutical Industry in the five ASEAN Countries in 1980. Aside from these studies and publications, interviews and consultation and position papers were submitted by various sectors such as Academe, the Professional Health Providers in government and private sector the Pharmaceutical Industry, – Government Organizations, Non-Governmental Organizations and Consumer Groups. All these were analyzed and synthesized by the Task Force resulting in the identification of the following 7 areas of concern: 1.) essential drug list 2 ) use of generic name, 3.) advertising and promotions 4.) procurement and self-sufficiency 5.) self-medication 6.) registration of pharmaceuticals and 7.) pricing. These 7 areas of concern were then discussed in depth in seminar-workshops and conferences. All in all, there were 25 position papers, 2 national seminar-workshops or conference

participated in by 99 individuals and resource persons representing 61 different organizations.

From these discussions was formulated the first statement of the Philippine National Drug Policy and its four component pillars. The statement was enunciated by Pres. Corazon Aquino on April 30, 1987 on the inauguration of the new buildings and laboratories of the Bureau of Food and Drugs of the Department of Health at Alabang.

## SUMMARY OF THE PHILIPPINE NATIONAL DRUG POLICY

### *GOAL*

The goal of the PNDP is to provide access for the majority of the population to essential drugs which are safe, efficacious and of high quality.

Towards this end, the following major problems had to be addressed: 1.) the presence of toxic or unsafe, inefficacious, and substandard drugs in the market, 2.) the unnecessary or inappropriate use of drugs, 3.) the high dependency on imported active ingredients and even excipients, for drug formulation, and 4.) the wasteful and inappropriate procurement of drugs by DOH.

### *FOUR COMPONENT PILLARS*

These 4 problem areas required major program initiatives which eventually were known as the four pillars of the Policy. These four pillars are interdependent and they mutually reinforce one another. For easy recall, the pillars were made to start with the letters Q, R, S, and T, the names of the ECG wave pattern familiar to health workers. As renamed, the four pillars are:

Q – Quality Assurance
R – Rational Drug Use
S – Self-reliance
T – Tailored Procurement

Each of the component pillars addresses the four major problem areas enumerated above.

### *KEY PLAYERS*

For the crafting of the policy statement declared by the President, principal credit goes to Undersecretary Mario Taguiwalo. For the refinement and fleshing out of the four pillars, credit goes principally to Dr. Alberto Romualdez, Jr. and later also to Dr. Quintin Kintanar and Manuel Dayrit.

### *IMPLEMENTATION*

Even before the PNDP and its four pillars were fully elaborated, implementation had already been started in late 1986 by the original core group of the task

force, namely Antonio Perlas, Natividad de Castro and Estrella Paje-Villar – then known as the "troika." In early 1987, Dr. Alberto Romualdez,Jr. was named as the official responsible for the National Drug Policy. Simultaneous activities were undertaken to achieve the goal of PNDP through its four pillars

*Quality Assurance*

Strengthening the Bureau of Food and Drugs (BFAD) – the government's regulatory agency – was the first order of the day. An Advisory Committee on BFAD was created. Its members were Dr. Cecile Gonzales, a pharmacologist, and Dr. Natividad de Castro and Prof. Leticia Gutierez, pharmacists. BFAD policies, standards and procedures were reviewed. In-house and external training of incumbent personnel was undertaken while new personnel were carefully selected to strengthen the human resource of BFAD This training was started in 1987 during the term of Dr. Romualdez as Assistant Secretary In-Charge and BFAD Director Catalina Sanchez. BFAD was further strengthened with the upgrading and revision of standards and requirements under Dr. Q. Kintanar starting July, 1988 and Dr. Cecile Gonzales, who became BFAD director in February of 1989.

Completed by the end of 1988 were the revised rules and regulations for obtaining a license to operate drug establishments and outlets (A.O. 56 s. 1989), the process of Drug registration (A.O. 67 s. 1989), and the process of review and evaluation of Questioned Drugs (A.O. 66 s. 1989). Copies of these A.O.'s are attached for reference as Annex A.

The new BFAD has already produced concrete results. The drug market has been cleansed of unsafe and inefficacious products with the withdrawal of 138 of the 265 banned, severely restricted, or disapproved drugs in other countries. Two manufacturing firms have been closed and the licenses to operate of 26 manufacturers have been suspended for major deficiencies in Current Good Manufacturing Practice Standards (CGMP).

*Rational Drug Use*

This pillar was tackled in both diagnostic and prescriptive ways. First, an analysis of national drug requirement and sales by Therapeutic Category as part of the RP-UNIDO Pharmaceutical Industry Development Study completed by Oct. 1988, showed a large unfilled gap of $ 44 B worth of drugs, while at the same time documenting an 16% irrational or unnecessary use of drugs such as vitamins, hormones and dermatologicals amounting to ₱1.5 B in 1987 (Table 1)

Then the National Drug Committee developed the first Philippine National Drug Formulary (PNDF), with the active participation of experts and specialists in academe, government, private sector and industry. From the PNDF (which contains 297 Core list Drugs and 262 Complementary List Drugs belonging to 22 therapeutic Sectors and 64 therapeutic Subcategories) have been derived the DOH Hospital Formulary for Secondary and Tertiary Hospitals and the Formulary of Primary Medical Care Drugs for Rural Health Units. These formularies shall

serve as a guide in the procurement of essential drugs by government and provide the best available scientific information and experience to the medical and pharmaceutical professions on the most important safe and effective drugs.

Lastly, to rationalize and put the use of drugs on a scientific basis, the Generic Acts of 1988 requires the use of the generic name of the active ingredient at every stage of the drug life from production, distribution, advertisement, prescribing, dispensing to consumption.

*Self-reliance*

This pillar is by its very nature a long-term program. However, some elements geared towards self-reliance had already started when the PNDP was formally declared in 1987.

For instance, there had been for about 10 years a well-coordinated productive R and D on medicinal plants under the National Integrated Research Program on Medical Plants (NIRPROMP) and funded by the Philippine Council for Health Research and Development (PCHRD). By 1987, NIRPROMP had already generated research results ready for commercial application. Moreover the previous Administration had built the infrastructure and bought equipment under a World Bank loan for three commercial processing and manufacturing facilities for medicinal plant products in three different regions. Thus, what we required to operationalize this plank of the self-reliance pillar was only to complete the delivery of necessary equipment, install them, and start the commercial production of medicinal plant products. The Cotabato plant started its operation in November of 1988 with no less than Pres. Corazon Aquino as guest of honor.

The Second Plank under the self-reliance pillar was the expansion and modernization of the Alabang Vaccine Production now under the Biological Production Service of the DOH. A major development plant study had earlier been completed by Intercare Consultancy Firm through PCHRD, funded by US-AID. This study was reviewed by an International Panel of Experts from UNIDO which confirmed many of the findings and conclusions of the Intercare study recommending the establishment of a Biologicals Central Control Authority & Laboratory and the building of a new production facility to replace the largely obsolete present vaccine production facilities at Alabang in support of the country's Expanded Program of Immunization.

The Third Plank of this pillar consists of longer-gestation projects identified by the RP-UNIDO Pharmaceutical Industry Development Study. These projects are for the local production of strategic pharmaceutical products using largely indigenous raw materials. Seven prospects requiring further feasibility or pilot studies have been identified, namely: a) Establishment of a multi-purpose fermentation pilot plant for antibiotics b) Establishment of a production plant for Penicillin and 6APA (6-Amino-Penicillanic Acid) c) Expansion of existing facilities for semi-synthesis of Ampicillin, Amoxycillin, Cloxacillin and Cephalexin d) Establishment of an Erythromycin derivatives & Rifampicin Production Plant e) Establish-

ment of a multi-purpose pilot plant for chemical synthesis f) Cultivation and processing of Cinchona and utilization to produce Quinine g) Upgrading of quality control facility and the Biologicals Production Services at Alabang.

*Tailored Procurement*

This pillar has been most amenable to immediate implementation and has already generated concrete impact. The Logistics and Procurement Service of the DOH was re-organized and new bidding policies and requirements and procedures were instituted to minimize graft and corruption, and effect cost reduction in drug procurement. In the initial two years of application, a 30% cost-saving in the expenditure for DOH drugs and medicines, amounting to about ₱270 M, was realized. The savings were used to purchase more drugs thereby fulfilling the ultimate goal of the PNDP of providing essential drugs to those who do not have access to them.

Further improvements can still be realized under this pillar by studying the real drug requirements of hospitals through a methodology involving an analysis of morbidity patterns, number of cases treated and applying the current accepted standards therapy on these cases using the essential drugs recommended in the PNDF. This way, the procurement can truly be tailored to the actual needs of patients and hospitals. A study in Sri-Lanka showed that this approach can cut the cost of medicines for in-patients by four-fold and out-patients by eight-fold.

In all these activities, the principle of dynamic flexibility adapting to the exigencies and changing environment as quickly and as much as necessary has been followed. A substantial measure of success has already been achieved in all four pillars of the PNDP.

## *THE GENERICS ACT OF 1988*

In a similar manner the evolution, crafting and implementation of the Generics Act of 1988 demonstrate the kind of preparation, hard work and meticulous attention to detail necessary in good Science and Technology policy-making.

### *IDEATION STAGE*

Again, the Generics Act of 1988 came serendipitously, even as an afterthought. Initially, it was thought that the PNDP and its four pillars were sufficient to effect the needed reform in the pharmaceutical system. Keen legislators in both the Senate and the House of Representatives, however, saw the need for a law to hasten this transformation as envisaged in the PNDP which the Department of Health quickly supported.

### *EVOLUTION*

From direct legislative mandate to promote or require the use of off-patent cheaper generic and essential drugs for the public sector, the idea evolved into a

broader legislative proposal based on the philosophy of rational and scientific use of drugs and medicines. The Generics Act in its final form gave the patient a role in the choice of the final product he shall buy, without deviating from the doctor's prescription.

## *CRAFTING OF THE GENERICS ACT OF 1988*

With the technical assistance given by the Department of Health, principally through Secretary Alfredo R.A. Bengzon, Dr. Alberto Romualdez Jr., the NDP Management Committee, and the DOH liaison officers in Senate (Dr. Cora Rivera) and the House (Mr. Dante P. Esquejo), the bills passed both houses reasonably smoothly. The principal authors were Sen. Orlando Mercado for the Senate and Congressman Narciso D. Monfort for the House of Representatives. However, a "killer" amendment was introduced in the House version which would allowed the prescribing doctor to write "No Substitution" or words to that effect. Because the law allows the physician to indicate the brand name, if he so desired, allowing the prescriber to write "No Substitution" would have nullified the participation by the patient in the final selection of the product to buy. The versions approved separately by both houses are attached as Annex B for reference and comparison.

Fortunately, alert members of the Joint Conference Committee who saw it, removed the "killer" amendment in the final harmonized version which was passed by the Senate and the House almost unanimously, and signed into law by Pres. Corazon C. Aquino on September 13, 1988 amid demonstrations and pickets for and against the law outside Malacañang Palace.

To show how much care was given this bill in both Houses of Congress and in the Joint Conference Committee, one high-level official or staff of DOH was assigned to each key legislator to explain the rationale of each provision and to ensure his continued support of the bill, throughout the entire legislative process. The Secretary even sent individual notes and called up key legislators. He went to the extent of asking the good offices of the President and the Executive Secretary to help preserve the progressive and reformist provisions of the Generics Act of 1988. Dr. Quintin L. Kintanar provided technical support in the final stages of the crafting of the harmonized version the Joint Conference Committee to ensure the integrity of its provisions.

## IMPLEMENTATION

The implementation strategy had to be formulated with the full participation of all affected and interested parties. First, the macro plan was developed in a two-day DOH Top Management Seminar-Workshop in October 1988. At this workshop, the need for thorough and participatory consultation in the preparation of the implementing guidelines of the Generics Act of 1988 was recognized.

## STAGGERED IMPLEMENTATION (DOH)

To have some experience with its implementation before applying it to all

sectors, the law was initially implemented in the home front- the Department of Health.

A Task Force headed by Dr. Quintin L. Kintanar prepared the various draft implementing guidelines. The first was the A.O. 51 – Implementing Guidelines for DOH Compliance to Generics Act of 1988. The draft guidelines were first revised and refined through the NDP Management Committee and DOH Executive Committee in October and then processed through a series of three national seminar-workshops held in November, December, 1988, and in January 1989. In turn, DOH National level key personnel, Regional level personnel, Provincial and District level personnel involved in drug transactions or use, were processed in these seminar-workshops culminating in a "Miting de Avance" on January 12, 1989. It was decided that all DOH Regions and units must implement A.O. 51 not later than March 1, 1989.

## OTHER GOVERNMENT DEPARTMENTS AND AGENCIES

Letters were sent to the Secretaries of other Government Departments which are significant users of drugs and meetings were held to inform these parties of the plan of implementation. The Commission on Audit later issued COA Circular 298 stating that all drug transactions in government must use generic terminology otherwise they shall not be passed in post-audit beginning March 1, 1989. In effect this meant full implementation of procurement using generic terminology by the entire government system.

## PHARMACEUTICAL INDUSTRY

An important element favoring compliance by prescribing doctors and dispensing pharmacists recognized in the Top Management seminar-workshop in October, 1988 was the availability of pharmaceutical products bearing generic names prominently, as provided by GA of 1988. Thus, the implementing guidelines on generic labelling (A.O. 55 s. 1988 as amended by A.O. 64 s. 1989) were formulated in consultation with manufacturers, traders who own the products, and other interested parties. After a lot of negotiations, including the holding of a series of cocktail parties for the Filipino group, the large companies and Transnational Companies (including the Americal Chamber of Commerce), the guidelines on Generic Labelling were published in December, 1988. They were to take effect at first by April 1, 1988, which deadline was moved back later to July 1, 1989 in time for the new BFAD – approved generic labels in production. However, to give time for inventories to be consumed, products bearing present or "old" labels already in the market shall be allowed to be sold up to the end of 1989.

Similarly, a series of consultations with Pharmaceutical Companies, Advertising Companies and other interested parties such as Non-Government Organizations (NGO) and Consumer groups were held to finalize the Implementing Guidelines on Advertising (A.O. 65 s. 1989 as amended by A.O. 69 s. 1989).

## PRIVATE PROFESSIONAL SECTOR

This sector had to be given special treatment because of its resistance to the Generics Act, particularly the provisions on generic dispensing on "substitution", and the penalty clauses among the doctors.

This resistance was not entirely unexpected as the doctors felt their turf was threatened. Under the law and the implementing guidelines on prescribing (A.O. 62 s. 1989) and dispensing (A.O. 63 s. 1989), the patient will now have the option to choose from among generically-equivalent products to that prescribed by the doctor. This absolute power to determine what the patient gets in medicine could mean less privileges and material rewards for industry.

To allow for adequate education and information and learning and adjustment time, the implementation for the private professional sector was scheduled in 3 phases:

Phase I – Education & Information Dissemination – March to May 1989
Phase II – Voluntary Compliance with Monitoring but Without Penalties – June to August 1989
Phase III – Full Implementation with Monitoring & Penalties Beginning September 1, 1989

These guidelines considered comments and suggestions coming from all affected and interested parties and were finalized only after a nationwide consultation with prescribers and dispensers in all 13 regions of the country in February 1989.

## OTHER PROVISIONS OF GA 1988

There are other provisions of the Law for which the implementing guidelines still remain to be formulated. These are:

| | | |
|---|---|---|
| Section 4b | – | Systems of incentives for manufacturers of essential generic drugs |
| Section 8 | – | Required Producting of Generic Drugs |
| Section 10 | – | Importation of Raw Materials by DOH for allocation to Filipino-owned or controlled companies for the manufacture of essential generic drugs |

An inter-agency committee chaired by the Board of Investment and with members from the Pharmaceutical Industry and other concerned government agencies have been meeting to package these incentives under Section 4b and define the implementing guidelines for Section 8 & Section 10.

For reference, copies of the GA 1988 are attached as Annex B2 and the various implementing guidelines already completed are attached as Annex B3.

## ORGANIZATIONAL STRUCTURE

Given the responsibility for the implementation of the PNDP and GA 1988

is the Assistant Secretary (Asec) for Standards and Regulations who has the mandate to create as many Implementation Teams or Working Groups as needed. Besides the NDP and GA 1988, he has also control and supervision over line agencies and program of DOH concerned with pharmaceuticals, such as the Bureau of Food & Drugs, the Biologicals Production Service, the Regional Herbal Pharmaceuticals Program in Tuguegarao, Tacloban, Cotabato and Davao and the Regional Mini Drug Laboratories in all twelve (12) regions. As of May, 1989, the organizational structure of the Asec for Standards and Regulations and PNDP is shown in Figure 1 with seventeen (17) different working groups.

## CONCLUDING STATEMENT

We have described the evolution, crafting and implementation of a landmark policy and legislation which have health, human rights and social justice implications as a model for Science and Technology policy-making. This experience points out the importance of a thorough research and study in coming up with an accurate and comprehensive situationer or diagnosis which is a prerequisite to good Science and Technology or other policy-making for that matter. It also demonstrates the need for democratic participation and consultation both during the formulation of the policy and its implementation. The Philippine National Drug Policy and the Generics Act of 1988 experience exemplifies Science and Technology policy-making, a policy with both immediate and long-term impacts on the life of our people.

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# AGRICULTURAL SCIENCES

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# EFFECTS OF VARYING LEVELS OF SABAWIL (*MUCUNA PRURIENS* (L.) D.C.) LEAF MEAL ON THE GROWTH PERFORMANCE OF BROILERS[1]

FRANCISCO M. BASUEL[2]
*Isabela State University*
*Echague, Isabela*

## ABSTRACT

The study was conducted at the College of Agriculture's Experimental Area, Isabela State University, Echague, Isabela, for a period of six weeks, using 225 Lohmann strain of broilers. It aimed to determine the effect of the different levels of sabawil leaf meal on the growth performance of broilers and to evaluate the economy of feeding the non-conventional feedstuff to broilers, at least, in terms of return above feed cost.

The study revealed that broilers feed with diet containing 5 percent sabawil leaf meal were the best in terms of body weight, gain in weight, feed conversion, and return above feed cost. The broilers on the 10 percent sabawil leaf meal diet were as good as those in the control, as far as the aforementioned parameters are concerned. On the dressing percentage, birds treated with the different levels of the experimental feedstuff were as good as the control group, although those treated with 5 and 10 percent levels were higher. Insignificant differences in the liver weight of broilers were also noted indicating that the feedstuff used had no toxic effect.

Therefore, it is advisable to incorporate sabawil leaf meal in broiler diet at 5 to 10 percent level.

## Introduction

The high cost of feeds is a problem among almost all broiler raisers in the world. If it remains unabeted, then the broiler industry will surely "close shop". This will aggravate malnutrition due to the widening of the protein gap in the diet of the burgeoning population and result in economic dislocation and unemployment in the countryside. Therefore, the search for an effective solution to the perennial problem on spiralling feed cost must be sustained. The use of non-conventional feedstuffs should be explored to enable the raisers to make use of the

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[1]A study-component of a research project on the Utilization of Nonconventional Feedstuffs in Poultry which is funded by ISU-PCARRD.

[2]Project Leader and Associate Professor, ISU, Echague, Isabela.

locally grown and available crop as substitute for expensive feedstuffs. If they do, the cost of feeds will be reduced and consequently, broiler industry will again be lucrative.

Sabawil, scientifically known as *Mucuna pruriens* (L.) DC., is one of the emerging leguminous crops which could be utilized for feeding broilers. It is a viny legume locally grown by the farmers, although not as extensively as other field legumes because it has not yet gained its socio-economic prominence as commercial crop. But the uniqueness of this crop is that it is cultivated for food. The green pods can be used as vegetable, while the mature seeds processed to a meal form are commonly used as substitute for coffee. Chemical analysis reveals that sabawil leaf meal contains 29.5 percent crude protein on a dry matter basis.

Whyte *et. al.* (1973) reported that a legume does not only contain a relatively high percentage of protein, but that the protein itself is of unusually good quality, thus making it important as constituent or supplement of animal feeds. Castillo (1986) also stated that leaf meal contains amounts of carotene and xantophyll which contribute to the yellow or orange pigmentation of chicken's breast, shanks, and egg yolks. Moreover, Cocjin and Paglinawan (1982) found out that broilers fed diet containing 10 percent leaf meals from *Ipomoea prescaprae* and *Canavalia lineata* were not significantly different from the control at the end of the feeding period.

In addition, Pataueg and Binag (1987) observed in their preliminary study that birds fed diets of 2, 4, 6 and 8 percent levels of sabawil leaf meal had body weight and gain in weight comparable with the control. They also noted that birds fed diets of 2 and 6 percent were better than those on the 8 percent level and comparable with those in the control. However, they claimed that birds fed with diet of 2 percent sabawil leaf meal had the highest return above feed cost.

The Sabawil leaves are not yet widely utilized as feedstuff precisely due to a dearth of information on their utility. Hence, this study was conducted to determine the effect of the varying levels of sabawil leaf meal on the growth and economic performance of broilers.

## MATERIALS AND METHODS

### *Construction of Experimental Broiler House*

A gable-type broiler house was constructed in the fishpond of the College of Agriculture of the Isabela State University, Echague, Isabela using locally available materials such as cogon, bamboo, buho and lumber. It was provided with three (3) pens, each with a dimension of 3 x 25 feet. The pens were equally sub-divided into five experimental units, each of which had a floor area of 15 square feet.

### *Procurement of Chicks*

A total of three hundred (300) Lehmann broiler chicks were purchased from

a reliable dealer in Santiago, Isabela. Of these, two hundred twenty-five (225) were used for the experiment.

*Preparation of Sabawil Leaf Meal*

The sabawil leaves were gathered from the production area of the College of Agriculture and from the nearby municipalities. They were dried for three (3) days and ground to a meal form with the use of an electric hammer mill in the Animal Nutrition Research Laboratory of the Cagayan Valley Agricultural Resources Research and Development in the aforementioned University.

*Formulation of Experimental Diets*

Five experimental diets were formulated for the study, four of which contained various levels 5, 10, 15 and 20 percent of sabawil leaf meal (SLM). The control diet, did not contain sabawil leaf meal but 5 percent ipil-ipil leaf meal (ILM) was incorporated in it for comparison purposes. The common feedstuffs used in the preparation of the experimental diets were white corn, rice bran, copra meal, soybean oil meal, fish meal, meat and bone meal, salt and afsillin. The minimum crude protein requirement of 20 percent was used as basis for the formulation of the different broiler diets. The composition and calculated nutrient contents of the various experimental broiler diets are presented in Table 1.

Table 1. The composition and calculated nutrient contents of the different experimental broiler diets

| *INGREDIENT* | *DIET 1 0% SLM* | *DIET 2 5% SLM* | *DIET 3 10% SLM* | *DIET 4 15% SLM* | *DIET 5 20% SLM* |
|---|---|---|---|---|---|
| Ground White Corn | 50.60 | 51.90 | 49.85 | 47.80 | 45.70 |
| Soybean Oil | 13.40 | 12.10 | 9.15 | 6.20 | 3.30 |
| Sabawil Leaf Meal | – | 5.00 | 10.00 | 15.00 | 20.00 |
| Ipil-ipil Leaf Meal | 5.00 | – | – | – | – |
| Rice Bran | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| Copra Meal | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| Fish Meal | 9.00 | 9.00 | 9.00 | 9.00 | 9.00 |
| Meat and Bone Meal | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Afsillin | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| TOTAL WEIGHT(K) | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated Crude Protein, % | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |

Table 1. Continuation

| *INGREDIENT* | *DIET 1 0% SLM* | *DIET 2 5% SLM* | *DIET 3 10% SLM* | *DIET 4 15% SLM* | *DIET 5 20% SLM* |
|---|---|---|---|---|---|
| Metabolizable Energy, kcal/kg | 2758.25 | 2774.27 | 2736.81 | 2699.36 | 2661.28 |
| Calcium (%) | 1.06 | 1.05 | 1.05 | 1.05 | 1.06 |
| Phosphorus (%) | 1.09 | 1.08 | 1.08 | 1.07 | 1.07 |
| Crude Fiber (%) | 4.13 | 4.07 | 4.69 | 5.31 | 5.93 |

*Experimental Design, Treatment and Distribution of Birds*

The Completely Randomized Design (CRD) was followed in the study. The different treatments were designated as follows:

Treatment 1 – Basal + 5% ILM (Control)
Treatment 2 – Basal + 5% SLM
Treatment 3 – Basal + 10% SLM
Treatment 4 – Basal + 15% SLM
Treatment 5 – Basal + 20% SLM

All the treatments were replicated thrice, constituting of 15 experimental units, each unit containing 15 chicks.

*Brooding Management*

The chicks were provided with an optimum temperature during the breeding period which lasted for 21 days. Principally, the supplemental source of heat was 50-watt bulbs.

*Feeding Management*

The birds were fed with the different experimental diets during the feeding trial period. *Ad libitum* feeding was done during the observation period.

*Providing of Drinking Water and Sanitation*

Clean, fresh water was made available to the birds at all times during the conduct of the feeding trial. Univite was added to the drinking water every time it was necessary.

Strict sanitation and hygiene was observed in all the experimental pens and their surroundings.

*Collection of Data*

The following were the data gathered:

1. *Body Weight.* The body weight of the birds was recorded upon the arrival

and thereafter, weekly weight was taken up to the sixth week.

2. *Feed Consumption.* The feed consumption of the birds in all the dietary treatments was determined. The data on the orts were subtracted from the feed offered to determine the actual feed consumption of the birds.

3. *Dressed Weight.* A male and a female broiler from each replication were dressed at the end of the experimental period. The data on the liveweight and dressed weight of each sample, with and without giblets, were recorded and these were the bases of determining the dressing percentage of the experimental birds.

4. *Liver Weight.* The data on the liver weight were gathered and these were the bases of determining possible toxic substance brought about by the sabawil leaf meal.

5. *Return Above Feed Cost.* The data on the cost of feeds and broilers produced were recorded and these were used in computing the return above feed cost.

6. *Other Observations.* The physical conditions of the birds were properly observed. Feathering and pigmentation of the skin and shanks were also noted.

## OBSERVATION AND DISCUSSION OF RESULTS

### *Observation*

Generally, the experimental birds were normal during the first two weeks of the observation period. On the third up to the fifth week of the experiment, the differences in physical appearance became noticeable. It was observed that the birds treated with 5 percent level of sabawil leaf meal grew faster and had a more rapid feather development. Those on the 10 percent sabawil leaf meal diet had almost similar growth with the control group.

More elaborate differences in physical appearance were noted on the sixth week of the study when birds in Treatment 2 with 5 percent sabawil leaf meal were observed to have the most impressive growth and feather development. The growth of birds in Treatment 3 (10% SLM) was as fast as those of the control group. Retarded growth and development were noticeable in birds on the other experimental diets.

In terms of the pigmentation of skin and shanks, the birds fed with diets of 5 percent sabawil leaf meal and the control diet had almost the same degree of yellow color; however, it was lighter than those in Treatments 4 and 5. The birds in Treatment 3 (10% SLM) had an intense yellow pigment.

### *Discussion of Results Body Weights*

The average initial and weekly body weights of birds fed with the different experimental diets are shown in Table 2. The initial body weights of the birds taken

immediately at the start of the experiment had no significant variations. However, on the first week of the observation period, highly significant differences in body weights of the birds were discernible. Birds fed with diets of 5 and 10 percent sabawil leaf meal with 96.07 and 89.29 grams, respectively, were significantly similar with those on the control diet. Lighter body weights were recorded in birds treated with 15 and 20 percent of the nonconventional feedstuff.

Table 2. Average initial and weekly body weight of birds fed with diets of different levels of sabawil leaf meal in grams

| *DIETARY TREATMENT* | *INITIAL WEIGHT*$^{NS}$ | *WEEKLY BODY WEIGHT* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *FIRST WEEK*** | *SECOND WEEK*** | *THIRD WEEK*** | *FOURTH WEEK*** | *FIFTH WEEK*** | *SIXTH WEEK*** |
| 1 (CONTROL) | 35.69 | 89.93$^{a}$ | 209.82$^{ab}$ | 391.69$^{a}$ | 666.35$^{a}$ | 960.47$^{a}$ | 1164.58$^{b}$ |
| 2 (5% SLM) | 37.09 | 96.07$^{a}$ | 225.22$^{a}$ | 409.00$^{a}$ | 666.71$^{a}$ | 959.80$^{a}$ | 1330.87$^{a}$ |
| 3 (10% SLM) | 36.11 | 89.29$^{a}$ | 186.93$^{bc}$ | 343.24$^{b}$ | 563.38$^{b}$ | 835.07$^{b}$ | 1124.69$^{bc}$ |
| 4 (15% SLM) | 36.67 | 78.80$^{b}$ | 147.27$^{d}$ | 261.58$^{c}$ | 425.95$^{c}$ | 639.13$^{c}$ | 895.18$^{d}$ |
| 5 (20% SLM) | 37.04 | 69.42$^{bc}$ | 113.82$^{c}$ | 185.48$^{d}$ | 285.49$^{d}$ | 426.13$^{d}$ | 629.15$^{c}$ |
| C. V. % | 2.98 | 4.77 | 5.63 | 5.68 | 3.25 | 2.13 | 2.48 |

NS – Not significant **Highly significant

On the second week of the experiment, the trend on the body weight was similar to that of the first week, although birds in Treatment 2 with 5 percent sabawil leaf meal with an average body weight of 225.22 grams were the only ones comparable with those on the control diet. Evidently, all the birds in the other treatments had significantly lighter body weight. In descending order, birds in Treatment 3 had an average body weight of 186.93 grams; those in Treatment 4 of 147.27 and those in Treatment 5 of 113.82 grams.

The pattern on the body weights of birds on the third week was observed up to the fifth week of the experimental periods. Birds in Treatment 2 with 5 percent sabawil leaf meal had an average body weight similar to those in the control. Significantly, lighter body weights were noted in birds treated with 10 percent sabawil leaf meal. So far, the lightest birds were apparent in Treatment 5 with 20 percent of the experimental feedstuff.

Amazingly, on the sixth week of the observation period. it appeared that birds fed with diet of 5 percent sabawil leaf meal with an average weight of 1330.87 grams were significantly the heaviest. Birds on the 10 percent sabawil leaf meal diet with an average weight of 1124.69 grams were statistically similar with those on the control diet with 1164.58 grams. The birds treated with higher levels of the non-conventional feedstuff had an inferior body weight.

Based on these findings, it could safely be stated that the growth performance of birds fed with diet of 5 percent sabawil leaf meal was better than that of the control birds given ration with 5 percent ipil-ipil leaf meal. The birds fed diet with 10 percent sabawil leaf meal performed as well as those on the control diet.

*Gain in Weight, Feed Consumption and Feed Conversion*

Table 3 shows the average gain in weight, feed consumption and feed conversion of broilers fed with diets of different levels of sabawil leaf meal which varied significantly ($P<0.01$). Birds in Treatment 2 with 5 percent sabawil leaf meal obtained an average gain in weight of 1293.78 grams which was significantly the highest. Those on the control diet and Treatment 3 had higher gain in weight than the rest of the dietary treatments.

Table 3. Average gain in weight, feed consumption and feed conversion ratio of broilers fed with diets of different levels of sabawil leaf meal and the control ration for six weeks

| *DIETARY TREATMENT* | *GAIN IN WEIGHT(G)*** | *FEED CONSUMPTION(G)*** | *FEED CONVERSION*** |
|---|---|---|---|
| 1 (CONTROL) | $1128.89^{b}$ | $2742.30^{ab}$ | $2.43^{c}$ |
| 2 (5% SLM) | $1293.78^{a}$ | $2744.60^{a}$ | $2.12^{c}$ |
| 3 (10% SLM) | $1088.58^{bc}$ | $2608.50^{c}$ | $2.39^{cd}$ |
| 4 (15% SLM) | $858.51^{d}$ | $2230.30^{d}$ | $2.60^{b}$ |
| 5 (20% SLM) | $592.11^{c}$ | $1700.00^{e}$ | $2.88^{a}$ |
| C. V. (%) | 2.51 | 1.96 | 1.82 |

**Highly significant

On the feed consumption, birds in Treatment 2 (5 percent SLM) had the highest with an average of 2744.55 grams, which was significantly comparable with the control diet. Lower feed intake was generally noted in birds treated with the higher levels of the experimental feedstuff possibly because the considerable fiber content made the feeds unpalatable to the birds.

The most efficient feed conversion was also noted in birds fed with diet of 5 percent sabawil leaf meal since they only needed 2.12 kilograms of feeds to produce a kilogram of broiler meat. Birds treated with 10 percent of the experimental feedstuff were as efficient as those on the control diet. The rest of the dietary treatments were less efficient.

*Dressing Percentage and Liver Weight*

The average dressing percentage and liver weight of broilers fed with diets of the different levels of sabawil leaf meal and the control ration are presented in

Table 4. The dressing percentage of broilers with giblets removed did not vary significantly. Although the statistical analysis revealed no significant differences among the treatment means, it was noted that broilers in Treatments 2 and 3 with an average of 67.43 and 66.91 percent, respectively, were higher in dressing percentage than those in the control group. They were followed by broilers in Treatments 4 and 5.

Table 4. Dressing percentage and liver weight of broilers fed with diets of different levels of sabawil leaf meal and the control ration at sixth week

| DIETARY TREATMENT | DRESSING PERCENTAGE$^{NS}$ Without Giblets | With Giblets | LIVER Weight(G)* |
|---|---|---|---|
| 1 (CONTROL) | 66.70 | 72.74 | $33.00^{abc}$ |
| 2 ( 5% SLM) | 67.43 | 73.72 | $34.67^{ab}$ |
| 3 ( 10% SLM) | 66.91 | 73.21 | $29.00^{abcd}$ |
| 4 ( 15% SLM) | 64.90 | 72.10 | $35.33^{a}$ |
| 5 ( 20% SLM) | 65.44 | 72.41 | $21.17^{d}$ |
| C. V. (%) | 2.76 | 2.34 | 15.74 |

NS – Not significant *Significant

Keeping the giblets intact with the dressed broilers still gave the same dressing percentage pattern as that of the broilers without giblets where Treatments 2 and 3 were higher than the control. Broilers in Treatments 4 and 5 had the least dressing percentage.

On the liver weight, significant differences ($P < 0.05$) among treatment means were noted. Except for the birds in Treatment 5 (20 percent SLM), all others in the different treatments had liver weights ranging from 29.00 to 35.33 grams which were comparable with those on the control diet with 33.00 grams. These findings revealed that incorporating sabawil leaf meal up to 15 percent level in broiler diet did not detrimentally affect the liver. This implies that sabawil leaf meal is not really toxic to the birds.

*Return Above Feed Cost*

The return above feed cost shown in Table 5 was evaluated by considering the value of broilers produced and the cost of feeds consumed. After deducting the cost of feeds from the value of broilers produced, it turned out that Treatment 2(5 percent SLM) had the highest return of ₱29.43. It was followed by Treatment 3 (10 percent SLM) with an almost ₱24.00 return above feed cost which was

higher than that of the control with ₱23.66. Treatment 4 and 5 had the lowest economic return of ₱19.10 and ₱13.31, respectively.

It is economically advisable to incorporate sabawil leaf meal at 5 to 10 percent level in broiler diet.

Table 5. Return above feed cost from broilers fed with diets of varying levels of sabawil leaf meal and the control ration in pesos

| *ITEM* | *DIETARY TREATMENT* | | | | |
|---|---|---|---|---|---|
| | *I (CONTROL)* | *II (5% SLM)* | *III (10% SLM)* | *IV (15% SLM)* | *V (20% SLM)* |
| Liveweight of broiler, kg. | 1.16 | 1.33 | 1.12 | 0.895 | 0.629 |
| Return per live broiler, P* | 38.28 | 43.89 | 36.96 | 29.54 | 20.76 |
| Amount of feed consumed, kg. | 2.74 | 2.74 | 2.61 | 2.23 | 1.70 |
| Cost of feed consumed, ₱ | 14.62 | 14.46 | 12.97 | 10.44 | 7.45 |
| Return above feed cost per broiler, ₱ | 23.66 | 29.43 | 23.99 | 19.10 | 13.31 |

*Selling price per kilo liveweight of broiler – ₱33.00

## SUMMARY, CONCLUSION AND RECOMMENDATION

*Summary*

The study was conducted for 6 weeks to determine the best level of sabawil leaf meal for broiler production and the economy of feeding sabawil leaf meal to broilers in terms of return above feed cost. The results are as follows:

1. The growth performance of broilers fed with diet containing 5 percent sabawil leaf meal was significantly better than those on the control diet. The broilers fed the diet with 10 percent of the nonconventional feedstuff was as good as that of the control.

2. Birds fed with diet of 5 percent sabawil leaf meal had the highest gain in weight. Those treated with 10 percent of the experimental feedstuff were comparable with those on the control diet. Both groups were higher in gain in weight than the rest of the dietary treatments.

3. The greatest amount of feed was consumed by birds fed with diet of 5 percent sabawil leaf meal, although statistically comparable with the control. Lower feed intake was observed in those given the higher levels of the experimental feedstuff.

4. Birds fed with diets of 5 percent sabawil leaf meal were found to be the most efficient, while those treated with 10 percent of the experimental feedstuff were as efficient as those on the control diet.

5. The dressing percentage of broilers, with and without giblets, on the sabawil leaf meal diets and the control diet did not vary significantly.

6. Birds fed with diets of 5, 10 and 15 percent sabawil leaf meal had liver weights similar with those on the control diet. Those in the 20 percent level had the lightest liver weight.

7. Broilers on the 5 percent sabawil leaf meal diet gave the highest economic return above feed cost while those in the 10 percent level had a return which was higher than those on the control diet.

*Conclusion and Recommendations*

Based on the foregoing findings, it could be inferred that the broiler diet with 5 percent sabawil leaf meal could tremendously influence the growth and economic performance of broilers. The growth of birds on the diet containing 10 percent level was comparable to that of the control, but gave a return higher than the latter. Although the diet with 5 percent sabawil leaf meal surpassed all the other treatments, the effect of the 10 percent level on the previously mentioned parameters is considerably large, thus making it advisable to include it as a part of the broiler diet. Therefore, the 5 to 10 percent level of sabawil leaf meal is highly recommended for incorporation in the broiler diet.

It is suggested, that a similar study be conducted on layers to find out the effects of levels of sabawil leaf meal on egg production and quality.

Also, research on the scientific culture of sabawil crop should be undertaken so that it could be massively produced and thereby ensure its availability as feedstuff for chickens.

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# FRESH COCONUT MEAT IN POULTRY RATIONS*

Bernabe B. Cocjin**, Fe C. Evangelio*** and Rola Brizuela Salidaga***

***West Visayas State University, Iloilo City*
****Visayas State College of Agriculture, Leyte*

## ABSTRACT

Almost 1/4 of all the coconuts in the world is produced in the Philippines. During periods of high supply of coconuts it would be better to feed coconut meat to farm animals for conversion into meat and eggs.

Three studies were conducted at the Visayas State College of Agriculture from April, 1983 to April, 1985 to determine the response of Mallard ducks, Muscovy ducks and broilers to fresh coconut meat supplementation in their diets.

Results showed that Mallard ducks on ration with coconut meat performed similarly with those on ration without coconut meat. Feed cost per dozen eggs was reduced by 28-30% with coconut meat. Feed cost per unit gain of Muscovy ducks was reduced by 32-37% by coconut meat supplementation. With broilers feed conversion, gain in weight and breast weigh were significantly improved by coconut meat supplementation. Return-above-feed cost increased with increasing level of coconut meat in the ration.

## INTRODUCTION

The Philippines produces almost 1/4 of all the coconut in the world with 377 million coconut trees producing 12 billion nuts per year (Banzon and Velasco, 1982). When there is a considerable drop in the price of copra, as in 1981-82, farmers tend not to harvest the nuts for processing. This situation of low price of copra, which has been cyclical, contributes to the misery of the rural poor.

One way of alleviating the situation is to feed the fresh nuts to farm animals for conversion into high-priced animal products like meat and eggs which are saleable in the market. It is common knowledge that coconut residue either as meal, flour or presscake ("sapal") is used as feed ingredient, but its large scale incorporation in non-ruminant's diet is limited due to its low digestibility (Hagensity, 1977), Gerpacio and Castillo (1974)) analysed the proximate composition of "sapal" or

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**Professor III and Director of Research, West Visayas State University, Iloilo City;
***Instructor and Research Assistants, respectively, Visayas State College of Agriculture, VISCA, Leyte.

"presscake" to be 90% dry matter, 6.1% crude protein, 5.0% ether extract, 34.7% crude fiber, 1.5% ash and 24.3% nitrogen-free extract.

In this study, whole grated mature coconut meat was tested as part of the rations of Mallard and Muscovy ducks while presscake or "sapal" was used as part of the rations of broilers. Mallard and Muscovy ducks were used because these are commonly raised in the rural areas and they belong to the species of poultry long neglected by research and development activities. Broilers were also used as test animals because of the potential of coconut meat as part of the broiler finishing rations.

### Time and Place of the Study

This study was condcucted from April, 1983 to April. 1985 at the Department of Animal Science and Veterinary Medicine, Visayas State College of Agriculture (VISCA), Leyte.

## OBJECTIVES

The objectives of this study were:

1. To determine the effect of supplementing layer ration with mature coconut meat on the egg production of Mallard ducks *(Anas boscas* Linn.).
2. To evaluate the effect of supplementing rations with mature coconut meat on the growth performance of Muscovy ducks *(Cairina moschata* Linn.).
3. To determine the growth response, carcass cut-up yield and financial returns from broilers fed rations supplemented with two levels of fresh coconut meat.

## Materials and Methods

*Study I.*

A total of 120 Mallard ducks layers, around 10 to 14 months of age, were used. They were distributed among 4 treatments replicated 6 times with 5 birds per pen. A pen is equivalent to one experimental unit. The floor dimension of a cage was 2 x 5 feet or an allowance of 2 square feet per bird.

The treatments used were:

A – Commercial layer ration (30 birds);
B – 50% Commercial ration + 50% Coconut meat (30 birds);
C – Formulated layer ration with 17% C. P. (30 birds);
D – 30% Formulated ration + 50% Coconut meat (30 birds)

Grated mature coconut meat was used. The milk was not extracted to shorten processing time before feeding the coconut meat. Samples of the coconut meat were analyzed for proximate composition. The period of feeding was 53 days. Feed and water were given *ad libitum*. The data gathered were statistically analyzed using the analysis of variance in a randomized complete block design. Treatment mean comparisons on parameters whose "F" values were significant at 5% level of probability were done using the DMRT (Gomez and Gomez, 1976).

*Study II.*

A total of 120 Muscovy ducklings (*Cairina moschata*) were distributed to 4 treatments replicated two times with 15 birds per experimental unit. The floor dimension of the cage was 2 x 8 feet, or an allowance of a little more than one square foot per bird.

The treatments used were:

A – Commercial broiler starter (22% C.P.) (30 birds);
B – 50% Commercial ration + 50% Coconut meat (30 birds);
C – Formulated broiler starter (22% C.P.) (30 birds);
D – 50% Formulated ration + 50% Coconut meat (30 birds)

Grated coconut meat was also used. Milk was not extracted. The feeding period was 90 days. Feed and water were given *ad libitum*. Analysis of variance in RCDO was the statistical tool used. The formulated rations in Studies I and II are found in Table 1.

Table 1. Formulated rations used in Studies I and II

| *Feed Ingredients* | *Parts by weight (kg.)* | |
|---|---|---|
| | *Layer ration* | *Broiler ration* |
| Rice bran | 34.1 | 28.4 |
| Cassava meal | 38.6 | 32.8 |
| Fish meal | 14.5 | 20.0 |
| Meat and Bone meal | 6.0 | 11.5 |
| Ipil-ipil leaf meal | 5.0 | 5.0 |
| Oyster shell | 0.5 | 0.5 |
| Vitamin-Mineral pre-mix | 1.0 | 1.0 |
| Salt | 0.3 | 0.3 |
| TOTAL | 100.0 | 100.0 |
| Calculated Analysis: | | |
| Crude protein, % | 16.97 | 22.00 |
| M. E., cal./kg. | 2406.52 | 2402.12 |
| Cost/kg., ₱ | 3.39 | 4.22 |

Study III.

A total of 135 day-old straight-run Pilch broiler chicks were used. These were distributed among 3 treatments replicated 3 times with 15 birds per pen. The floor dimension of the pens used was 2 x 5 feet, or a floor space allowance of 1.5 square feet per bird.

The treatments used were:

A – Control (Broiler ration without coconut meat) (45 birds);
B – Broiler ration with 30% coconut meat (45 birds);
C – Broiler ration with 50% coconut meat (45 birds).

The birds, from 7 to 49 days of age, were fed with the ration treatment. Milk-extracted coconut presscake ("sapal") was used because of the availability of this material from a study on the barangay-based wet method of coconut oil production from coconut milk. Feed and water were provided to the birds *ad libitum*. The ration treatments used in Study III are found in Table 2.

Table 2. Ration treatments used in Study III

| *Feed Ingredients* | *Parts by weight (kg.)* | | |
|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ |
| Ground yellow corn | 50 | 22 | 10 |
| Rice bran | 20 | 14 | 5 |
| Soybean oil meal | 8 | 15 | 15 |
| Ipil-ipil leaf meal | 5 | 5 | 5 |
| Fish meal | 8 | 10 | 10 |
| Meat and Bone meal | 8 | 3 | 4 |
| Vitamin-Mineral pre-mix | 1 | 1 | 1 |
| Coconut meat (presscake) | 0 | 30 | 30 |
| TOTAL | 100 | 100 | 100 |
| Calculated Analyses: | | | |
| Crude protein, % | 20.51 | 21.19 | 20.56 |
| M. E., kcal/kg. | 2784.10 | 2716.78 | 2708.20 |
| Calcium, % | 1.19 | 0.98 | 1.09 |
| P (avail.), % | 0.88 | 0.68 | 0.55 |
| Price/kg. ration, ₱ | 6.40 | 5.95 | 5.87 |

At the end of the study, 9 birds per treatment were dressed for the measurements of carcass and cut-up data.

The data were statistically analyzed using the analysis of variance in RCBD. Treatment mean comparisons were done using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1976).

## Observations and Results

*Study 1.*

Table 3 shows the results of the proximate composition of the mature coconut meat submitted for analysis in the laboratory.

It appears that the technicians at the laboratory dried the samples submitted before conducting the analysis. Fresh mature coconut meat usually contains an average of 30% moisture, while the result showed an average of only 3.80% moisture. On dry matter basis, the crude protein content of the samples submitted was 2.34%, whereas the calculated crude protein content of the fresh mature coconut meat given to the birds was 1.64% (as-fed basis).

Table 3. Average composition of proximate analyses of mature coconut meat (as-analyzed basis)

| *Composition* | *Average* |
|---|---|
| Moisture, % | 3.80 |
| Crude Protein, % | 2.25 |
| Crude Fat, % | 61.30 |
| Crude Fiber, % | 8.80 |
| Ash, % | 2.00 |

Table 4 shows the average feed consumed, percent egg production (hen-day), average egg production per bird, feed conversion, gain in weight and weight of eggs of Mallard ducks fed with the experimental rations.

Table 4. Biological responses of Mallard duck layers to rations with and without fresh coconut meat (53 days feedings)

| *Ration Treatments* | *Average Feed Consumed Kg.* | *% Egg Prodn. (hen-day)* | *No. of Eggs Produced per bird* | *Feed Conversion* | *Gain in wt. kg.* | *Average wt. of Eggs gm.* |
|---|---|---|---|---|---|---|
| Commercial | 9.15[a] | 35.66[ns] | 18.9[ns] | 1.23[ns] | 0.43[ns] | 62.10[ns] |
| 50% Commercial + 50% Cocomeat | 9.10[a] | 23.20 | 12.3 | 1.36 | 0.86 | 61.30 |
| Formulated | 7.50[b] | 31.13 | 16.5 | 1.55 | 0.01 | 56.22 |
| 50% Formulated + 50% Cocomeat | 8.10[b] | 24.89 | 11.3 | 1.76 | 0.08 | 59.98 |

Table 4. Continuation

| *Ration Treatments* | *Average Feed Consumed* | *% Egg Prodn. (hen-day)* | *No. of Eggs Produced per bird* | *Feed Conversion* | *Gain in wt. kg.* | *Average wt. of Eggs gm..* |
|---|---|---|---|---|---|---|
| C.V., % | 1.87 | 16.46 | 14.2 | 15.45 | 36.78 | 5.51 |

abValues in column with different superscripts vary significantly from each other (P0.05).
nsNo significant differences between values in column (P0.05).

The data showed that rations with 50% coconut meat were as palatable as one without coconut meat. Commercial feed-based rations were more palatable than the counterpart formulated feed-based ones. Percent egg production on hen-day basis did not vary significantly between treatments. Likewise, statistical analysis did not show significant differences in the number of eggs produced per bird, feed conversion, gain in weight and average weight of the eggs of Mallard ducks between treatments. The high coefficient of variation in gain in weight is expected of Mallard ducks which are egg-type birds.

Table 5 shows the percent broken eggs produced from Mallard ducks fed with the ration treatments.

Table 5. Percent broken eggs produced from Mallard ducks fed rations with and without fresh mature coconut meat

| *Ration Treatments* | *Average & Broken Eggs* |
|---|---|
| Commercial | 2.25 |
| 50% Commercial | 5.88 |
| Formulated | 17.19 |
| 58% Formulated | 4.62 |

ns Values in column are not significantly different from one another (P20.05).

Although the values for the average broken eggs did not vary significantly between treatments because of limited number of observation, the data demonstrate the impairment of calcium metabolism due to the high fat content of diets supplemented with fresh coconut meat.

*Study II.*

Table 6 shows the average feed consumption, gain in weight and feed efficiency of Muscovy ducks fed ration with and without fresh coconut meat.

Statistical analyses showed that there were no significant differences between treatments on average feed consumption, gain in weight and feed efficiency of Muscovy ducks fed ration with or without fresh coconut meat. It was observed however, that feed consumption per bird increased from an average of 5.00 kg. in the first 45 days to 10.49 kg. in the second 45 days of feeding. Average gain in weight was 33.6 grams per day during the first 45 days and 11.2 grams per day during the second 45 days.

Feed efficiency was much better for birds fed during the first 45 days (3.33) than during the second 45 days (10.60).

Table 6. Average food consumption, gain in weight and Feed efficiency of Muscovy ducks fed ration with and without coconut meat (90 days)

| *Ration Treatments* | *Ave. Feed Consumption kg.* | *Ave. Gain in Weight kg.* | *Ave. Feed Efficiency* |
|---|---|---|---|
| Commercial | 8.34[na] | 1.46[na] | 6.56[ns] |
| 50% Commercial + 50% Cocomeat | 8.30<br>8.30 | 1.34<br>1.34 | 7.16<br>7.16 |
| Formulated | 7.18 | 1.10 | 7.34 |
| 50% Formulated + 50% Cocomeat | 7.27 | 1.18 | 6.78 |
| C. V., % | 2.32 | 3.38 | 2.69 |

[ns]Values in column do not differ significantly from one another (P50.05).

## COST ANALYSIS:

Table 7 shows the feed cost to produce a dozen eggs of Mallard ducks and kilogram gain in weight of Muscovy ducks.

Table 7. Feed cost analyses on Mallard and Muscovy ducks performance as influenced by fresh mature coconut meat incorporation

| *Ration Treatments* | *Cost to Produce a Dozen Mallard Ducks Eggs (₱ –)* | *Cost to Produce a kg. Gain of Muscovy Ducks (₱)* |
|---|---|---|
| Commercial | 6.77 | 38.84 |
| 50% Commercial + 50% Cocomeat | 4.76 | 26.56 |

Table 7. Continuation

| *Ration Treatments* | *Cost to Produce a Dozen Mallard Ducks Eggs (₱–)* | *Cost to Produce a kg. Gain of Muscovy Ducks (₱)* |
|---|---|---|
| Formulated | 5.25 | 30.98 |
| 50% Formulated + 50% Cocomeat | 3.79 | 19.39 |

N.B. Based on the observation that 3 nuts = 1 kg. meat; commercial layer ration = ₱5.50/kg; commercial broiler ration = ₱5.92/kg; 1 nut = ₱0.50

It was demonstrated that at the prevailing prices of the feeds used, supplementing the feeds with 50% coconut meat resulted in economical feed cost to produce a dozen eggs or a kilogram gain of Muscovy ducks. Based on the performance of the Mallard ducks, the price of the nut must be ₱1.48 each for the supplemented commercial ration to equal the cost of producing a dozen eggs by birds on all-commercial ration. In the case of the Muscovy ducks, the price of the nut must be ₱1.64 each for the supplemented commercial ration to equal the cost of producing a kilogram gain. In the case of formulated rations, the price of the coconut must be ₱0.86 per nut in the layer ration and ₱1.41 in the Muscovy duck ration for the cost of the supplemented rations to equal the feed cost of production of all-mesh formulated rations.

When the price of the nuts is lower than that in the conditions cited above, then it would be advisable to supplement 50% fresh coconut meat in the rations for Mallard and Muscovy ducks.

*Study III.*

Table 8 shows the biological and economic responses of broilers to ration treatments with different levels of fresh mature coconut meat (presscake).

The data show that final liveweight, gain in weight, feed consumption and feed efficiency were better obtained from broilers fed with rations with the presscake of mature coconut meat (sapal) than these fed with the control ration (sapal). There were no significant differences in carcass weight, dressing percentage, weight of gibblets (liver, gizzard plus proventriculus and heart), and weight of back with rib and tail, thigh, drumstick, feet, and heart, and neck observed between treatments.

However, significant differences were observed in the weights of breasts and the wings of the broilers. Weights of breasts and wings of birds given ration containing presscake of mature coconut meat (sapal) were significantly higher than those in the control treatment.

The incorporation of the coconut meat in the ration of broilers also reduced the cost of the formulated ration; thus, it was observed that the return-above-feed cost per live and dressed bird was highest in birds given ration with 50% presscake of fresh coconut meat (sapal).

Table 8. Biological and economic responses of broilers to ration treatments with different levels of fresh mature coconut meat (milk-extracted)

| *Parameters* | *Treatments* | | | |
|---|---|---|---|---|
| | *Control* | *30% Cocomeat* | *50% Cocomeat* | *C. V. %* |
| Final liveweight, kg. | 0.91[b] | 0.99[a] | 1.03[a] | 1.50 |
| Gain in weight, kg. | 0.79[c] | 0.86[b] | 0.91[a] | 1.32 |
| Carcass weight, kg. | 0.72[ns] | 0.79 | 0.82 | 5.39 |
| Feed consumed, kg. | 2.26[b] | 2.40[a] | 2.49[a] | 1.15 |
| Feed efficiency, F/G | 2.86[c] | 2.79[b] | 2.74[a] | 0.55 |
| Dressing percentage | 71.66[ns] | 72.98 | 73.96 | 1.47 |
| Percent Livebility | 97.77[ns] | 95.55 | 97.77 | 4.58 |
| Weight of meat cuts: | | | | |
| Back (rib + tail), gm. | 127.88[ns] | 137.99 | 136.44 | 6.15 |
| High, gm. | 109.88[ns] | 118.44 | 124.33 | 8.15 |
| Drumstick, gm. | 90.99[ns] | 98.10 | 102.22 | 8.45 |
| Feet, gm. | 54.88[ns] | 55.33 | 54.99 | 8.31 |
| Head and neck, gm. | 92.88[ns] | 94.22 | 97.22 | 9.17 |
| Breast, gm. | 151.77[b] | 191.33[a] | 194.66[a] | 4.41 |
| Wings, gm. | 79.66[b] | 87.88[a] | 92.44[a] | 4.08 |
| Return-above-feed cost per bird, ₱ | 0.48 | 2.60 | 3.67 | |

[abc]Means in the row with different letter superscript vary significantly from one another ($P<0.05$)

[ns]Means in the row are not significantly different from one another ($P>0.05$).

It is very profitable therefore to incorporate the presscake of fresh mature coconut meat (sapal) in broiler rations, provided the formulated ration meets the requirements of the birds for crude protein and energy.

## Recommendations

The results show that it was most economical to add even up to 50% fresh mature coconut meat in the rations of Mallard ducks, Muscovy ducks and broiler chickens. However, the impairment of calcium was evident in the poor shell quality of the eggs laid by the Mallard ducks fed with 50% coconut meat in the ration.

The development of the breast and wings of the broilers was improved at higher levels of coconut meat (presscake) supplementation. Returns per broiler raised on rations with fresh coconut meat (presscake) increased with increasing level of coconut meat in the ration. It is advisable, however, to start feeding the fresh mature coconut meat to broilers at the age of three weeks.

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# ELECTROPHORETIC ANALYSIS OF GENETIC VARIATION IN BROWN PLANTHOPPER *NILAPARVATA LUGENS* (STAL) (HOMOPTERA: DELPHACIDAE) AND GREEN LEAFHOPPER *NEPHOTETTIX VIRESCENS* (DISTANT) (HOMOPTERA: CICADELLIDAE) IN THE PHILIPPINES

C. G. DEMAYO*, R.C. SAXENA*, AND A.A. BARRION**

**International Rice Research Institute*
***Genetics Laboratory, University of the Philippines at Los Baños*

## ABSTRACT

Allozyme polymorphisms at 26 gene loci of *N. lugens* and *N. virescens* revealed polymorphism in 11 and 14 loci, respectively. *N. lugens* from 10 localities in the Philippines significantly differ in allelic frequency at 4 polymorphic gene loci. For *N. virescens*, heterogeneity of gene frequencies among 7 local populations was detected at three loci. Partitioning the total variation within and between local populations using the Shannon information index, H and Nei's modification of Wright's F-statistics demonstrated that most of the genetic diversity among population of different localities existed as within-subdivision diversity.

Genetic differentiation of the population susceptible and resistant rice varieties was also observed, emphasizing the importance of host plants as biotic factors associated with the genetic structure of the two species. The potential role of host plants in the process of speciation through biotype or host race formation in *N. lugens* and *N. virescens* is discussed.

## Introduction

Host-plant resistance has been used successfully for a number of species of agricultural importance. But sometimes it has been rendered unstable due to the evolution of a pest's nullifying effects on the resistance genes in host plants. Good examples are the two most serious insect pests of rice in tropical Asia, the brown planthopper, *Nilaparvata lugens* (Stal), and the green leafhopper, *Nephotettix virescens* (Distant). *N. lugens* causes "hopperburn" or complete wilting and drying of rice plants (Dyck and Thomas 1979) and transmits the grassy stunt (Ling 1972) and ragged stunt virus diseases (Ling 1967, Ling 1977, Ling *et. al.*, 1981, IRRI 1978). On the other hand, *N. virescens* damages rice plants by excessive feeding and transmission of tungro and other virus diseases (Pathak, 1968).

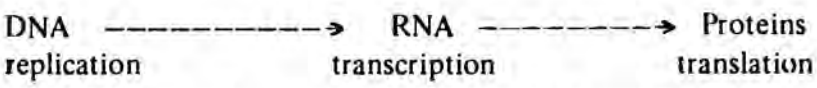
Evolution of insect pest populations which are capable of surviving on and damaging the rice plant with known genes for resistance has been observed in *N. lugens* and are referred to as biotypes. Based on varietal reactions, three rice-infesting biotypes have been recognized at IRRI (Seshu and Kaufmann 1980, IRRI 1976, 1982). Biotype 1 population survives on and damages varieties without genes for resistance; biotype 2 thrives on varieties carrying *Bph* 1 resistance gene, in addition to those susceptible to biotype 1; and biotype 3 can multiply on varieties having *bph* 2 resistance gene, in addition to those susceptible to biotype 1. These three *N. lugens* populations do not damage rice varieties with *Bph* 3 or *bph* 4 resistance gene, and the variety Ptb 33 carrying two unidentified genes. Recently, another biotype infesting a weed grass, *Leersia hexandra* Swartz, was identified on the IRRI farm.

There are *N. virescens* that can survive on and damage varieties of known resistance. Some populations can damage the variety TN1 (lacking resistance gene); others can attack Pankhari 203 (*Glh* 1 resistance gene), IR8 (*Glh* 3 resistance gene), and TAPL (*Glh* 6 resistance gene) (Heinrichs and Rapusas 1984, 1985).

Colonization and subsequent differentiation of insect pests to resistant crops pose problems for plant breeders, chemists, systematists and crop managers. The applied and theoretical aspects of the problem need urgent attention. Failure to recognize the evolution and differentiation of insect pests in nature can have far-reaching and frustrating consequences in pest management (Diehl and Bush 1984).

Studies on genetic differentiation in insect populations infesting rice host plants with known genes for resistance are fundamental in crop and pest management. They provide necessary tools for analyzing pest-cultivar relationships that in turn serve as in programs of breeding pest resistance varieties. Knowledge of the interrelationships between the rice host and pests is helpful to entomologists and plant breeders in combatting pests and at the same time maintaining genetic diversity in rice crop.

In the past, problems were encountered in determining and quantifying the existence of genetic variability of a given population. Traditional methods in genetics led to more questions than answers because traditional genetics only accounted for observable variant traits but not those traits which did not show any variability. However, with the advent of electrophoretic techniques and advances in molecular genetics, most of these problems have been avoided. With electrophoresis, the nature of the genes can be examined by studying the nature of gene-products. The principle follows the concept of the central dogma of molecular biology wherein;




Using electrophoresis, the genotypes of individuals and frequencies of homozygotes and heterozygotes can be determined. The assessment of genetic variation and variability makes use of allozymes (multiple molecular forms of the enzyme coded by alternative forms of a gene) (Lewontin 1974).

The technique has found increasing uses in studies of genetic variation of natural populations (Johnson *et al.*, 1966, Ayala and Powell 1972. Ayala *et al.*, 1972, Selander and Johnson 1973). Among phytophagous insects, genetic variation associated with host plants has been demonstrated within populations of several phytophagous insect species (Edmunds and Alstad 1978, Mitter and Futuyma 1979, Guttman *et al.*, 1981). Genetic differences in host utilization also have been documented in conspecific populations of a few species (Heslop-Harrison 1927, Painter 1951, Dethier 1954, Singer 1971, Knerer and Atwood 1973, Philipps and Barnes 1975, Hsiao 1978, Hsiao and Fraenkel 1968), although with a few exceptions (Hatchett and Gallun 1970, Gould 1979, Mitter *et al.*, 1979).

Using electrophoresis, we studied the genetic variations in *N. lugens* and *N. virescens* populations in the Philippines.

## MATERIALS AND METHODS

### *A. Starch Gel Electrophoresis*

Adults of *N. lugens* and *N. virescens* were collected from host plants and stored frozen at - 70°C. Homogenates were prepared by grinding individual insects in wellson a spot-plate with 15ul of homogenizing solution (0.0086M Tris-0.0046M Histidine buffer, pH 8) using a glass rod. Distilled water or 0.1% mercaptoethanol could also be used as a homogenizing solution. Whatman filter paper (No.#3) pieces (eby 9mm) were used to adsorb the homogenates and were inserted directly into the gel slot. Horizontal starch gel electrophoresis was conducted at 4°C and 40mA/ gel slab. The starch gel was prepared using 14% starch (SIGMA) and 0.0086M tris - 0.0046M histidine buffer, pH 8. After electrophoresis, the gels were sliced horizontally and stained following the procedures of Shaw and Prassad (1970) and Brewer (1970).

The genetic basis of biochemical variants was tested by single pair crosses from a sample of the two pest populations. However, in cases where this was not done, the genetic interpretation of the electrophoretic pattern was based on the principles described by Harris (1980), Harris and Hopkinson (1976), and also the published enzyme structure by Klotz (1967), Darnall and Klotz (1975) and Ward (1977).

Each gene locus was analyzed based on the number of genes sampled, the number of alleles, the number of bands in the heterozygotes, and whether the locus was polymorphic or monomorphic.

### *B. Statistical Analyses*

Heterozygosity at each locus was estimated by direct count of heterozygotes (HO) and also by calculating the frequency of heterozygotes expected (He) at

Hardy-Weinberg equilibrium ($He = 1-\Sigma pi^2$), where pi was the estimated frequency of the ith allele in the population).

The goodness-of-fit of observed genotypic proportions to expected proportions was tested by Chi-square ($X^2$) analysis performed for each of the polymorphic loci tested. When more than two alleles were detected at a locus, the genotypes were pooled into three classes (homozygotes for the commonest allele, heterozygotes for the commonest and another allele, and all other genotypes) to circumvent problems in the Chi-square test when expected frequencies of some classes of genotypes were low. Average deviations of genetic proportions from expected values were estimated using fixation index (Fis) (Wright, 1969). Heterogeneity of gene frequencies among host plants was tested using the method of Workman and Niswander (1970). The formula for computing the Chi-square statistics was as follows:

$$X^2 = \frac{\Sigma(2Ni)\ pi^2 - p\Sigma(2Ni)pi}{\bar{p}\bar{q}}$$

where p and q denote the weighted means of the alleles pi and qi (i.e.,$p = \Sigma((Ni/N)$ pi).

In general, if there are k alleles at a locus, the $X^2$ value for the corresponding r x k contigency table is given $X^2 = 2N$ ( oj/pj), where pj and oj denote the mean and variance of the frequencies of the jth allele. Thus the genic contingency $X^2$ is a function of the total sample size and the mean and variance of the gene frequencies.

Two methods were employed in partitioning the amount of genetic variation among populations of the two species. These were the Shannon information index, H (Lewontin 1972) and Nei's (1977) modification of Wright's F-statistics. Genetic similarity among insects collected from their different hosts was measured by the coefficient of genetic identity (Nei 1972), where I = Jxy/jxjy and the genetic distance $D = -\log_e$. The key to these two formulae is given as: xi = frequency of the ith allele in population X; yi = frequency of the ith allele in population Y; $jx = \Sigma xi^2$; $jy = \Sigma yi^2$; $jxy = \Sigma xiyi$; Jx, Jy, Jxy are arithmetic means of jx,jy and jxy; ly = normalized identity of genes with respect to one locus; I = normalized identity of genes with respect to all loci; D = genetic distance between X and Y.

## RESULTS

### I. Genetic Variation in *N. lugens*

Starch gel electrophoresis of 43 enzyme synthesizing *loci* of *N. lugens* revealed 26 active loci in which 11 were polymorphic, while the rest were monomorphic.

The genetic structures of *N. lugens* populations sampled from 10 localities in the Philippines, namely: Albay, Camarines Sur, Isabela, Laguna, Mindoro, Negros

Occidental, North Cotabato, Palawan, South Cotabato and Zamboanga del Sur were investigated. The determinations of allelic frequencies of 6 populations were concentrated on four polymorphic loci such as adenyl kinase (AK), alkaline phosphatase (ALKP), isocitric dehydrogenase (IDH) and malate dehydrogenase (MDH). The allele frequencies in the AK locus of *N. lugens* from the 3 localities of Isabela, Laguna and Zamboanga were not determined yet.

Comparison of the genetic structure of the 10 local populations of *N. lugens* in the Philippines revealed significant deviations from expected proportions at one or more sites in 2 of the 4 loci investigated (Tables 1 to 4). Significant deficiencies in heterozygotes were seen in ALKP for one population (Table 1) and in IDH for 3 populations (Table 2). Significant heterogeneity of gene frequencies among localities was found in all four loci (Tables 1 to 4 ). Partitioning the total variation within and between populations using Shannon information index, H and Nei's modification of Wright's F-statistics revealed that most of the variability occurred within populations (= 91%) (Table 5).

*N. lugens* from different rice hosts TN1, Mudgo, ASD7. amd *L. hexandra* were not well-differentiated; coefficient of genetic identity exceeded 0.99. However, when loci were analyzed individually, *N. lugens* from TN1, Mudgo, ASD7, and *L. hexandra* Swarta were significantly differentiated in five loci (Table 6). TN1 and Mudgo populations were differentiated in all five loci (Table 6). TN1 and Mudgo populations were differentiated in all five loci, TN1 and ASD7 populations in three loci, TN1 and *L. hexandra* populations in four loci, Mudgo and ASD7 populations in two loci, Mudgo and *L. hexandra* populations in four loci, ASD7 and *L. hexandra* populations in five loci (Table 7).

II. Genetic Variation in *N. virescens* Populations

Allozyme polymorphisms at 26 enzyme loci of *N. virescens* revealed that 14 out of the 26 possessed more than one allelic form. Four loci ( ALKP, EST-A, IDH, and MDH) were investigated in 7 local populations of *N. virescens* sampled from Camarines Sur, Isabela, Laguna (IRRI and Pila), North Cotabato, Quezon and Zamboanga del Sur. Significant deviations from Hardy-Weinberg equilibrium were observed in 3 loci (MDH, IDH, and EST-A). Significant deficiencies in heterozygotes were observed in MDH for 2 populations (IRRI and Quezon), in ALKP for 1 population (IRRI), and in esterase for 6 out of the 7 populations (Tables 8 to 11). Significant spatial heterogeneity of gene frequencies among 7 local populations of *N. virescens* was detected at three loci (Table 8 to 11). Partitioning the total variability observed within and between populations revealed that most of the variability was found within populations of *N. virescens* (Table 12).

Analysis of the gene pool of *N. virescens* from different host plants, such as TN1, P203, IR8, and TAPL were analyzed. Genetic differentiation was observed in 3 of the 5 loci (Table 13). Between TN1 vs. IR8 and P203 vs IR8, significant differentiation existed at three loci. TN1 vs P203, TN1 vs TAPL, and P203 vs TAPL were significantly differentiated at two loci, while IR8 vs TAPL differed in just one locus (Table 14).

Table 1. Allele frequencies for alkaline phosphatase (ALKP) locus in ten *N. lugens* populations

| *Locality* | *Alleles* | | | *Genes (No.)* | $X^{2a}$ | *Significance* |
|---|---|---|---|---|---|---|
| | *97* | *100* | *103* | | | |
| Albay | 0.024 | 0.976 | – | 42 | 0.020 | 0.886 |
| Isabela | – | 0.956 | 0.043 | 48 | 0.100 | 0.7521 |
| Laguna | – | 1.0 | – | 91 | 0 | |
| Mindoro | 0.007 | 0.964 | 0.029 | 70 | 0.094 | 0.759 |
| Negros Occ. | 0.050 | 0.928 | 0.022 | 88 | 2.856 | 0.091 |
| N. Cotabato | 0.033 | 0.967 | – | 30 | 0.030 | 0.861 |
| Palawan | 0.0085 | 0.983 | 0.0085 | 59 | 0.020 | 0.886 |
| S. Cotabato | 0.004 | 0.988 | 0.008 | 133 | 0.378 | 0.539 |
| Zamb. Sur | – | 1.0 | – | 92 | 0 | |

$X^{2b}_{p} = 47.841, < 0.001, df = 16$

[a]Test the goodness-of-fit of observed genotypic proportions to the proportion expected according to Hardy-Weinberg Law

[b]Tests the homogeneity of gene frequencies among *N. lugens* populations using the method of Workman and Niswander (1970)

Table 2. Allele frequencies in the adenyl kinase (AK) locus in seven *N. lugens* populations

| *Locality* | *Alleles* | | | | | *Genes (No.)* | $X^2$ | *Significance* |
|---|---|---|---|---|---|---|---|---|
| | *94* | *97* | *100* | *103* | *106* | | | |
| Albay | 0 | 0.361 | .633 | 0 | .006 | 93 | 1.99 | 0.158 |
| Cam. Sur | 0 | .033 | .889 | .078 | 0 | 45 | 0.71 | 0.399 |
| Mindoro | .020 | .259 | .721 | 0 | 0 | 120 | 0.509 | 0.024 |
| Negros Occ. | .020 | 0 | .976 | 0 | .004 | 128 | 0.081 | 0.776 |
| N. Cotabato | .008 | 0 | .988 | .004 | 0 | 129 | 0.021 | 0.884 |
| Palawan | 0 | 0 | 1.00 | 0 | 0 | 100 | 0 | |
| S. Cotabato | 0 | 0 | 1.00 | 0 | 0 | 34 | 0 | |
| | $X^{2b} = 391.36, P < .001, df = 24$ | | | | | | | |

[a]Tests the goodness-of-fit of observed genotypic proportions expected according to Hardy-Weinberg Law
[b]Tests the homogeneity of gene frequencies among populations based on the method of Workman and Niswander (1970)

Table 3. Allele frequencies for isocitric dehydrogenase (IDH) in ten *N. lugens* populations

| *Locality* | *Alleles* | | | | | | *Genes* | $X^{2a}$ | *Significance* |
|---|---|---|---|---|---|---|---|---|---|
| | *97* | *100* | *103* | *106* | *109* | *112* | | | |
| Albay | 0.050 | 0.851 | | | 0.099 | | 111 | 51.170 | $7.6 \times 10^{-11}$ |
| Cam. Sur | | 1.000 | | | | | 45 | 0 | |
| Isabela | 0.021 | 0.958 | 0.021 | | | | 24 | 0.02 | 0.887 |
| Laguna | 0.005 | 0.979 | 0.016 | | | | 92 | 0.024 | 0.878 |
| Mindoro | 0.021 | 0.913 | 0.008 | | 0.058 | | 120 | 0.117 | 0.733 |
| Neg. Occ. | 0.006 | 0.994 | | | | | 158 | 0.081 | 0.7753 |
| N. Cot. | 0.023 | 0.845 | 0.019 | 0.019 | 0.085 | 0.013 | 132 | 61.040 | $7.6 \times 10^{-11}$ |
| S. Cot. | 0.128 | 0.625 | 0.037 | 0.024 | 0.186 | – | 148 | 21.040 | $3.7 \times 10^{-6}$ |
| Palawan | 0.037 | 0.963 | | | | | 109 | 0.157 | 0.692 |
| Zamb. Sur | | 1.000 | | | | | 92 | 0 | |
| | | $X^{2b} = 386.655, P < 0.001, df = 45$ | | | | | | | |

[a]Tests the goodness-of-fit of observed genotypic proportions to the proportion expected according to Hardy-Weinberg Law
[b]Tests the homogeneity of gene frequencies among *N. lugens* populations using the method of Workman and Niswander (1970)

Table 4. Allele frequencies of malate dehydrogenase (MDH) in 10 local populations of *N. lugens*

| *Locality* | *Alleles* | | | *Genes* | $X^{2a}$ | *Significance* |
|---|---|---|---|---|---|---|
| | *97* | *100* | *103* | *(No.)* | | |
| Albay | 0.0335 | 0.895 | 0.0725 | 89 | 1.21 | 0.27 |
| Cam. Sur | 0 | 1.0 | 0 | 45 | 0 | |
| Negros Occ. | 0 | 1.0 | 0 | 158 | 0 | |
| Isabela | – | 1.0 | – | 96 | 0 | |
| Laguna | | 0.989 | 0.011 | 91 | 0.01 | 0.919 |
| Mindoro | 0.042 | 0.920 | 0.038 | 120 | 0.89 | 0.345 |
| N. Cotabato | 0.034 | 0.965 | – | 129 | | |
| Palawan | – | 1.0 | – | 109 | 0 | |
| S. Cotabato | 0.002 | 0.998 | – | 233 | 0.005 | 0.942 |
| Zamb. Sur | 0.006 | 0.961 | 0.033 | 92 | 0.14 | 0.707 |
| | $X^{2b} = 110.67$b, $P < 0.001$, $df = 18$ | | | | | |

[a]Tests the goodness-of-fit of observed genotypic proportions expected according to the Hardy-Weinberg Law
[b]Tests the homogeneity of gene frequencies among populations based on the method of Workman and Niswander (1970)

Table 5. Partitioning of genetic variability in a population of *N. lugens* using the Shannon information index, H (Lewontin, 1972), and Nei, (1977) modification of F statistics

| *Locus* | *Shannon Index* | | | *Nei Method* | | | |
|---|---|---|---|---|---|---|---|
| | *Hepop* | *Hgrp* | *% within groups* | *Ht* | *Hs* | *Dst* | *Gst* |
| ALKP | 0.138 | 0.120 | 98.20 | 0.052 | 0.051 | 0.001 | 0.019 |
| AK | 0.424 | 0.266 | 84.29 | 0.205 | 0.165 | 0.040 | 0.195 |
| IDH | 0.399 | 0.278 | 87.86 | 0.164 | 0.145 | 0.017 | 0.106 |
| HDH | 0.143 | 0.114 | 97.10 | 0.053 | 0.051 | 0.002 | 0.039 |
| Mean | 0.276 | 0.195 | 91.86 | 0.1185 | 0.103 | 0.015 | 0.089 |
| | | | | | | | % = 91.02 |

Table 6. Allele frequencies in five protein loci of *N. lugens* populations infesting different host plants

| *Locus* | *Population* | *Genes (No.)* | *Allele Frequencies* | | | $X^{2*}$ | *df* |
|---|---|---|---|---|---|---|---|
| | | | *1* | *2* | *3* | | |
| AK | TNI | 96 | 0.224 | 0.776 | – | 24.60b | 3 |
| | Mudgo | 77 | 0.072 | 0.928 | – | | |
| | ASD7 | 235 | 0.228 | 0.772 | – | | |
| | *L. hexandra*188 | | 0.147 | 0.853 | | | |
| ALKP | TNI | 258 | 0.160 | 0.838 | 0.002 | 75.04b | 3 |
| | Mudgo | 109 | 0.023 | 0.977 | 0 | | |
| | ASD7 | 249 | 0.024 | 0.002 | | | |
| | *L. hexandra* | 164 | 0.107 | 0.888 | 0.006 | | |
| IDH | TNI | 322 | 0.930 | .907 | 0 | 160.11b | 6 |
| | Mudgo | 101 | 0 | 1.000 | 0 | | |
| | ASD7 | 228 | 0.011 | 0.989 | 0 | | |
| | *L. hexandra* | 254 | 0.061 | 0.856 | 0.083 | | |
| MDH | TNI | 238 | 0.954 | 0.046 | – | 30.72b | 3 |
| | Mudgo | 189 | 0.990 | 0.010 | – | | |
| | ASD7 | 149 | 0.991 | 0.041 | – | | |
| | *L. hexandra* | 200 | 1.000 | 0 | – | | |
| PGI-2 | TNi | 138 | 0.018 | 0.583 | 0.398 | 195.04b | 6 |
| | Mudgo | 378 | 0.012 | 0.904 | 0.084 | | |
| | ASD7 | 102 | 0.005 | 0.868 | 0.128 | | |
| | *L. hexandra* | 333 | 0.047 | 0.668 | 0.285 | | |

*Test the homogeneity of gene frequencies among populations based on the method of Workman and Niswander (1970).

[a]Significant at 5.0% level.

[b]Significant at 0.1% level.

Table 7. Results of homogeneity tests* of gene frequencies between population of *N. lugens* infesting different host plants at five polymorphic loci, indicating level of significant heterogeneity

| | *ENZYME LOCI* | | | | |
|---|---|---|---|---|---|
| | *AK* | *MDH* | *PGI-2* | *ALKP* | *IDH* |
| TNI vs. Mudgo | 14.97b | 9.36b | 144.67b | 27.91b | 20.22b |
| df | (1) | (1) | (2) | (2) | (1) |
| TNI vs. ASD7 | 0.01* | 5.68a | 45.31b | 55.47b | 32.33b |
| df | (1) | (1) | (2) | (2) | (1) |
| TNI vs. *L. hexandra* | 5.27a | 18.87b | 14.29b | 5.53a | 57.97b |
| df | (1) | (1) | (2) | (2) | (2) |
| Mudgo vs. ASD7 | 18.38b | 0.018 | 4.31a | 0.44* | 2.24* |
| df | (1) | (1) | (2) | (2) | (1) |
| Mudgo vs. *L. hexandra* | 5.61a | 4.02a | 120.43b | 15.01b | 32.43b |
| df | (1) | (1) | (2) | (2) | (2) |
| ASD7 vs. *L. hexandra* | 8.83b | 3.61* | 31.66b | 26.36b | 58.56b |
| df | (1) | (1) | (2) | (2) | (2) |

a = significant at 5.0% level
b = significant at 0.1% level
* = based on comparison of allelic frequencies according to the method of Workman and Niswander (1970)

Table 8. Observed phenotypes and allelic frequencies in alkaline phosphatase locus in 7 populations of *N. virescens*

| *Population* | *Genes (No.)* | *Allele frequencies* | | | $X^{2a}$ | *Significance* |
|---|---|---|---|---|---|---|
| | | *1* | *2* | *3* | | |
| Cam. Sur | 56 | – | .982 | .018 | .02 | .887 |
| Lag. (IRRI) | 239 | .006 | .981 | .013 | 47.5 | <.0001 |
| Lag. (Pila) | 72 | .028 | .972 | – | .06 | .804 |
| Isabela | 179 | .006 | .994 | – | .019 | .890 |
| N. Cotabato | 135 | .011 | .985 | .004 | .03 | .862 |
| Quezon | 67 | .009 | .991 | – | .02 | .887 |
| Zambo. Sur | 143 | .013 | .981 | .006 | .05 | .822 |

$X^{2b} = 18.09, P > 0.1$ df = 12

[a]Tests the goodness-of-fit of observed genotypic proportions expected to the Hardy-Weinberg law

[b]Tests the homogeneity of gene frequencies among populations based on the method of Workman and Niswander (1970)

Table 9. Observed phenotypes and allelic frequencies in esterase locus in 7 population of *N. virescens*

| *Population* | *Genes (No.)* | *Allele frequencies* | | | $X^{2a}$ | *Significance* |
|---|---|---|---|---|---|---|
| | | *1* | *2* | *3* | | |
| Cam. Sur | 58 | .388 | .612 | – | 5.58 | .0182 |
| Isabela | 98 | .202 | .798 | – | 24.45 | < .0001 |
| Lag. (IRRI) | 176 | .102 | .483 | .415 | 61.34 | < .0001 |
| Lag. (Pila) | 45 | .300 | .700 | – | .46 | .5001 |
| N. Cotabato | 76 | .553 | .447 | – | 13.06 | .0003 |
| Quezon | 67 | .200 | .791 | .009 | 32.01 | < .0001 |
| Zamb. Sur | 12 | .151 | .817 | .032 | 38.10 | < .0001 |
| | $X^{2b} = 490.78, P < .001$ df = 12 | | | | | |

[a]Tests the goodness-of-fit of observed genotypic proportions expected to the Hardy-Weinberg law

[b]Tests the homogeneity of gene frequencies among populations based on the method of Workman and Niswander (1970)

Table 10. Observed phenotypes and allelic frequencies in isocitric dehydrogenase locus in 7 populations of *N. virescens*

| *Population* | *Genes (No.)* | *Allele frequencies* | | | $X^{2a}$ | *Significance* |
|---|---|---|---|---|---|---|
| | | *1* | *2* | *3* | | |
| Cam. Sur | 56 | – | .982 | .018 | .02 | .887 |
| Isabela | 191 | – | .987 | .013 | .032 | .858 |
| Lag. (IRRI) | 254 | .138 | .860 | .002 | 2.50 | .114 |
| Lag. (Pila) | 45 | – | .978 | .022 | .022 | .882 |
| N. Cotabato | 137 | .018 | .967 | .015 | 1.04 | .308 |
| Quezon | 67 | – | 1.000 | – | – | |
| Zamb. Sur | 142 | – | .997 | .003 | .02 | .879 |
| | | $X^{2b} = 173.47$, $P < 0.001$ df = 12 | | | | |

[a]Tests the goodness-of-fit of observed genotypic proportions expected to the Hardy-Weinberg law
[b]Tests the homogeneity of gene frequencies among populations based on the method of Workman and Niswander (1970)

Table 11. Observed phenotypes and allelic frequencies in malate dehydrogenase locus in 7 populations of *N. virescens*

| *Population* | *Genes* | *Allele frequencies* | | | $X^{2a}$ | *Significance* |
|---|---|---|---|---|---|---|
| | | *1* | *2* | *3* | | |
| Camarines Sur | 58 | – | 1.00 | – | – | – |
| Isabela | 167 | – | .953 | .047 | .126 | .723 |
| Laguna (IRRI) | 175 | – | .974 | .026 | 5.73 | .017 |
| Laguna (Pila) | 45 | – | .978 | .022 | .022 | .882 |
| North Cotabato | 137 | – | 1.00 | – | – | – |
| Quezon | 55 | .045 | .864 | .091 | 32.52 | < .0001 |
| Zamboanga Sur | 95 | – | 1.00 | – | – | – |
| | $X^{2b} = 102.82$, $P < .001$ df = 12 | | | | | |

[a]Tests the goodness-of-fit of observed genotypic proportions expected to the Hardy-Weinberg law
[b]Tests the homogeneity of gene frequencies among populations based on the method of Workman and Niswander (1970)

Table 12. Partitioning of genetic variability in a population of *N. virescens* using the Shannon information index, H (Lewontin, 1972), and Nei' (1977) modification of F statistics

| *Locus* | *Shannon Index* | | | *Nei Method* | | | |
|---|---|---|---|---|---|---|---|
| | *Hpop* | *Hgrp* | *% within groups* | *Ht* | *Hs* | *Dst* | *Gst* |
| ALKP | .093 | .086 | 99.3 | .0322 | .0319 | .0003 | .0093 |
| AK | .743 | .647 | 90.4 | .414 | .4810 | .0670 | .1390 |
| IDH | .162 | .124 | 96.2 | .064 | .0590 | .0050 | .0780 |
| MDH | .161 | .129 | 96.8 | .064 | .0609 | .0030 | .0470 |
| Mean | .289 | .247 | 95.68 | .144 | .158 | .019 | .0683 |
| | | | | | | | % = 93.2 |

Table 13. Allele frequencies in five protein loci of *N. virescens* populations

| *Locus* | *Population* | *Genes (No.)* | *Allele Frequencies* | | | $X^{2*}$ | *df* |
|---|---|---|---|---|---|---|---|
| | | | *1* | *2* | *3* | | |
| IDH | TNI | 254 | 0.138 | 0.860 | 0.002 | 100.07b | 3 |
| | P203 | 124 | 0.016 | 0.984 | | | |
| | IR8 | 132 | 0 | 1.0 | 0 | | |
| | TAPL | 124 | 0 | 1.0 | 0 | | |
| ALKP | TNI | 237 | 0.007 | 0.989 | 0.004 | 57.78b | 3 |
| | P203 | 124 | 0 | 1.0 | 0 | | |
| | IR8 | 198 | 0 | 0.940 | 0.060 | | |
| | TAPL | 124 | 0 | 1.0 | 0 | | |
| MDH | TNI | 175 | – | 0.994 | 0.006 | 4.57 | 3 |
| | P203 | 124 | – | 1.0 | 0 | | |
| | IR8 | 132 | – | 1.0 | 0 | | |
| | TAPL | 124 | – | 1.0 | 0 | | |
| EST-A | TNI | 176 | – | 0.396 | 0.604 | 97.10b | b |
| | P203 | 93 | – | 0.597 | 0.403 | | |
| | IR8 | 81 | – | 0.759 | 0.241 | | |
| | TAPL | 153 | – | 0.725 | 0.275 | | |
| AK | TNI | 187 | – | 0.006 | 0.994 | 3.61 | 3 |
| | P203 | 100 | – | – | 1.0 | | |
| | IR8 | 100 | – | – | 1.0 | | |
| | TAPL | 100 | – | – | 1.0 | | |

*Tests the homogeneity of gene frequencies among GLH populations based on the method of Workman and Niswander (1970)
a = significant at 5% level
b = significant at 0.1% level

Table 14. Results of homogeneity tests* of gene frequencies between GLH populations at five polymorphic loci, indicating level of significant heterogeneity.

| | *Enzyme loci* | | | | |
|---|---|---|---|---|---|
| | *IDH* | *MDH* | *EST-A* | *ALKP* | *AK* |
| TNI vs P203 | 28.66b | 1.49 | 19.76b | 2.75 | 1.21 |
| TNI vs IR8 | 40.71b | 1.59 | 58.50b | 26.25b | 1.21 |
| TNI vs TAPL | 38.723b | 1.49 | 1.56b | 2.75 | 1.21 |
| P203 vs IR8 | 4.26a | 0 | 10.32b | 15.45b | 0 |
| P203 vs TAPL | 4.00a | 0 | 8.66b | 0 | 0 |
| IR8 vs TAPL | 0 | 0 | 0.63 | 15.45b | 0 |

*Based on comparison of allelic frequencies according to the method of Workman and Niswander (1970)
a = significant at 5% level
b = significant at 0.1% level

## Discussion

Population of *N. lugens* and *N. virescens* possess diverse gene pools. Significant heterogeneity of gene frequencies among 10 local populations of *N. lugens* was detected at 2 loci, while in 7 populations of *N. virescens*, it occurred at 3 loci. For example, in *N. lugens*, the frequency of the most common allele in IDH ranged from as high as 1.0 to as low as 0.63 in South Cotabato, while at ALKP, the frequency of the most common allele ranged from 0.96 in Zamboanga del Sur to 0.99 in South Cotabato . On the other hand, in *N. virescens*, the frequency of the most common allele at EST-A ranged from as high as 0.82 in Zamboanga del Sur to as low as 0.45 in North Cotabato, while in ALKP, the most common allele ranged from 0.98 in Zamboanga del Sur to 0.99 in North Cotabato. The magnitudes of these genic differentiation among local populations of *N. lugens* and N. *virescens* were comparable to the host-specific populations of both species.

Despite distinct variations in gene frequencies in some polymorphic loci between populations of either *N. lugens* or *N. virescens*, the overall computed coefficients of genetic identity (Nei, 1972) of 0.99 among *N. lugens* and *N. virescens* from different localities and from their different hosts showed that these populations are closely related to one another. They are simply infraspecies. Early stages of differentiation may not be associated with substantial genetic change (Prakash *et al.*, 1969, Lewontin 1974, Bush 1975). The differences earlier observed in *N. lugens* host plant relationships such as differences in varietal reactions (Oka 1978, Seshu and Kaufmann 1980, IRRI 1982), host-mediated differential responses (Saxena and Pathak 1977), morphological differences (Saxena and Rueda 1983), cytological differences (Saxena and Barrion 1982, 1983a, b) and a certain degree of reproductive isolation (Saxena *et al.*, 1984) all substantiate the existence of genetic differences in the gene pool of the populations (this study). A combination of all these events may contribute to the accumulation of host-adapted gene complexes conducive to genetic events likely to be associated with the evolution of *N. lugens* and *N. virescens* virulent populations or biotypes. The genetic differences detected among *N. lugens* and *N. virescens* may be attributed to selection by the host plants, to population structure, or to a combination of both. Nevertheless, in each case, the subdivision of the populations detected is conducive to genetic events likely to be involved in speciation (Wright 1940, Wilson 1975, Bush *et al.*, 1977, White 1978). Infestations of resistant cultivars and other non-rice hosts such as *L. hexandra* can lead to possible founding populations by genotypes that may not be random samples of the original population. This is a kind of situation that may lead to rapid speciation of groups not specifically adapted to different environments (Templeton 1980a, b). Should these populations be structured along host lines, a host race or biotype may evolve preadapted for life in its new habitat (Sturgeon and Mitton 1982).

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# HEALTH SCIENCES

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# μ-CONOTOXINS: SPECIFIC BLOCKERS OF MUSCLE SODIUM CHANNEL

Lourdes J. Cruz and Gloria P. Corpuz
*Marine Science Institute, College of Science, U.P. Diliman*

## ABSTRACT

The voltage sensitive sodium channel is one of the key components of excitable membranes involved in the transmission of impulses in nerve and muscle. It is a medically important macromolecule not only because of its central physiological role but also because it is the target of a number of toxins including the paralytic shellfish poisons (PSP), puffer fish toxin (tetrodotoxin) and μ-conotoxins from the venomous marine snails, *Conus*. Although derived from very different biological sources, the three toxins have the same mechanism of action, they block the sodium channel and compete for binding at the same site on the protein.

μ-Conotoxins are the most potent peptide toxins known to block sodium channels; saxitoxin and tetrodotoxin are both complex polycyclic guanidinium compounds. Thus, given present technology, μ-conotoxins are much more amenable to chemical synthesis, chemical modification and radiolabeling than the two other toxins. In fact, μ-conotoxin GIIIA has recently been chemically synthesized and a biologically active, radioiodinated derivative prepared (Cruz *et al.* Biochemistry 28:3437, 1989; LeCheminant *et al.*, Trans. Nat. Acad. Sci. Tech. Phil., 10:423-430, 1989). The radiolabeled derivative binds specifically to a membrane protein with molecular weight in the size range of sodium channels and it competes with tetrodotoxin for binding to an elextroplax membrane preparation. The binding competition between tritiated derivative of μ-conotoxin and saxitoxin has similarly been demonstrated by Yanagawa *et al.* (J. Neurosci 7: 1498, 1987) This specific competition between saxitoxin and μ-conotoxins is now being developed as the basis for a sensitive biochemical assay to analyze paralytic shellfish poisons produced by the toxic dinoflagellates responsible for red tide.

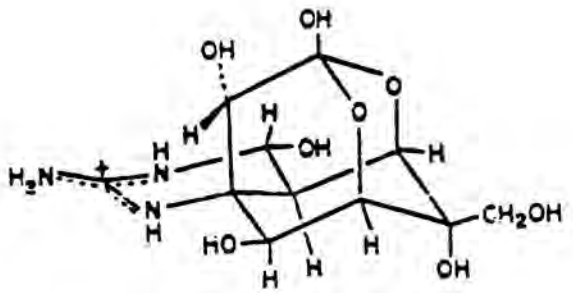
In contrast to saxitoxin and tetrodotoxin which block both nerve and muscle sodium channels to about the same extent, μ-conotoxins are highly specific for the muscle channel subtype. This unique specificity has made μ-conotoxins the choice pharmacological agents for applications in medicine and physiology requiring a quiescent muscle system while maintaining normal synaptic activity.

## Introduction

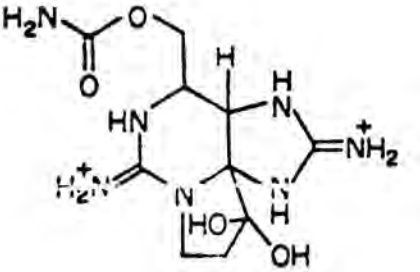
The voltage sensitive sodium channel is one of the key elements of excitable membranes. In most multicellular organisms, it plays a fundamental role in the generation and propagation of action potentials. This protein is a medically im-

portant macromolecule not only because of its central physiological role but also because it is the target of a number of lethal neurotoxins which either inhibit or enhance sodium ion transport (1,2,3). The most potent sodium channel blockers are the paralytic shellfish poisons (PSP) such as saxitoxin, the puffer fish toxin (tetrodotoxin) and $\mu$-conotoxins from the venomous marine snails, *Conus*. Although chemically different and derived from unrelated biological sources (Fig. 1), $\mu$-conotoxins are the only peptides (7); tetrodotoxin and the paralytic shellfish poisons, such as saxitoxin, are all polycyclic guanidinium compounds (8). Thus, given present technology, $\mu$-conotoxins are much more amenable to chemical synthesis, chemical modification and radiolabeling than the two other toxins (9).

The physiological activity and chemical properties of $\mu$-conotoxins make them very suitable for development as biochemical reagents for the detection and quantitation of PSP. The basis for such a method is the specific competition among the sodium channel blockers. However, in order to develop $\mu$-conotoxin GIIIA as a convenient reagent, relatively large amounts of the toxin must be available. To remedy the current shortage of pure toxin, we have devised a quicker method for the isolation of $\mu$-conotoxins. (Although $\mu$-conotoxin GIIIA has recently been chemically synthesized, the method for folding of the synthetic peptide to give the active conformer needs improvement. Moreover, facilities for peptide synthesis are not yet available in the Philippines.) Radioiodinated $\mu$-conotoxin GIIIA with high specific activity has also been prepared recently. This paper describes our preliminary binding studies and cross-linking experiments to ascertain specificity of radiolabeled toxin derivatives for the voltage sensitive sodium channel in preparation for their development as tools for the quantitation of PSP levels in food and biological samples.




A. Tetrodotoxin




B. Saxitoxin

Arg.Asp.Cys.Cys.Thr.Hyp.Hyp.Lys.Lys.Cys.Lys.Asp.Arg.Gln.Cys.Lys.Hyp.Gln.Arg.Cys.Cys.Ala*

C. $\mu$-Conotoxin GIIIA

Figure 1. Structures of potent blockers of voltage sensitive sodium channels. Saxitoxin is one of the paralytic shellfish poisons which are closely related guanidinium compounds. The asterisk at the end of the peptide indicates an amidated carboxyl end.

## Materials and Methods

μ-Conotoxin GIIIA was isolated from *Conus geographus* according to the method of Cruz *et al.* (7) or as described below. Radiolabeled μ-conotoxin GIIIA, $^{125}$I-3[4-hydroxyphenyl] propionyl GIIIA ($^{125}$I-HPP-GIIIA, 0.44 mCi/nmole) was prepared as described previously (9). The membrane preparation from the electric organ of *Electrophorus electricus*, a rich source of muscle type sodium channels, was generously provided by Dr. Edward Moczydlowski of Yale University, Connecticut, U.S.A. All biochemicals were from Sigma Chemical Corporation, St. Louis, Missouri.

*Modified method for the preparation of μ-conotoxins.* Lyophilized crude venom from *Conus geographus* was extracted several times with 0.1 M ammonium acetate, pH 6.8 and then several times with 1.1% acetic acid by repeated suspension in the solvents, sonication and centrifugation at 10,000 rpm using a Sorvall SS-34 rotor. Ammonium acetate extracts were pooled and used directly for high pressure liquid chromatography but pooled HAc extracts were lyophilized and resuspended in the minimum amount of 0.1M ammonium acetate, pH 6.8 prior to chromatography. The isolation procedure involves fractionation through a molecular sieve followed by a series of runs through C18 reverse phase columns. As a second step, 5-ml aliquots of the extracts were chromatographed through a preparative BioSil TSK-125 HPLC column (Biorad) eluted with 0.1M ammonium acetate, pH 6.8. Fractions were bioassayed by intraperitoneal injection to 10-gram mice (Swiss Webster strain) and peaks which caused paralysis and death were further fractionated using a semi-preparative Vydac C18 reverse phase HPLC column eluted with a 1.2%/min gradient of acetonitrile in 0.1% trifluoroacetic acid (TFA). Pure toxin was obtained by rechromatography of active peaks on an analytical Vydac C18 reverse phase HPLC column eluted with shallower gradients (0.06 to 0.3%/min) of acetonitrile in 0.1% TFA.

*Binding assay.* Assay for the binding of μ-conotoxin to the receptor site on the sodium channel was done as developed previously (6) using electroplax membrane preparation from *E. electricus.* The reaction mixture in a total volume of 200 μL contained 0.32M sucrose, 5mM HEPES/Tris buffer, pH 7.4, 45 mM KCl and about 50 μg protein of membrane preparation. Pre-incubation of control samples with unlabeled toxin (GIIIA or tetrodotoxin) and other unlabeled ligands was done for 30 minutes on ice. The final concentration of unlabeled toxins and ligands was 5 μM, except for lysozyme which was 0.2 mg/mL. Radiolabeled toxin was then added to all tubes and reaction mixtures were incubated for 30 minutes at room temperature. Samples were filtered through Whatman GF/C under vacuum and washed three times with 2.0 mL of wash medium containing 16 mM choline chloride, 15 mM $CaCl_2$, 5 mH HEPES/Tris, pH 7.4 and 0.2 mg/mL BSA. A Packard Multi-Prias gamma counter was used to determine radioactivity of filters.

*Cross-Linking of radiolabeled GIIIA to sodium channels (9).* A reaction mixture containing about 150 μg of membrane protein and 0.2 pmol of radioiodinated GIIIA in 200 μL of 20 mM NaHEPES, pH 7.5 was incubated for 30 minutes at room

temperature. To determine nonspecific binding, the same amount of membrane protein in buffer was preincubated with 0.1 nmol of unlabeled native GIIIA for 30 minutes at 0°C before radioiodinated GIIIA was added. Toxin receptor complexes were cross-linked according to the procedure of Pilch *et al.* (10) then analyzed by SDS-PAGE electrophoresis by the method of Laemmli (11) using 4-15% gradient gels. Pellets were dissolved with PAGE sample buffer containing 80 mM DTT just before electrophoresis.

## Results

Until recently, the method used for isolating μ-conotoxins from extracts of *C. geographus* venom consisted of molecular sieving through a very long column of Sephadex G-25 followed by a series of HPLC runs through reverse phase columns (7). For the first step alone, it took a week to standardize the column and chromatograph the venom extract. We therefore devised a quicker way for purifying

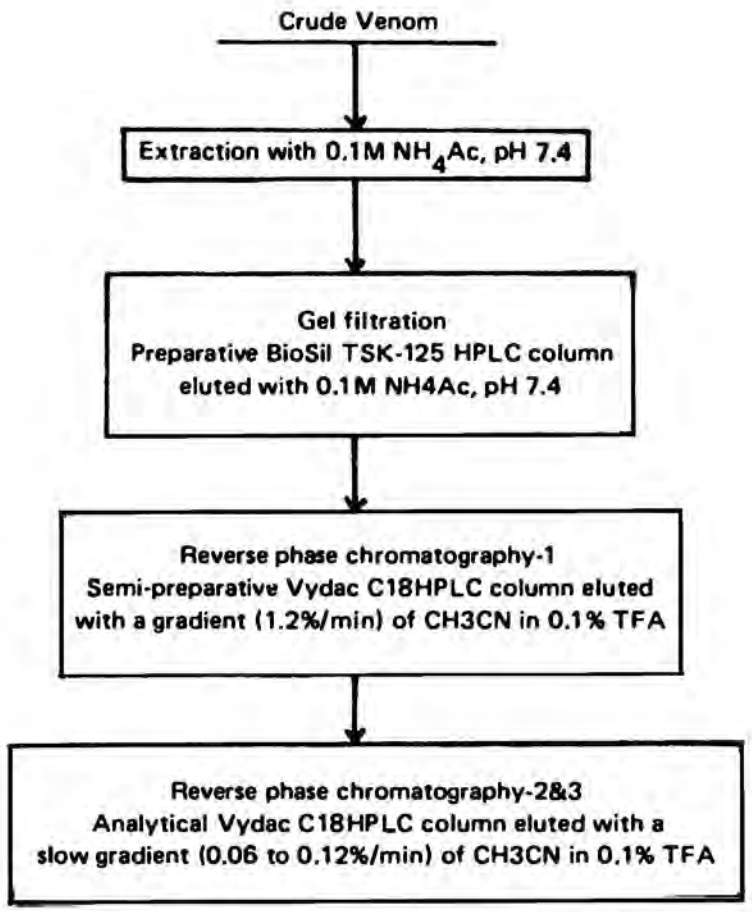



Figure 2. Schematic diagram of revised method for isolation of μ-conotoxins from *C. geographus* venom.

μ-conotoxins as shown in Fig. 2. By substituting an HPLC run for the regular gel filtration chromatograph in the second step, molecular sieving of crude venom extracts can be accomplished in a couple of hours. Subsequent steps involving reverse phase HPLC columns were also modified; a quick subfractionation on a semi-preparative C18 column using a fast gradient (1.2%/min) of acetonitrile in 0.1% TFA is followed by one or two purification steps on analytical C18 column eluted with shallower gradients (0.06 to 0.3%/min) of acetonitrile in 0.1% TFA. This method has now been adapted as a general strategy for the isolation of not only μ-conotoxins but also of other biologically active compounds from *Conus* venom. With the availability of more μ-conotoxins through a faster purification method, supply of the toxin will no longer be limiting.

For binding and immunological assays, radioiodinated reagents have been preferred because of the very high specific activities one can obtain with $^{125}I$ and the ease in determining radioactivity of gamma-emitters. Scintillation cocktails are not necessary and quenching is not a problem. However, since μ-conotoxins do not have tyrosine or histidine residues which can be radioiodinated the Bolton-Hunter reagent has been used to prepare 3-[4-hydroxyphenyl] propionyl derivatives of GIIIA. Five derivatives were found to be biologically active but as a first attempt, the most abundant one was iodinated to give $^{125}I$-HPP-GIIIA (9). This derivative was used for binding and cross-linking studies. To biochemically characterize the receptor, radiolabeled μ-conotoxin GIIIA was bound to membrane preparations and cross-linked to the receptor using a bivalent crosslinker, disuccinimidylsuberate. As shown in Figure 3, electrophoresis of cross-linked material under denaturing conditions indicated a specifically labeled band with a relative molecular weight greater than 200,000 which is the expected result if cross-linking of radiolabeled GIIIA were occurring at the sodium channel.

Table 1 shows the effect of various unlabelled ligands on the binding of radioiodinated μ-conotoxin GIIIA. It is clear that the two sodium channel blockers, GIIIA and tetrodotoxin completely inhibit binding of GIIIA to the receptor or ion channel. On the other hand, the other positively charged conotoxins which have different physiological action do not significantly affect the binding of labeled GIIIA; lysozyme, a positively charged protein, similarly does not significantly affect the binding of μ-conotoxin. These data indicate that radioiodinated μ-conotoxin GIIIA binds specifically to the guanidinium site on the sodium channel which is not bound by other positively charged peptides or protein. Although we did not test competition of GIIIA with saxitoxin (due to a very limited supply of standard saxitoxin), the binding competion between tritiated derivative of μ-conotoxin and saxitoxin has recently been demonstrated by Yanagawa *et al.* (5). The above findings indicate the feasibility of using μ-conotoxin GIIIA as a research tool for the guanidinium binding site on the sodium channel and as a biochemical reagent for the quantitation of PSP levels in food and biological samples.

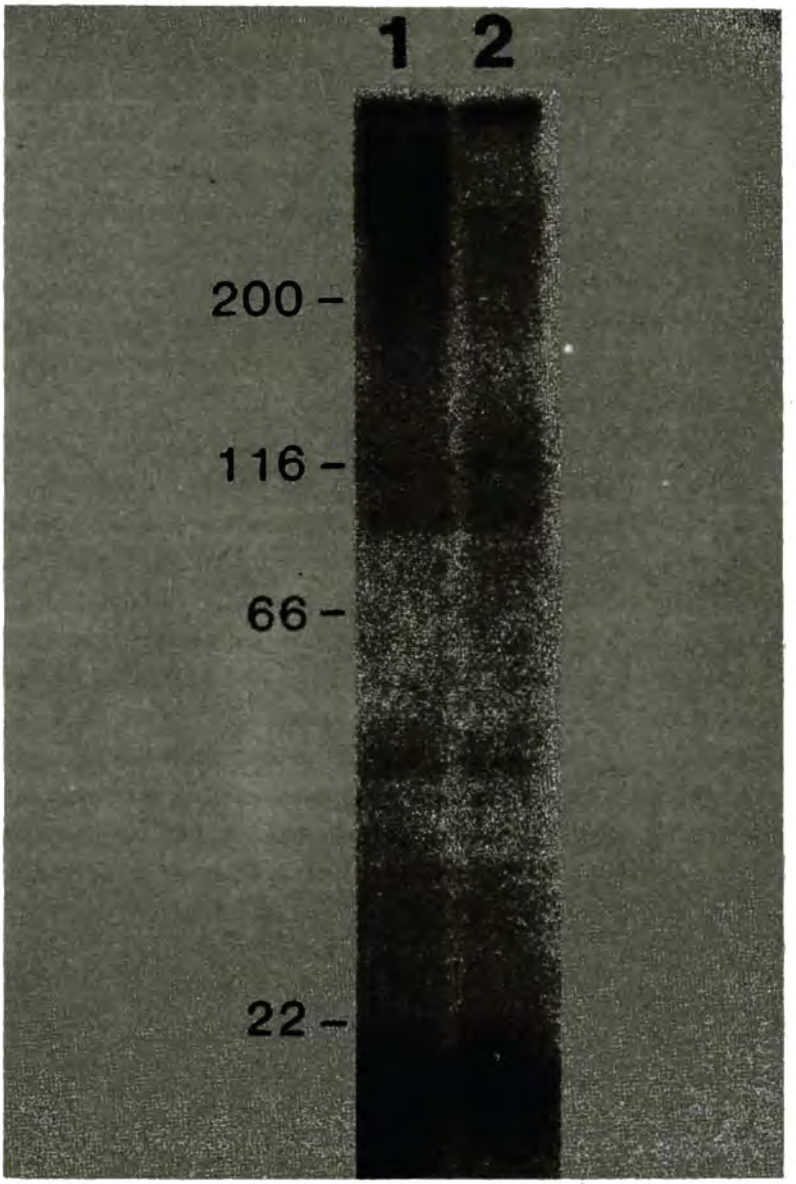



Figure 3. Cross-linking of radiolabeled μ-conotoxin GIIIA to membrane preparation from eel electroplax. Lane 1: Membrane incubated with $^{125}$I-3[4-hydroxyphenyl] propionyl GIIIA. Lane 2: Membrane preincubated with unlabeled GIIIA before addition of labeled toxin.

Table 1. Competition between radiolabeled μ-conotoxin GIIIA and various unlabeled ligands

| *Unlabeled Ligand* | *% Binding of 125I-HPP GIIIA* | *Target* |
|---|---|---|
| None | 100 | |
| μ-Conotoxin GIIIA | 0 | Voltage sensitive sodium channel |
| Tetrodotoxin | 0 | Voltage sensitive sodium channel |
| ω-Conotoxin GVIA | 96 | Voltage sensitive calcium channel |
| α-Conotoxin MI | 80 | Acetylcholine receptor |
| α-Conotoxin GI | 92 | Acetylcholine receptor |
| Lysozyme | 83 | Bacterial cell wall |

## Discussion

So far, the following have already been accomplished toward the development of μ-conotoxin GIIA as an analytical tool for saxitoxin and other paralytic shellfish poisons: 1. Alternative methods for the preparation of pure toxin have been established, one a quicker way for the isolation of μ-conotoxins from crude *C. geographus* venom and another, the chemical synthesis of μ-conotoxin GIIIA 2. A biologically active radiolabeled derivative of μ-conotoxin GIIIA has been prepared by first making a 3[4-hydroxyphenyl] propionyl derivative using the Bolton-Hunter reagent and then labeling one of the derivatives with iodine-125; 3. A radioiodinated derivative of μ-conotoxin GIIIA has been demonstrated to compete specifically with the sodium channel blocker, tetrodotoxin. Tritiated GIIIA has been demonstrated by Yanagawa *et al* (5) to also compete with saxitoxin To pursue the development of this promising reagent we plan to radioiodinate the four other biologically active hydroxyphenyl-propionyl derivatives of GIIIA and compare their binding characteristics. Relative affinities of the labeled derivatives for the ion channel/receptor complex will be determined. The one with the highest affinity will be chosen for standardizing binding curves of radiolabeled. GIIIA and its displacement from the receptor by saxitoxin. Conditions to maximize sensitivity and to ensure linearity of the assay will be studied. The assay based on the competition between guanidinium toxins and μ-conotoxins promises to be a very sensitive and specific one for PSP.

μ-Conotoxin, saxitoxin and tetrodotoxin differ in their tissue specificities. The guanidinium toxins have high affinities for skeletal muscle and nerve sodium channels but low affinities for heart type sodium channels. On the other hand, μ-conotoxin GIIIA is very active only on skeletal muscle type; it is at least 1000-fold less active on nerve versus muscle sodium channels and it also has a low affinity for cardiac muscle sodium channels (7,9). This unique specificity has made μ-conotoxins the choice pharmacological agents for applications in medicine and physiology requiring a quiescent muscle system while maintaining normal synaptic activity.

## Acknowledgments

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# INHIBITORY EFFECTS OF SOME AMINO ACIDS ON SOMATIC AND GERM CELL GENOTOXICITY OF SOME ANTICANCER AGENTS

Clara Y. Lim-Sylianco and A.P. Guevarra
*Institute of Chemistry, College of Science*
*University of the Philippines*
*Diliman, Q. C.*

**ABSTRACT**

Adriamycin, basulfan, chlorambucil, cyclophosphamide and mitemycin C are anticancer agents which are very reactive with DNA of cancer cells However, these anticancer agents also react with the DNA of normal cells. Therefore, they are gentoxic to both cancer and normal cells.

These anticancer agents induce the formation of micronucleated polychromatic erythrocytes in bone marrow cells of experimental mice; hence, they are somatic cell genotoxins. Their genotoxicity to germ cells were observed when they reduced the fertility index, and increased the percentage dead implants and percentage of females with resorptions.

Cysteine, asparatic acid, glutamic acid, arginine, histidine and lysine reduced the formation of micronucleated polychromatic erythrocytes induced by these anticancer agents. These amino acids increased the fertility index, reduced the % dead implants and % of females with resorptions.

Therefore, these amino acids inhibit both the somatic and germ cell genotoxicity of adriamycin, busulfan, chlorambucil, cyclophosphamide and mitomycin C.

## Introduction

Adriamycin is an antitumor antibiotic that interacts with DNA through a free radical mechanism (Myers *et al.* 1976). Busulfan is an anticancer drug used for the treatment of granulocytic leukemia (Haut and Abbott, 1961). Chlorambucil is used for chronic lymphocytic leukemia (Sadler *et al.* 1976) while cyclophosphamide is used for almost every kind of solid tumor (Brookes and Lawley, 1961). Mitomycin C is an anticancer antibiotic which is a bifunctional alkylating agent of DNA (Waring, 1981: Iyer and Sybalski, 1964).

Antimutagenic activities of amino acids have been reported. Histidine inhibited the mutagenic activity of dimethylnitrosamine (Torralba and Sylianco, 1987). Cysteine reduced the genotoxicity of mexaform, an antidiarrheal agent (Flores and Sylianco, 1984). Gebhart (1974) observed a reduction of the mutagenic

activity of nitrogen mustard by cysteine. Clarke and Shankol (1975) reported the antigenotoxic effects of methionine against *Saccharomyces pombe*.

In this study, antigenotoxic effects of cysteine, aspartic acid, glutamic acid, arginine, histidine and lysine against adriamycin, busulfan, chlorambucil cyclophosphamide and mitomycin C are reported.

## Experimental Methods

Somatic cell genotoxicity of adriamycin, busulfan, chlorambucil, cyclophosphamide, and mitomycin C was studied using the micronucleus test (Schmid, 1975). Each anticancer agent was administered orally by gavage 30 hours and 6 hours before the mouse was sacrificed. The animal was killed by cervical dislocation and the femur removed. The bone marrow cells from the femur were flushed out using fetal calf sorum. The cell suspension was centrifugal and the supernatant was discarded. Slides were prepared of the cells. After staining, the number of micronucleated polychromatic crythrocytes were counted under the microscope.

The effects of the amino acids were also studied using the micronucleus test. The amino acid solution was administered by gavage right after the anticancer agent. The reduction n the formation of micronucleated polychromatic crythrocytes was used as a measure of the inhibitory effect of the amino acid.

Germ cell genotoxicity of the anticancer agents was studied using the dominant lethal test (Generoso, 1980). The anticancer agent was administered orally by gavage to a male mouse and which was mated with two virgin females after six days. The first day of pregnancy was marked by the appearance of vaginal plugs. On the 18th day of pregnancy, the females were sacrificed and the uterus removed very carefully. Dead implants, live implants and resorption sites were recorded. The effects of the amino acids were also monitored using the dominant lethal test by administering the amino acid to the male mouse right after the anticancer agent was given.

## Results and Discussion

Table I shows the formation of micronucleated polychromatic crythrocytes by adriamycin and the reduction by cysteine, aspartic acid, glutamic acid, arginine histidine and lysine. The structure of adriamycin is shown as follows:

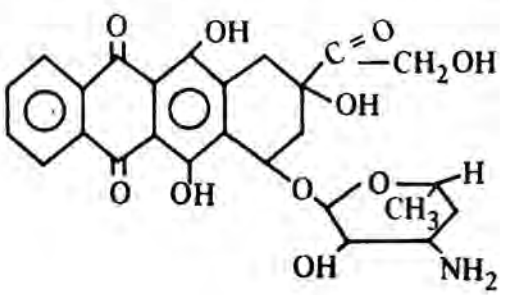


Table 1. Inhibitory effects of some amino acids on the formation of micronucleated polychromatic erthrocytes induced by adriamycin

| | *No. of micronucleated polychromatic eryhrocytes per thousand* |
|---|---|
| Negative control, distilled water | 2.11 ± 0.15 |
| Adriamycin alone (0.5 mg/kg) | 12.03 ± 1.32 |
| plus cysteine * | 1.14 ± 0.38 |
| aspartic acid | 2.22 ± 0.28 |
| glutamic acid | 2.76 ± 0.45 |
| arginine | 3.12 ± 0.52 |
| histidine | 3.98 ± 0.11 |
| lysine | 3.46 ± 0.23 |

* each amino acid was administered in a dose 50 mg/kg

The best reduction was given by cysteine, an established free radical scavenger. The effect of adriamycin on the DNA of cancer cells has been attributed to the formation of free radicals (Myers *et al.*, 1976). The basic, as well as the acidic, amino acids can bind adriamycin through hydrogen bonding interactions which could inhibit the metabolism of adriamycin to genotoxic free radicals.

Table 2 shows the inhibitory effects of some amino acids on the genotoxicity of busulfan to bone marrow cells. The structure of busulfan is given:

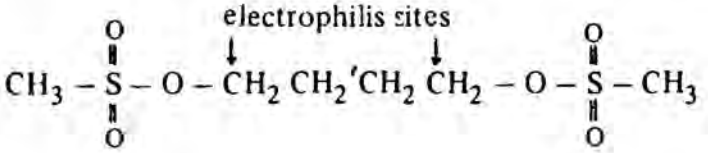



Table 2. Inhibitory effects of some amino acids on the formation of micronucleated polychromatic erythrocytes induced by busulfan

| | *No. of micronucleated polychromatic eryhrocytes per thousand* |
|---|---|
| Negative control, distilled water | 1.45 ± 0.26 |
| Busulfan alone, 5 mg/kg | 16.89 ± 2.06 |
| plus cysteine * | 2.09 ± 0.11 |
| aspartic acid | 2.38 ± 0.09 |
| glutamic acid | 2.13 ± 0.12 |
| arginine | 1.28 ± 0.54 |
| histidine | 3.28 ± 0.32 |
| lysine | 2.12 ± 0.07 |

* 50 mg/kg – dose of amino acids

Cysteine, aspartate, and glutamate can interact with its electrophilic sites because of their nucleophilic groups. The basic amino acids can bind the oxygens of busulfan through ionic interactions. These interactions can inhibit the reactivity of busulfan with bases of DNA.

Busulfan is metabolized to a bifunctional alkylating agent of DNA. The alkylating ability could be responsible for the fragmentation of chromatin material resulting in the formation of micronucleated polychromatic erythrocytes. After telophase when the nucleus is expelled, some fragments will be left behind forming micronuclei in the cytoplasm of the cells.

The effects of the amino acids on the genotoxicity of chlorambucil to bone marrow erythrocytes are shown in Table 3. The effects of the basic amino acids on the reduction of the formation of micronucleated polychromatic erythrocytes are more pronounced than the effects of acidic amino acids and cysteine. The structure of chlorambucil is shown:

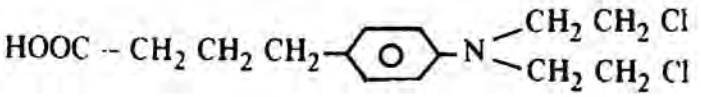


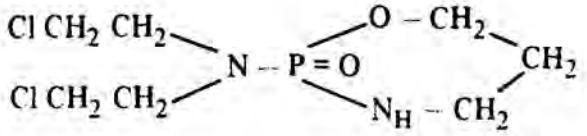

Table 3. Inhibitory effects of some amino acids on the formation of micronucleated polychromatic erythrocytes per thousand as induced by chlorambucil

| | *No. of micronucleated polychromatic eryhrocytes per thousand* |
|---|---|
| Negative control, distilled water | 1.87 ± 0.14 |
| Chlorambucil alone, 1 mg/kg | 10.87 ± 1.23 |
| plus cysteine * | 3.28 ± 0.21 |
| aspartic acid | 3.21 ± 0.43 |
| glutamic acid | 3.24 ± 0.67 |
| arginine | 1.09 ± 0.08 |
| histidine | 2.02 ± 0.11 |
| lysine | 1.23 ± 0.31 |

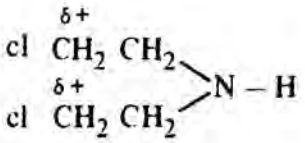
* dose of amino acids – 50 mg/kg

When the carboxyl group is ionized, it can effectively form ionic bonds with the positive groups of the basic amino acids, such as the guanidyl group of arginine, the opsilon amino group of lysine and the protonated imidazole ring of histidine. Cysteine, asparatate and glutamate can give nucleophilic groups that can interact at electrophilic sites of chlorambucil.

The structure of cyclophosphamide is shown as follows:



It is metabolized to yield:



which is a bifunctional alkylating agent. Cysteine, aspartate, and glutamate have nucleophilic sites which can interact with the electropholic sites of cyclophosphamide or its active metabolite. The basic amino acids can form hydrogen bonds with the unmetabolized or with the metabolized product. The reduction of the formation of micronucleated polychromatic crhtyrocytes as shown by cysteine, aspartate, glutamate, arginine, histidine and lysine are shown in Table 4.

Mitomycin C is an anticancer antibiotic that is metabolized to a bifunctional alkylating agent of DNA. Its structure is shown:

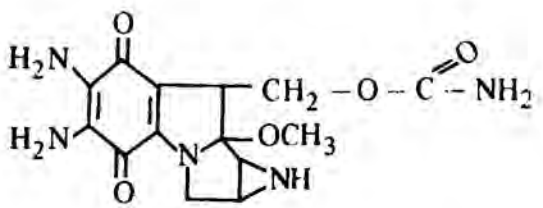


Table 4. Inhibitory effects of some amino acids on the formation of micronucleated polychromatic erythrocytes induced by cyclephosphamde

| | *No. of micronucleated polychromatic erythrocytes per thousand* |
|---|---|
| Distilled water, negative control | 1.45 ± 0;09 |
| Cyclophosphamide alone, 8 mg/kg | 8.79 ± 0.12 |
| plus cysteine * | 1.21 ± 0.09 |
| aspartic acid | 1.31 ± 0.08 |
| glutamic acid | 1.28 ± 0.11 |
| arginine | 2.14 ± 0.32 |
| histidine | 2.64 ± 0.12 |
| lysine | 2.28 ± 0.08 |

* dose of each amino acid – 50 mg/kg

It gives a carbocationic center upon metabolism, which will be trapped by amino acids that can give nucleophilic centers like cysteine, aspartate and glutamate. Other types of interaction like hydrogen bonding can account for the effects of the basic amino acids which can inhibit the metabolism of mitomycin C to an active alkylating agent.

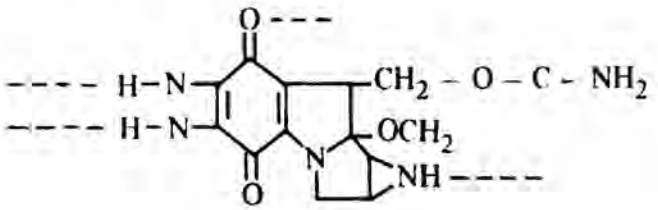


Table 5. Inhibitory effects of some amino acids on the formation of micronucleated polychromatic erythrocytes induced by mitomycin C

| | *No. of micronucleated polychromatic erythrocytes per thousand* |
|---|---|
| Negative control, distilled water | 1.09 ± 0.05 |
| Mitomycin alone, 5 mg/kg | 9.88 ± 0.96 |
| plus cysteine * | 1.02 ± 0.67 |
| aspartic acid | 1.21 ± 0.27 |
| glutamic acid | 1.11 ± 0.38 |
| arginine | 2.11 ± 0.34 |
| histidine | 2.44 ± 0.45 |
| lysine | 2.33 ± 0.12 |

* dose of each amino acid – 50 mg/kg

The anticancer agents studied induce low fertility index, high percentage of dead implants and high percentage of females with resorptions. The fertility index is enhanced while % dead implants and % females with resorptions are reduced. Cysteine, aspartate, glutamate, arginine, histidine, and lysine are administered with the anticancer agent (Tables 6, 7, 8, 9, and 10). This suggests that the amino acids reduce the reactivity of the anticancer agents with the DNA of the germ cells. The amino acids inhibited the genotoxicity of the anti cancer agents not only on the bone marrow erythrocytes but also on germ cells.

Table 6. Inhibitory effects of some amino acids on germ cell genetoxicity of adriamycine

| | *FI* % | *DI* % | *FR* % |
|---|---|---|---|
| Control | 94 | 7 | 10 |
| Adriamycin alone * | 40 | 56 | 81 |
| plus cysteine ** | 94 | 9 | 25 |
| aspartic acid | 91 | 10 | 32 |
| glutamic acid | 89 | 8 | 22 |
| arginine | 78 | 23 | 26 |
| histidine | 81 | 23 | 31 |
| lysine | 76 | 19 | 26 |

FI – fertility index
DI – dead implants
FR – females with resorptions
* 1 mg/kg
** 50 mg/kg

Table 7. Inhibitory effects of some amino acids on germ cell genotoxicity of busulfan

| | *FI* % | *DI* % | *FR* % |
|---|---|---|---|
| Control | 96 | 8 | 8 |
| Busulfan alone * | 38 | 34 | 79 |
| plus cysteine ** | 94 | 11 | 23 |
| aspartic acid | 91 | 17 | 32 |
| glutamic acid | 92 | 15 | 29 |
| arginine | 87 | 23 | 34 |
| histidine | 89 | 21 | 29 |
| lysine | 91 | 19 | 32 |

* 5 mg/kg
** 50 mg/kg
FI – fertility index
DI - dead implants
FR – females with resorptions

Table 8. Inhibitory effects of some amino acids on germ cell genotoxicity of chlorambucil

| | *FI* % | *DI* % | *FR* % |
|---|---|---|---|
| Control | 96 | 3 | 9 |
| Chlorambucil alone * | 32 | 56 | 84 |
| plus cysteine ** | 78 | 21 | 43 |
| aspartic acid | 82 | 26 | 39 |
| glutamic acid | 79 | 31 | 42 |
| arginine | 92 | 11 | 18 |
| histidine | 89 | 15 | 21 |
| lysine | 91 | 12 | 15 |

* 2 mg/kg
** 50 mg/kg
FI – fertility index
DI – dead implants
FR females with resorptions

Table 9. Inhibitory of some amino acids on germ cell genotoxicity of cyclophosphamide

| | *FI* % | *DI* % | *FR* % |
|---|---|---|---|
| Control | 92 | 4 | 7 |
| Cyclophosphamide * | 42 | 32 | 78 |
| plus cysteine * | 91 | 8 | 10 |
| aspartic | 89 | 11 | 15 |
| glutamic | 92 | 6 | 11 |
| arginine | 79 | 23 | 24 |
| histidine | 76 | 19 | 27 |
| lysine | 79 | 11 | 12 |

* 8 mg/kg
** 50 mg/kg
FI – fertility index
DI – dead implants
FR – females with resorptions

Table 10. Inhibitory effects of some amino acids on germ cell genotoxicity of mitomycin C

| | *FI* % | *DI* % | *FR* % |
|---|---|---|---|
| Control | 97 | 5 | 7 |
| Mitomycinalone* | 54 | 43 | 38 |
| plus cysteine ** | 94 | 8 | 11 |
| aspartic | 93 | 11 | 15 |
| glutamic | 92 | 9 | 10 |
| arginine | 87 | 21 | 17 |
| histidine | 84 | 27 | 20 |
| lysine | 88 | 25 | 21 |

* 5 mg/kg
** 50 mg/kg
FI – fertility index
DI – dead implants
FR – females with resorptions

## Conclusion

Cysteine, aspartate, glutamate, arginine, histidine and lysine inhibited the genotoxicity to bone marrow erythrocytes and to germ cells as induced by adriamycin, busulfan, chlorambucil, cyclophosphamide and mitimycin C.

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# EPIDEMICS OF PARALYTIC SHELLFISH POISONING IN THE PHILIPPINES, 1988-1989 LESSONS IN SCIENCE AND PUBLIC POLICY

Manuel M. Dayrit, M.D., MSc.
Nino Ismael S. Pastor, M.D.
Ivy L. Gopez, M.D.
Maria Consorcia Quizon, M.D.
Nicolas B. Bautista, Jr. M.D.
Felilia M. White RN, BSN
Vivian S. Lofranco, M.D.
Rosario Marilyn S. Benabaye, M.D.
Mark E. White, M.D., FACPM

## Introduction

Red tide is a generic term used to describe any abnormal discoloration of sea water due to the active proliferation of dinoflagellates which are unicellular photosynthetic algae. Halstead commented that the earliest reference is found in the Bible in Exodus 7: ". . . when all the water that were in the river were turned to blood. . . ." (1). This ancient reference dates back to 1491 BC during Moses's lifetime. Red tides have been reported in many continents including North America, Europe, Africa, Australia, and Asia within the last 2 centuries (2). This environmental phenomenon is correlated with the human illness called paralytic shellfish poisoning or PSP.

The first known human case of PSP occurred in 1689 in a 20-year-old girl (1). The earliest epidemic of PSP was documented in 1793 among the expeditionary crew of Captain George Vancouver after a meal of mussels obtained off the coast of British Columbia, Canada. The medical literature has abundant references to outbreaks of mussel poisoning in temperate and tropical countries.

In the Philippines, there were anecdotal reports of a PSP outbreak in the Sulu archipelago between 1976 and 1977 during a red tide affecting Borneo particularly Sabah and Brunei, but there were no formal scientific studies (Dr. F. Valeza, Department of Health, personal communication). The first documented Philippine PSP epidemic occurred in Catbalogan, Western Samar. It began on June 20, 1983 and lasted until the 3rd week of February 1984. There were 691 cases with 14 deaths (3).

Other PSP reports included:

* in late July 1983, in Bulan, Sorsogon: 48 cases, 1 death (4),
* in August 1983, in Mati, Davao City: 1 case (5),

* in September 1983, in Juban, Sorsogon: 1 death (4),
* in September 1983, in Barra, Capiz: 8 cases. 1 death (4),
* in September 1983, in Ticao Island, Masbate, 27 cases, 2 deaths (4),
* between May and August 1987, in West Samar: 202 patients, 5 deaths (6).

As of year 1987, in the Philippines 976 cases and 25 fatalities were documented (4, 5, 6).

This report documents PSP outbreaks between July 9, 1988 and February 14, 1989 in seven affected provinces in five islands Bataan, Cavite, Metropolitan Manila, Western Samar, Negros Occidental, Capiz, and Cebu. The first of the series of these epidemics occurred in Samar on July 9, 1988 followed by Bataan on August 19, 1988; Metropolitan Manila on September 6, 1988; Cavite on September 9, 1988; Negros Occidental and Capiz on December 14, 1988 and Mactan, Cebu on February 1, 1989. All these epidemics were investigated by trainees of the Field Epidemiology Training Program of the Department of Health.

Unique diagnostic and public policy issues became prominent during the efforts to investigate and control the outbreaks. For example, loans granted by the Department of Agriculture to fishermen to soften the economic impact of the outbreak may have resulted in overreporting of symptoms among the residents. Furthermore several warnings about the dangers of red tide affected all fishermen and fish vendors indiscriminately, even if there was a patchy distribution of the areas affected by red tide. This paper will summarize the epidemiological monitoring and control aspects of the 1988 to 1989 outbreaks with the view to future courses of action.

## Method of the Investigation

*Case definition.* The symptoms of PSP result from the action of the toxin on the sodium channels of nerve cells. The channel is blocked by the toxin preventing the entry of sodium ions into the cell. This leads to persistent depolarization seen grossly as muscle paresis that progresses to paralysis.

A case of PSP was defined as a previously healthy person who suddenly developed at least two of any of the following motor defects: defects in gait, dysphagia, diplopia, dysphonia, prostration, dyspnea paralysis, weakness and at least two of any of the following sensory abnormalities: numbness, paresthesia, feeling hot (feverish), pruitus, dysthesia, light headedness, and short tongue sensation.

Cases with a history of cerebrovascular accidents, exposure to alcohol (at least 5 bottles of beer or 1 pocket size bottle of rhum) and exposure to insecticides were excluded from the list.

*Case finding.* Case-finding consisted of reviews of hospital records and a community search.

We collected hospital records of patients suspected to be PSP victims. Their addresses were obtained and located. Then a house-to-house search for additional patients was made. When a person claimed to be a "red tide victim" he/she was personally interviewed. Investigators were not able to collect gastric acid contents.

The interview consisted of open and closed-ended questions regarding the name, age, sex, address of patients, including the meal, symptoms following the meal, and methods of preparation of food. The question on food preparation methods included such items as source of sea food, storage, cooking, and seasoning (eg: vinegar, and salt).

*Case-control study.* Cases were matched with controls. A control was defined as a healthy person who was of the same age and lived in the same household or neighborhood as the patient.

The diets of the case and control were compared using the McNemar's paired test analysis at the 95% level.

*Sampling of sea food.* Attempts to identify food contaminated with the toxin were unsuccessful.

The investigators requested interviewees to identify the actual physical location of the sources of shellfish (eg. on the beach) and appropriate specimens were sent to the Bureau of Food and Drugs laboratory.

*Mouse Inoculation tests.* Marine fisheries products were collected and tested for the presence of the toxins.

The test followed the protocol recommended by the American Organization of Analytical Chemists (AOAC) for biotoxin testing. It consisted of adjusting the pH of the shellfish extracts between 3 and 4.5 and injecting the acidified extract into the peritoneum of mice weighing between 17 and 22 grams. The death time after intraperitoneal injection was compared to Sommer's Table in the AOAC manual in order to derive the amount of toxin present in the sample.

Any sample containing greater than 80 micrograms of toxin (about 400 mouse units) per 100 grams of sample was considered not fit for human consumption. A mouse unit was the amount of poison titrated to kill a 20-gram mice in 15 minutes.

*Identification of the causative dinoflagellate.* The Bureau of Fisheries and Aquatic Resources identified the causative dinoflagellate through regular phytoplankton surveys in established key stations in the affected areas. The survey consisted of concentrating the dinoflagellates in sea water with fine mesh plankton nets towed by a boat. The concentrated sea water sample was then placed on a Sedgewick-Rafter counting slide and the number of all types of dinoflagellates per cross-sectional area on the counting slide was calculated. The species of phytoplankton was also identified and its population estimated. Density of a species of phytoplankton was estimated using area and volume relations between sea water, net and counting slide.

The identity of the dinoflagellate was validated by Mr. Jay Maclean from the International Center for Living and Aquatic Resources, Manila.

*Analysis of Shellfish Extracts.* Shellfish extracts from Bataan, Cebu, and West Samar were shipped to Dr. Sherwood Hall of the US Food and Drugs Administration Seafood Toxin Laboratory in Washington, DC for analysis. Shellfish extracts from Bataan were also analyzed in the Muara Fish Landing Laboratory in Brunei by Dr. Yasukatsu Oshima.

Both laboratories used the High Performance Liquid Chromatograph (HPLC) to determine the toxin composition of shellfish from the 1988 Philippine PSP outbreaks.

## Results

*Survey.* Four hundred and thirty-seven patients were interviewed but only 224 or 51% fit the case definition adopted in these investigations. The earliest case was reported from West Samar on July 9, 1988 and the last case from Cebu on February 14, 1989 (see Table I).

There were 101 male and 123 female cases. Their ages ranged from 1 to 78 years old with a mean age of 29 years (see Figure I). The overall case-fatality ratio was 6 per 100 cases with 14 deaths: 1 in Limay, Bataan; 2 in Malabon, Metro Manila; 1 in Rosario, Cavite; 4 in Victorias, Negros Occidental; 1 in Roxas City, Capiz; and 5 in Mactan, Cebu.

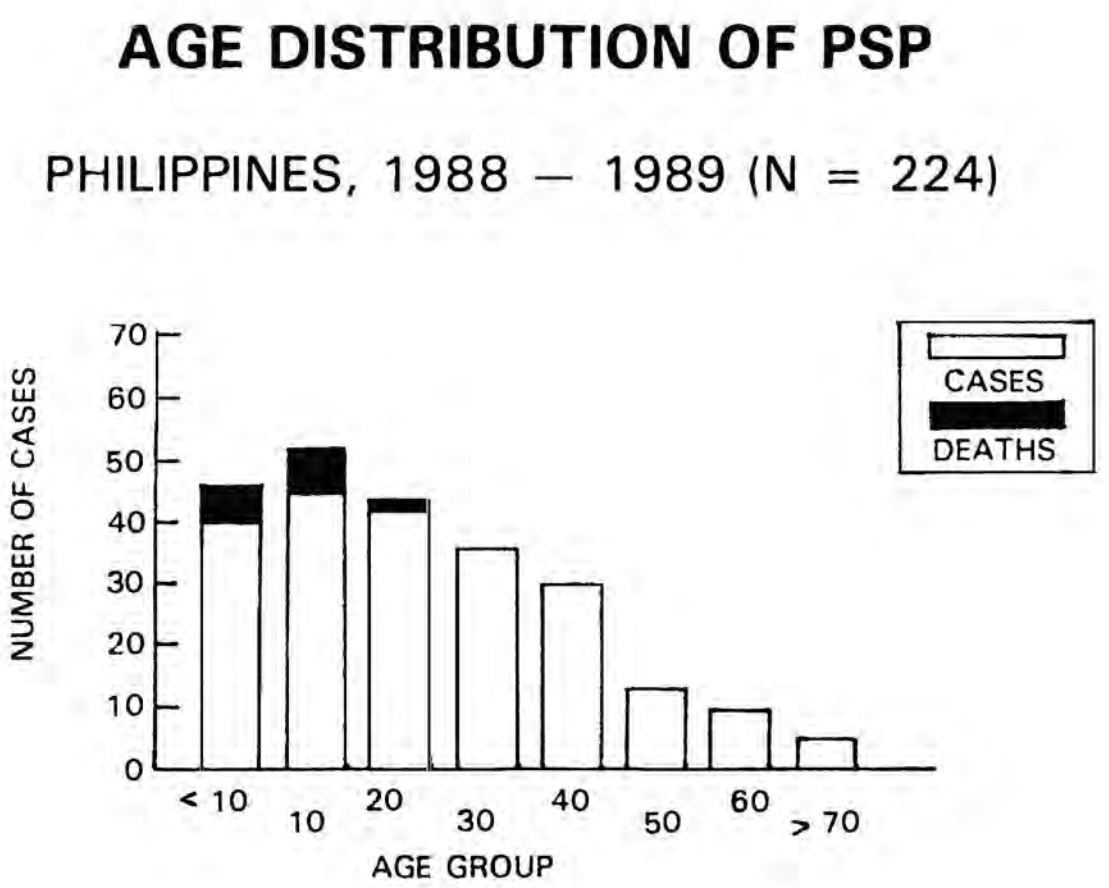
Differences in the manner by which the patients procured the offending seafood were noted. The epidemics in Bataan, Manila, Cavite, Negros and Cebu were all due to mussels or scallops obtained from farms. The epidemic in West Samar was due to mussels that were gathered from beaches and in Capiz to mussels from floating bamboo poles (see Table I).

Table 1. Profile of Six Philippine PSP Outbreaks (1988 – 1989)

| *Province* | *Cases* | *Deaths* | *Epidemics Start* | *Dates End* | *Toxin ** | *Transvector* | *Mean Onset (Hrs)* |
|---|---|---|---|---|---|---|---|
| Bataan | 44 | 1 | 08/19/88 | 09/30/88 | 1005 | Cultured mussels | 4 |
| Manila | 14 | 2 | 09/06/88 | 09/30/88 | 80 to 90 | Cultured mussels | 4 |
| Cavite | 8 | 1 | 09/09/88 | 09/10/88 | 40 to 100 | Cultured mussels | 15 |
| W. Samar | 22 | 0 | 07/09/88 | 09/05/88 | 164 | Wild mussels | 4 |
| Negros | 109 | 4 | 12/14/88 | 12/19/88 | 47 | Cultured mussels | 9 |
| Capiz | 3 | 1 | 12/14/88 | 12/14/88 | (? ) | (? ) | (? ) |
| Cebu | 24 | 5 | 02/01/89 | 02/14/89 | 2,000 | Cultured scallops | 6 |
| Total | 224 | 14 | | | | | |

*Micrograms of toxin per 100 gms. of sample obtained initially
(? ) Mussels causing illness came from floating bamboo poles

Figure 1




Onset of symptoms ranged from minutes to as long as 34 hours, with a median incubation period of 5.6 hours and a mean of 7 hours (see Table I).

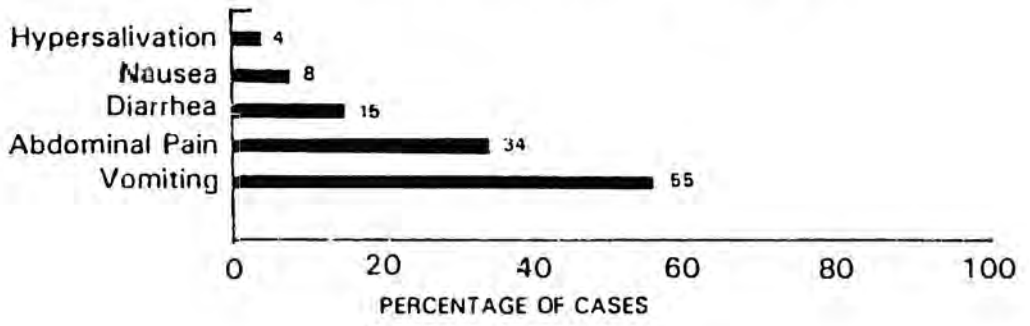
The illness affected the gastrointestinal, the neurologic and gastrointestinal systems (see Figure II). Generally, the illness began with vomiting followed by numbness with or without paresthesia of the oral cavity that descended to the upper then lower extremities. The patient then complained of a light-headed sensation which was followed by a motor defect that began with inability to walk followed by dyspnea, then dysphonia and dysphagia. A variety of gait defects were reported including drunken gait, scissors gait and ataxia. Death was due to respiratory failure.

*Case-control-study*. People who ate green bay mussels were 16 times more likely to get PSP than those who ate other food items, including vegetables, meat, and rice eaten with the sea food. People who ate scallops were 8 times more likely to develop PSP than those who ate other foods. Shrimps and fish did not pose a risk for PSP (see Table II).

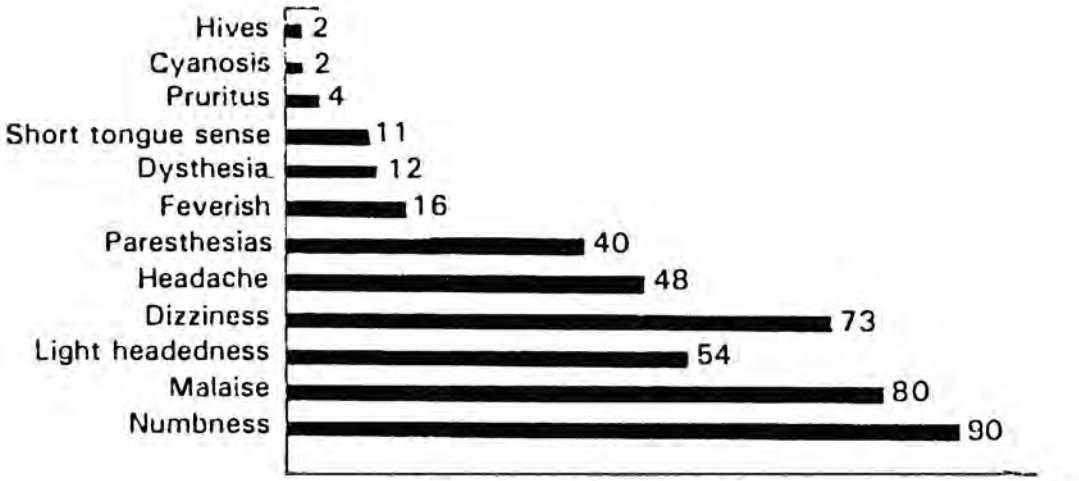
Figure 2

# SYMPTOMS OF PSP CASES

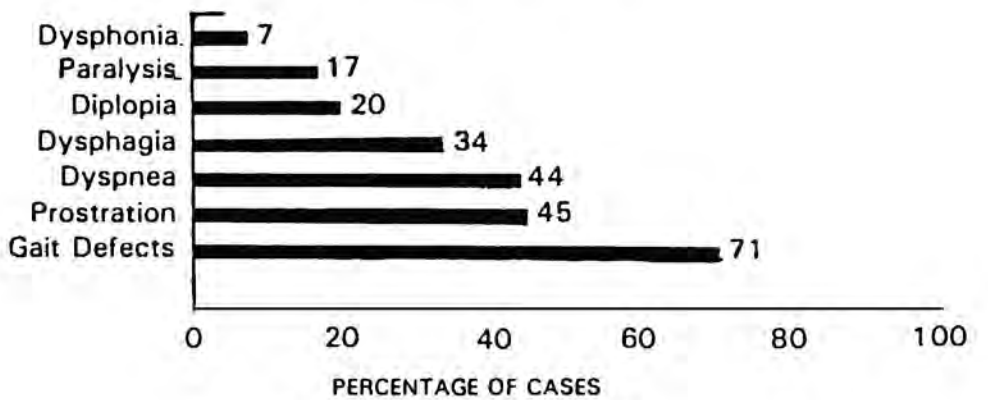
## PHILIPPINES, 1988 – 1989 (N = 224)




**GASTRO INTESTINAL**




**SENSORY DEFECTS**




**MOTOR ABNORMALITIES**

Table 2. PSP Transvectors

| *Transvector* | *Odds Ratio* | *95% Confidence Interval* |
|---|---|---|
| Asstd. Fish | 0.1 | 0.1 to 0.2 |
| Shrimps | 0.5 | 0.2 to 1.2 |
| *Perna Viridis* | 16.3 | 9.8 to 27.3 |
| *Chlamys Sp.** | 8.3 | 1.9 to 40.0 |

*Scallops from one epidemic only (Cebu)

*Mouse Inoculation tests.* The levels of toxicity of the mussels ranged between 47 to 1005 micrograms per 100 grams of meat. The level of toxicity of scallops was 2000 micrograms per 100 grams for meat. Assorted fish gills contained 38 micrograms per 100 grams of sample, while assorted fish guts did not contain any detectable levels.

The duration at which shellfish remained toxic varied among different areas. Mussels from Limay and Orion in Bataan, the western side of Manila Bay, remained toxic for 71 days after initial sampling on August 29, 1988 based on records of BFAR and BFAD. Mussels from Cavite, the eastern side of Manila Bay, remained toxic for 24 days after initial sampling on September 2, 1988. Mussels from Metropolitan Manila farms remained toxic for 14 days after initial sampling on September 19, 1988. The duration of shellfish toxicity in Capiz, Negros Occidental, Cebu, and West Samar could not be determined.

*Identification of phytoplankton.* Biologists from the Bureau of Fisheries and Aquatic Resources positively identified *Pyrodinium bahamense var compressum* from sea water concentrates obtained from key stations all over the country (Mr. Gonzales, Miss Maala, Bureau of Fisheries and Aquatic Resources, personal communication).

*Analysis of Shellfish Extracts.* The results of the HPLC analysis on 10 microliters of shellfish extracts from Bataan revealed a toxin composition of neosaxitoxin (43.7 MU/ml), saxitoxin (3.77 mU/ml) and decarbamoylsaxitoxin (7.21 MU/ml). Gonyaulotoxin 5 may have been also present, but we failed to discriminate this from artifacts.

## Conclusions

In the Philippines between 1988 and 1989, six outbreaks of PSP due to *Pyrodinium bahamense var. compressum* were confirmed from seven provinces. People who ate green bay mussels and scallops were at risk of acquiring PSP. The toxins from these products were saxitoxin, neosaxitoxin, and decarbamoylsaxitoxin.

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