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### The Vigorous Hybrid: A History of the Chemistry and Biology Research Institute

### **David Siminovitch**

Research Branch Agriculture Canada

Historical Series No. 30 1986

## One hundred years of progress

The year 1986 is the centennial of the Research Branch, Agriculture Canada.

On 2 June 1886, *The Experimental Farm Station Act* received Royal Assent. The passage of this legislation marked the creation of the first five experimental farms located at Nappan, Nova Scotia; Ottawa, Ontario; Brandon, Manitoba; Indian Head, Saskatchewan (then called the North-West Territories); and Agassiz, British Columbia. From this beginning has grown the current system of over forty research establishments that stretch from St. John's West, Newfoundland, to Saanichton, British Columbia.

The original experimental farms were established to serve the farming community and assist the Canadian agricultural industry during its early development. Today, the Research Branch continues to search for new technology that will ensure the development and maintenance of a competitive agri-food industry.

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On 1 April 1986 the Chemistry and Biology Research Institute was amalgamated with the Ottawa Research Station to form the Plant Research Centre.

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#### Chapter 1 ORIGIN AND FUNCTIONS

The Chemistry and Biology Research Institute (CBRI) is part of the Institutes Directorate of the Research Branch and is located in the K.W. Neatby Building of the Central Experimental Farm in Ottawa. The institute is responsible for national programs of research that require centralization of personnel, service facilities, and equipment. The Chemistry and Biology Research Institute's name conveys both its history and character because it is an amalgamation of the research disciplines of chemistry and biology as they relate to agriculture. The institute also includes a group of microbiologists who originally made up the Microbiology Research Institute, which was created from the old Bacteriology Division and was the first entity in the Department of Agriculture to undertake research on microbes and their role in agriculture. That division was created in 1923 as an integral part of the Central Experimental Farm.

From a scientific staff of 13 in the Microbiology Research Institute in 1966, CBRI now has a staff of 34 scientists who conduct research in a wide range of problems basic to our understanding of the chemical and biological principles They also provide service to underlying agriculture. agricultural institutions across Canada. The activities are conducted under seven main programs. These are as follows: the development of effective and environmentally acceptable methods of weed and fungus control by pesticides; the development of analytical methodology to establish safe levels of fungal toxins in food and feedstuffs and to provide effective measures for decontamination and control; the increase of the nitrogen-fixing capability of forage legumes through selection and genetic engineering of the bacterial symbiont and improvement of the host plant; the epidemiology and transmission of virus and mycoplasma diseases of plants in relation to disease incidence, management, and control; the efficient use of soil nitrogen and the prevention of soil organic matter losses; the mineralogy of Canadian soils; and the provision of new knowledge on the mechanisms of cold acclimation, freezing injury, and overwintering damage in relation to the development of crop plants resistant to environmental stresses. The institute also provides a comprehensive analytical chemistry service and an electron microscope service for Research Branch establishments across Canada.

With roots in a number of organizations that previously represented the department's research responsibilities in microbiology, plant biology, and chemistry and nourished by new people and ideas that came with the various amalgamations, CBRI is uniquely qualified to carry out the multiple aims of its mandate. Four events marked the evolution of CBRI. The first was the transfer of the Bacteriology Division to the Microbiology Research Institute; the second was the merger, in April 1967, of the Plant Research Institute with the Microbiology Research Institute to form the Cell Biology Research Institute; the third was the integration in 1972 of the Analytical Chemistry Research Service with the Cell Biology Research Institute; and the fourth was the transfer of a group of scientists to CBRI from the newly created Land Resource Research Institute, formerly known as the Soil Research Institute.

#### Microbiology Research Institute

The Microbiology Research Institute is regarded as the founding organization of CBRI. It provided the nucleus of personnel around which the Cell Biology Research Institute and its successor organization were built. The Bacteriology Division, which came before the Microbiology Research Institute and was created in 1923 as an integral part of the Central Experimental Farm, had already made a significant mark in the history of soil microbiology, as recorded by its first director, Allan Grant Lochhead. The formation of the Microbiology Research Institute from the staff of scientists in the Bacteriology Division was more a transition than a transformation. Under the stewardship of Dr. Lochhead, the division had an excellent start. A kind and unassuming man, Dr. Lochhead had a keen sense of humor and an enthusiasm for science. He and his staff made an invaluable contribution to soil microbiology. His dedication and knowledge attracted young scientists and influenced those around him.

While Dr. Lochhead was its director, the division was not confined to basic research in soil microbiology. The work was extended to include the microbiology of milk, cheese, butter, mastitis, fruits and vegetables, eggs, and poultry. In addition, wartime projects were taken on. In fact, for a time, the division was designated as the Division of Bacteriology and Dairy Research.

Dr. Lochhead was born in Galt, Ont. He attended high school in Guelph and Montreal, obtaining a B.Sc. and an M.Sc. in chemistry at McGill University. He then went to Leipzig to begin doctoral studies, which were completed only in 1919 at McGill, after returning from Germany where he had been interned during the war. He then lectured at Macdonald College, and after 3 years of industrial experience took up studies at the University of Alberta with the distinguished biochemist, J.B. Collip. When the Division of Bacteriology was created by the Dominion Department of Agriculture, Dr. Lochhead was appointed head of the new laboratory in 1923. His research in soil bacteriology and that of his staff, especially their study of the rhizosphere, allowed for the development of a greater understanding of the interactive behavior of all the living things in soils. This work earned the laboratory world-wide recognition. Allan Grant Lochhead was elected a fellow of the Royal Society in 1940 and received the Flavelle medal of the society in 1958. The University of Giessen made him an honorary Doctor of Agriculture in 1963.

The work of Dr. Lochhead's laboratory in soil microbiology brought honor to Canadian science. It is of interest to note that Michael Timonin, a member of Dr. Lochhead's team that pioneered research on the rhizosphere and a one-time student of the famous school of Selman Waksman at Rutgers University, is now still active in microbiological research, although he is over 80 years old. I.L. (Scotty) Stevenson is the last of Dr. Lochhead's colleagues remaining on the staff of the present institute.

Allan Grant Lochhead retired in 1955, and the direction of the Bacteriology Division passed to Harry Katznelson. In 1959, when the Research Branch was formed and the Microbiology Research Institute was created as part of the Institutes Directorate, Dr. Katznelson became its head also. Born in the Soviet Union, Dr. Katznelson immigrated to Vancouver in 1920. After receiving his B.S.A. degree from the University of British Columbia and his M.S. degree from Washington State University, he completed his Ph.D. at Rutgers University under Nobel laureate S.A. Waksman. He was also a postdoctorate fellow at Cornell University and Muellhaupt scholar in biology at Ohio State University. Later, in 1951, while on postdoctorate leave, Dr. Katznelson carried out research at Stanford University with the outstanding authority on microbial physiology, C.B. Van Neil. He is the author of more than 120 scientific papers and was elected a fellow of the American Association for the Advancement of Science in 1959 and a fellow of the Royal Society of Canada in 1962. He represented the Department of Agriculture at the United Nations International Conference on Peaceful Uses of Atomic Energy held in Geneva in 1958. He was also chairman of the microbiology section of the Ninth International Botanical Congress held in Montreal a year later.

The history of the Microbiology Research Institute is essentially a story of the objectives and traditions of the Bacteriology Division. The function of the new institute was to conduct basic research on microorganisms of importance to agriculture in relation to biochemistry, genetics, physiology, nutrition, taxonomy, and ecology. To perform this function the institute was divided into four sections and programs, with its 19 scientists distributed among the various sections. In the Biochemistry Section, with R.M. Hochster, S.M. Lesley, and N.B. Madsen, emphasis was placed on the enzymology of bacteria, fungi, and viruses that induced plant tumors. Elucidation of the tumour-inducing principle of the crown gall bacterium Agrobacterium tumefaciens was one of the section's major objectives. The research activities included the biochemistry of this organism as a model for tumor induction. In the Genetics and Taxonomy Section, I. Takahashi, V.N. Iyer, and G.B. Landerkin concentrated on soil bacteria with special emphasis on transformation, transduction, conjugation, bacteriophage,

mutation rates, and mutagens in relation to plant pathogenicity, symbiotic effectiveness, nutrition, and classification. Other activities included genetic mapping and coding as well as studies on base sequences and transformation. In the Physiology and Nutrition Section, I.L. Stevenson, D.C. Gillespie, and H.B. Gunner, studied the large and important group of soil bacteria, Arthrobacter spp. Studies were conducted on the morphology, growth, nutritional requirements, and synthetic activities of these organisms, on cell wall constituents, and on protoplast formation. Inorganic transformations mediated by Arthrobacter spp. were also examined. The Plant-Microbe Interrelationships Section, with J.W. Rouatt, F.D. Cook, and E.A. Peterson, concentrated on the bacterial and fungal flora in the rhizosphere and on the influence of these organisms on plant growth. Investigations were also made of the symbiotic relationships between legumes, nonlegumes, and nodule-producing microorganisms. Gains or losses of nitrogen due to the activities of free-living nitrogen or denitrifying bacteria were also studied. Thus, a nitrogen-fixation program in CBRI was in place long before the creation of the institute. Assisting in all this work were six scientists visiting the institute, either in the capacity of National Research Council of Canada postdoctorate fellows or as students.

From 1962 to 1964 Dr. Katznelson maintained the organization that the institute had before 1962, except that the Plant-Microbe Interrelationships Section was renamed the Ecology Section. Meanwhile, three new scientists, N.J. Hahn, C.S. Stachow, and G.W. Skyring joined the division, and four scientists, H.B. Gunner, N.B. Madsen, I. Takahashi and F.D. Cook left to accept university appointments. G.B. Landerkin moved to the Food Research Institute. Although the general orientation of research activities had not changed appreciably, the institute could claim some new developments. These new developments included the isolation of new carbohydrates from culture media of Agrobacterium tumefaciens; some resolution of the mechanism of action of the antibiotic mitomycin and new information on the mechanisms of transformation and transduction in Bacillus subtilis; the finding from cytological studies of striking alterations in the cell wall structures of Arthrobacter spp. during their life cycles; the possible production of a new antibiotic from a soil-borne bacterium; and new information on the mechanism of sugar uptake by resting cells of Agrobacterium tumefaciens. In addition, the institute tested the possibility of using gamma rays as a means of controlling various microorganisms that cause diseases of the honey bee.

On 10 February 1965, the institute experienced a great loss in the death of Harry Katznelson. During the time of his association with the Bacteriology Division and the Microbiology Research Institute, Dr. Katznelson had, through his own work and that of his staff, succeeded in maintaining and increasing the prominent position of soil bacteriology that the division and the institute occupied in the world community of science and agricultural microbiology.

Following Dr. Katznelson's death, R.M. Hochster was appointed director of the Microbiology Research Institute, a position he held until 1967, when he became the director of the newly formed Cell Biology Research Institute. In the intervening years, the institute carried on with its original activities and continued to make significant contributions to the field of agricultural microbiology. Following the acquisition of several electron microscopes, under the stimulus of I.L. (Scotty) Stevenson and J.T. Slykhuis, the institute was also able to establish a communal Electron Microscope Centre located in the basement of the K.W. Neatby Building. Dr. Stevenson put electron microscopy to good use in studies on Arthrobacter spp. These studies proved to be seminal and were cited in detail in the Proceedings of the International Congress of Microbiology held in Mexico in 1971. Dr. Stevenson provided strong evidence from RNA, DNA, and changes in intracellular structures during growth and morphogenesis that there was transient discoordination of cell synthesis and cell division during sphere to rod morphogenesis in these bacteria. The institute was able to list these contributions along with others on rhizosphere interactions, bacterial transformations, and transductions; on bacterial sugar metabolism and transport, bacterial RNA, and DNA metabolism; and on the development of a new antibiotic myxin, effective against a wide variety of bacteria, fungi yeasts, and actinomycetes. Finally, in 1969, upon the amalgamation of the Microbiology Research Institute with the Plant Research Institute to form the Cell Biology Research Institute, scientists started to explore new areas of interest, but without abandoning the objectives of the former Microbiology Research Institute. The scientists of the Microbiology Research Institute who were involved in the amalgamation were R.M. Hochster, I.L. Stevenson, J.W. Rouatt, E.A. Peterson, G.W. Skyring, D.C. Gillespie, N.S. Hahn, R.M. Behki, Jordan Ingram, S.M. Lesley, Ches Stachow, and V.N. Iyer. Karl Ivarson, who had joined the Microbiology Research Institute in 1956, was transferred to the newly formed Soil Research Institute.

#### Soil Research Institute and Analytical Chemistry Research Service

Created from the Chemistry Division of the Science Service in 1959, both the Soil Research Institute and the Analytical Chemistry Research Service were to provide the primary sources

for development of several of the major programs of CBRI. The Chemistry Division came into existence in 1887, even before the Science Service was formed and only a year after the Dominion Experimental Farm was established in 1886. The first director of the farm, William Saunders, appointed Frank Shutt to the position of chief chemist and charged him with developing and heading an agricultural chemistry laboratory. Planning for construction of the laboratory, which was to contain a chemistry unit, started at once, and the building that was to house the division's staff for 70 years was completed in 1895 and still stands. These were proud beginnings for CBRI, not only with respect to its early roots, but also to the pioneering accomplishments of Dr. Shutt and his colleagues, which were From the very start, the Chemistry Division was the numerous. only chemical service available to thousands of farmers in Canada, who were given helpful information and practical advice. This service included many soil analyses and studies that were necessary to increase soil productivity and fertility. The analyses were essential in directing and advising settlers, since data were collected with regard to composition of virgin soils from every large district and part of the country. Recommendations could then be made on the use of fertilizers, manures, and soil amendments. The division undertook many critical examinations in the field of animal nutrition. Also, many chemical analyses were performed to improve plant nutrition and pasture management. The selection of wheat of superior quality and early ripening was dependent on reliable protein analyses, contributed to the reputation that Canadian wheats enjoyed in world markets, and constituted significant economic returns for Canada. The record of these achievements is documented in detail in the Division of Chemistry annual reports, published from 1886 to 1936, which are housed in the Department of Agriculture Library in the Sir John Carling Building, Ottawa.

The early traditions of the Chemistry Division were adopted by the Science Service when the service, into which the Chemistry Division was absorbed, was established in 1937. In those days, only a handful of chemists were on staff, and they not only fulfilled the heavy demands of their positions, but they also published 100 scientific papers by 1943. At that time, the management of the division had passed to C.H. Robinson, and in 1949 he was succeeded by J.C. Woodward. Ιn their hands, the Chemistry Division underwent a major staff expansion largely because of the efforts and foresight of Dr. Woodward, who was responsible for the recruitment of many able scientists. These scientists were to play a significant role later in the evolution of the Research Branch and its various institutes. Upon the establishment of the Research Branch, the Chemistry Division had 41 professionals and 65 technicians on staff. As a result, the division easily paced the world

scientific explosion that was taking place: from 1943 to 1958 the division contributed well over 400 scientific papers. The duties and responsibilities, meanwhile, had multiplied and diversified greatly, with research being conducted in many new areas, without reducing the work of the service analytical program. Contributions were made on various analyses of plants, animals, soils, and pesticides. The division was the first to apply radioactive trace methodology in agricultural research. This work was carried out by B.B. Migicovsky, who later became the assistant deputy minister of the Research Branch. In an effort to better organize the work of the division, Dr. Woodward divided it into a number of sections (analytical chemistry, animal chemistry, plant chemistry, and soil chemistry) according to the general nature of their programs.

In 1955 A.R.G. Emslie became director of the division, after Dr. Woodward assumed new administrative duties in the department. In 1959, when the Research Branch was formed, the division was fragmented, and its members were incorporated into various research institutes that were established at that time: the Animal Research Institute, the Genetics and Plant Breeding Research Institute, the Soil Research Institute, and the Plant Research Institute. The fate of only three of these former sections of the Chemistry Division and their respective members are of direct concern to the history of the CBRI. These are the Soil Chemistry Section, the Analytical Chemistry Section, and the Plant Chemistry Section. In 1959 the staff of the Soil Chemistry Section together with the soil scientists of the Central Experimental Farm were grouped to form the Soil Research Institute, bringing together all the soil scientists in Ottawa under one umbrella. The whole of the Analytical Chemistry Section became the independent Analytical Chemistry Research Service, and three members of the Plant Chemistry Section, two of whom eventually became members of CBRI, were absorbed into the organization formed from the Botany Division, namely the Plant Research Institute.

#### Soil Research Institute

The expanded Soil Research Institute now had 30 scientists and was headed by Peter Stobbe. The breadth and diversity of knowledge generated by the merger of various disciples allowed for a broad program covering many areas ranging from cartography and soil surveys to physical chemistry of soils. The chemists of the former Chemistry Division made up two new sections and included F.J. (Fred) Sowden, H. Morita, and M. Schnitzer (soil organic matter); J.E. Brydon, S.A. Foreman, H.M. Rice, and N. Miles (soil mineralogy); R.C. Turner (physical chemistry); J.R. Wright (soil genesis and classification); and R. Halstead and A. Maclean (soil fertility). H.S. Atkinson, director of the Soils Section of the Chemistry Division, had already retired. The remaining members of the institute were engaged in various programs on soil fertility, soil surveys, soil classification, and cartography. These programs and the organization of the institute remained unaltered until 1967, when Peter Stobbe retired and J.S. Clark, who had joined the Physical Chemistry Section in 1962, took over as director of the institute.

At the time of Dr. Clark's appointment, the Soil Biochemistry Section, with F.J. Sowden, H. Morita, and K.C. Ivarson, and the Humic Acid Chemistry Section, with M. Schnitzer and S.P. Mathur, were already established. In 1973, Dr. Clark instituted a major reorganization of the institute reflecting a new orientation and emphasis in the program. The chemists were now all assembled in two sections entitled Soil Resource Research and Soil Conservation. The institute also consisted of the Soil Resource Inventory, the Cartography Section, and a number of soil survey sections representing different locations in the country. In 1974 a further reorganization took place, and the chemists were assigned to the Nutrient and Waste Management in Soils Section, the Organic Soils Section, and the Active Fraction and Soil Behavior Section. The Soil Resource Inventory, the Cartography Section and other soil survey sections were left intact, but the new Water and Nutrient Transport in Soils Section was established.

Thus the organization of the Soil Research Institute stood until 1978, when the personnel and research programs associated with soil organic matter chemistry, soil nitrogen, and soil clay mineralogy were transferred to CBRI. The scientists transferred were Morris Schnitzer, Don Gamble, Karl Ivarson, H. Kodama, Suku Mathur, Kaz Morita, John Ross, Surinder Singh, and Norman Miles. The remainder of the Soil Research Institute was reorganized and became the Land Resource Research Institute.

During the 19 years of the Soil Research Institute's existence, a number of chemists left, and others were transferred to CBRI. Jim Wright became director of the Kentville Research Station. Ron Halstead became the research coordinator of resources of the Planning and Evaluation Directorate, and Jim Bryden joined the Department of Environment. Harry Rice, Al Maclean, Fred Sowden, and Bob Turner--members of both the Chemistry Division and the Soil Research Institute--all retired. The contributions of these people and their colleagues had left an indelible international record of achievement in soil chemistry. Jim Wright was well known for his work in soil genesis. Bob Turner almost changed the rules in ionic equilibria in soils through his study of the influence of calcium and aluminum on soil pH. J.S. Clark also contributed to these equilibrium investigations. Fred Sowden pioneered the chemistry of nitrogenous compounds, especially amino acids in soils. M. Schnitzer did much in unraveling the complex chemistry of humic acid components of soil. Jim Bryden, H. Kodama, John Ross, Norman Miles, and Harry Rice, armed with a battery of sophisticated X-ray equipment, transformed the mineralogy section into a national center for the study of clays and other soil minerals. Karl Ivarson probed and resolved many problems of the effect of microbial activity on mineral transformations in soils. Ron Halstead and Al Maclean made many contributions to understanding of mineral nutrition of plants and soil fertility and were consultants to agriculturists in this field. S. Singh and H. Morita participated actively in research on the physical chemistry and organic compounds of soil.

#### Analytical Chemistry Research Service

Like the Soil Research Institute, the Analytical Chemistry Research Service was also launched in 1959 with the formation of the Research Branch and originated from the Analytical Chemistry Section of the Chemistry Division of the Science Service. The professional staff of the section at that time consisted of R.B. (Bob) Carson, its chief, I.(Al) Hoffman, D.A. (Dunc) Shearer, and Joyce MacIntosh. The technicians included Stu Skinner, Walter Jopkiewiez, George Morris, Art Brossard, Jacques Langevin, Aurel Labelle, Bob Westerby, Bobby Anderson, and Connie Sherwood.

The new Analytical Chemistry Research Service was directed toward the development of new methods of analysis of various agriculturally related products, such as animal- and plantderived components and foods as well as inorganic and organic pesticide residues. Particular attention was paid to new techniques and instruments as they became available. Over the years, the Chemistry Division had built up a background of knowledge in the analytical aspects of inorganic, organic, and physical chemistry and in pesticide residue methodology, which was available to Research Branch scientists. In addition, chemical analyses were provided to enable authorized projects to be undertaken at branch establishments where complete laboratory facilities were not available. In pursuing these functions, projects undertaken by the service in the years before amalgamation with CBRI included thin-layer and gas chromatographic methods for the detection and analyses of pesticide residues and the determination of trace metals, such as selenium, and plant micronutrients. Other projects included metal complexing reactions, moisture and nitrogen analyses in a vast variety of agricultural products, amino-acid analyses of proteins, as well as the detection and determination of insect

pheromone components. Also, a microanalytical service laboratory, available to all Research Branch establishments, was set up and supervised by George Morris for routine carbon, hydrogen, and sulfur analyses. New physical methods with new instrumentation were developed and adapted for the microdetermination of organic and inorganic residue contaminants. The new physical instruments, including an infrared spectrophotometer and a mass spectrophotometer, were housed and maintained for the benefit of all the institutes of the branch in Ottawa. In the capable hands of Stu Skinner, these instruments were to prove formidable weapons as diagnostic tools for the analysis of pesticide residues and for the identification and determination of organic agricultural products.

To implement all the services, additional chemists were hired. These included H.V. (Vic) Morley, R. (Purky) Purkayastha, Milan Ihnat, and R. Greenhalgh. In 1971, when the Analytical Chemistry Research Service was transferred to the Cell Biology Research Institute, Bob Carson had retired and Al Hoffman had resigned. Joyce MacIntosh also left to study library science and eventually joined the staff of the departmental library in Ottawa, later becoming its assistant director.

#### Plant Research Institute

Since the history of the Plant Research Institute is recorded in detail elsewhere, we need only mention here that several of its members came from the Plant Chemistry Section of the Chemistry Division. These were Fred Johnson, head of the section, Dave Siminovitch, Claude Sirois, and Herbert Stern. Dr. Siminovitch was brought to the Science Service by Jim Woodward in 1950 to conduct research on winterhardiness of plants. This work was expanded in the Cell Biology Research Institute and later in CBRI, and eventually evolved into what is now the Stress Physiology Program.

#### Chapter 3 THE FLEDGLING INSTITUTE (1967-1971)

On 1 April 1967 all members of the existing professional and technical staff of the Microbiology Research Institute were merged with members of the Plant Biochemistry, Phytopathology and Virology sections of the Plant Research Institute to create the Cell Biology Research Institute. The integration of the knowledge of plant biologists with that of microbiologists, with the common purpose of studying the living processes of cells in general, marked the beginning of the Chemistry and Biology Research Institute. R.M. Hochster was appointed director of the Cell Biology Research Institute. Dr. Hochster was internationally recognized for his biochemical investigations on respiratory and enzymatic processes in bacteria and was well prepared to assume the duties of directing an integrated unit whose mandate was to study the properties and activities of living cells. He had a Ph.D. in biochemistry from McGill University and had been a research scientist at the National Research Council of Canada before joining the Department of Agriculture in 1956. Dr. Hochster quickly took up the task of organizing and planning a program for the institute.

To begin with, Dr. Hochster established the Biochemistry, Cryobiology, and Cytology sections, in association with the Electron Microscope Centre, and the Microbial Ecology, Physiology, Phytopathology, and Virology sections. The Biochemistry Section consisted of R.M. Hochster, head of the section, Ram Behki, Jordan Ingram, Stan Lesley, and Ches Stachow, all of whom were former members of the Microbiology Research Institute. The other members of the Biochemistry Section, Clarence Madhosingh and John Shaw came from the Plant Research Institute. I.L. (Scotty) Stevenson, head of the Cytology Section and the Electron Microscope Centre, and Jim Rouatt, E.A. (Pete) Peterson, and G.W. Skyring, who made up the Microbial Ecology Section, had also all been part of the Microbiology Research Institute. The rest of the members of the new institute came from the Plant Research Institute. These were David Siminovitch, head of the Cryobiology Section and its only member; Ross Pringle, Ed Schneider, and Claude Sirois, (Physiology Section); Vic Wallen, P.K. Basu, Lloyd Seaman, and Murray Sutton (Phytopathology Section); and John Slykhuis, Lloyd Chiykowski, Yogesh Paliwal, and Ramesh Sinha (Virology Section). In addition to the staff members, the institute had a number of visiting scientists who were National Research Council of Canada postdoctoral fellows as well as a graduate student, Keith Pomeroy, who joined the institute in 1968. There were a number of departures from the institute in the first year of its operation. These included D.C. Gillespie, who was transferred to the Fisheries Research Board in March 1967; N.S. Hahn, who

resigned in June 1967; and V.N. Iyer, who resigned to join Carleton University.

In its first 3 years of operation (1967-1969), the structure and organization of the institute remained largely unaltered except for some additions, which included R.W. (Dick) Miller (Biochemistry Section); G.H. (Geoff) Haggis (Cytology Section and Electron Microscope Centre); Keith Pomeroy (Cryobiology Section); and W.C. (Clive) James (Phytopathology Section). Also in 1968, Z. Polak joined the institute and stayed for 2 years. J.Y. Yiu, who came in 1967, stayed until 1969. During these 3 years, the new institute claimed exciting achievements and developments in research in all areas of its program. With new emphasis in the phytopathology program on estimation of crop losses due to disease, and bolstered by the addition to the team of Clive James, who was renowned for his work on crop losses in England, a national crop loss assessment program was initiated in 1968. An important consequence of the work of the section headed by Vic Wallen was the demonstration of the power of aerial-infrared photography as a tool in the study of losses due to plant disease. A new, significant development in the study of plant disease was the implication, by the head of the section, Ramesh Sinha, of mycoplasma cells in clover phyllody diseases of asters and other plants. In continuation of their earlier studies in the Microbiology Research Institute on the antibiotic myxin, the biochemists were able to relate the antibiotic action of myxin to its effects on the synthesis of DNA. Some new properties of the respiratory systems of the tumorogenic bacterium Agrobacterium tumefaciens were disclosed. Disc electrophoresis of proteins and DNA analyses combined with numerical taxonomy and serological techniques were successfully applied to classification of soil bacteria and fungi in the rhizosphere. With the establishment of the Electron Microscope Centre, under the supervision of Scotty Stevenson and Geoff Haggis, the facilities of the center played an ever-increasing part in support of studies in a wide variety of biological disciplines, such as insect vectors and host tissues in virology, fine structure of bacteria of nematode cell membrane, and of replicating DNA, sites of fungal infections in plant disease, and mutagenic effects. In studies of tobacco mosaic virus, it was shown that the uncoating of protein from the virus occurred within minutes after the infection. New insights were obtained on the chemistry of the host-specific toxin of the casual agent of the leaf blight of certain corn hybrids. New evidence of the role of replication of membranes in the cells of plants undergoing frost hardening was found in electron microscope and metabolic studies. Research, in conjunction with industry, on the use of protein-based firefighting foams for the protective insulation of tomato and strawberry crops against frost damage aroused world-wide interest.

In 1970, following 3 years of stable organization, R.M. Hochster decided on a significant realignment of all of the sections. Sections were abolished, subdivisions were eliminated, and common interest groups were established around four main programs. Larger and more active research units emerged with no increase in staff. The reorientation was required because of transfers of a number of members of the institute to the Ottawa Research Station. These transfers included Vic Wallen, Lloyd Seaman, Clyde James, P.K. Basu, and John Slykhuis. At the same time, Chris Andrews and Fergus Macdowall were added to what was now the Cryobiology Section; Ed Schneider and Claude Sirois of the previous Physiology Section were also added to the Cryobiology Section. This section, now enlarged from two to five members, was renamed the Frost Hardiness and Dormancy Section. Ross Pringle, the only remaining member of the Physiology Section, was transferred to the newly established Agricultural Microbiology Section. The Physiology, Biochemistry, Microbial Ecology, Virology, and Phytopathology sections were abolished. I.L. (Scotty) Stevenson of the Electron Microscope Centre was also assigned to the Agricultural Microbiology Section. The Electron Microscope Centre continued with Geoff Haggis in charge. The Host-Parasite Relationship Section was the last section formed and included Ram Behki and Stan Lesley, formerly of the Biochemistry Section, and Ramesh Sinha, Lloyd Chiykowski, and Yogesh Paliwal, of the Virology Section.

In the meantime, Ian de la Roche, a National Research Council of Canada postdoctoral fellow, was seconded to the institute by arrangement with the University of Ottawa.

Four sections were now in place, and although the scientists in the institute were operating under new section titles, there was no radical change in the direction of programs. Except for some new developments and results, research continued along lines already well established in the first 3 years of the institute's existence. A significant development in the fall of 1970 was the building and opening of an extension to the Electron Microscope Centre to provide a properly integrated laboratory for use by all research scientists who required the facilities, regardless of whether they worked locally or outside Ottawa. In the same year, the Electron Microscope Centre introduced for the first time the use of cryofracture for the study of ultrastructure of cells. In the Agricultural Microbiology Section, the unique application of phages specific to various bacteria, in order to type and classify strains of bacteria of importance in agriculture, was meeting with considerable success. Also under the umbrella of the Agricultural Microbiology Section, research continued on the use of electrophoresis of proteins in the study of the taxonomy of bacteria. Further studies on the mode of action of the antibiotic myxin demonstrated that it acted by degradation of

the DNA template in susceptible bacterial cells. A comprehensive research program on the properties of key enzymes and cellular membranes of filamentous fungi was activated, with implications for control of important plant pathogens. This program included an assessment of the roles of membrane composition and structure, enzyme induction, and the formation of reactive oxygen species in the survival of pathogenic Fusarium species. The Frost Hardiness Section developed a new procedure for testing the freezing tolerance of seeds, and researchers were able to demonstrate that the ability of dormant seeds to survive freezing in winter was dependent on low seed hydration. Electron microscope studies supported by results of lipid analysis, showing increase in polar lipids and phospholipids in the winter hardening of plants, again pointed to a significant relationship between frost hardiness and membrane replication. The Host-Parasite Relationship Section demonstrated the effectiveness and promise of the use of tetracycline antibiotics for control of mycoplasmas, now established as the disease agents in clover phyllody and other diseases.

#### Chapter 4 CHEMISTRY AND BIOLOGY RESEARCH INSTITUTE

The Cell Biology Research Institute had just begun to function when on 23 March 1971 it entered an entirely new phase in its development with the announcement by the director general, B.B. Migicovsky, of the establishment of the Chemistry and Biology Research Institute. The new institute was formed by the amalgamation of the existing Analytical Chemistry Research Service with the Cell Biology Research Institute. The director of the Cell Biology Research Institute, R.M. Hochster, was named director of the new Chemistry and Biology Research Institute and was charged with the task of bringing the two units together.

Derived as it was from components of the old Chemistry Division, the Analytical Chemistry Research Service brought with it a strong complement of professional and technical staff who, under Robert Carson and later Israel (Al) Hoffman, had played such a significant role in providing analytical services not only to the Central Experimental Farm but to the department as a Thus the experience in analytical chemistry first gained whole. in the Chemistry Division and later in the Analytical Chemistry Research Service was transferred to a research-oriented organization while preserving the analytical services necessary to agriculture. In this sense, the creation of the institute crystallized the best hopes of F.T. Shutt, the first dominion chemist, and his successors, such as Jim Woodward, who had foreseen the important role that chemistry would play in providing essential analytical services to the department and in conducting future basic research in agriculture. The responsibilities of the new institute were greatly broadened and established in two major areas, research and service. An advantage of the union was the availability of a chemical library already in place in the K.W. Neatby Building. The library contained all the books and periodicals, housed originally in the old Chemistry Division Building, that were transferred to the Neatby Building at the time of dissolution of the division. M.N. Reynolds, director of the Library Division, decided that a field library was necessary to provide on-site facilities to the Chemistry and Biology Research Institute and its Analytical Chemistry Service. Under the able and dedicated supervision of Marcel Charette, the K.W. Neatby Library has proved to be indispensable, not only to the CBRI, but to all the establishments located in the building.

R.M. Hochster wasted no time in undertaking the complex task of drafting a plan and program for the new institute. The research program was developed first, in an effort to solve problems in three important areas. Plant productivity was to be increased through the understanding of the complex plant-bacteria-fungi relationships in plant diseases, thereby developing systems for control of disease. The significant components of the interactions of host plants and their parasites (viruses, mycoplasmas, bacteria, and fungi) were to be determined, and the parasitic action in eliciting susceptibility or resistance in plant cells was to be explained. A better understanding of the winter survival of plants through research on frost hardiness as well as of the related processes of dormancy in cereal seeds and fungal spores was to be achieved. The former Cell Biology Institute was already engaged in research along these lines, and so the sections were left Thus, the Agricultural Microbiology Section, with intact. Clarence Madhosingh, Dick Miller, E.A. Peterson, Ross Pringle, Jim Rouatt, and Scotty Stevenson, studied plant-bacteria-fungi relationships; the Frost Hardiness and Dormancy Section, with Chris Andrews, Fergus Macdowall, Keith Pomeroy, Ed Schneider, Dave Siminovitch, and Claude Sirois, investigated problems of plant winter survival and dormancy; and the Host-Parasite Relationship Section, with Ramesh Sinha, Lloyd Chiykowski, Yogesh Paliwal, Stan Lesley, and Ram Behki, studied the interaction of host and parasites.

The service program was designed to provide service to all branch establishments through four specialized groups. Two of these groups were to perform new functions within the institute. The Analytical Chemistry Service was to provide a broad spectrum of comprehensive analyses and a capability for development of new analytical methods for chemical elements, residues, and constituents of agricultural materials. The Instrumentation Centre was to be developed and managed to provide knowledge and service for sophisticated and expensive scientific equipment such as mass spectrometers, nuclear magnetic and electron paramagnetic resonance units, atomic absorption spectroscopes, and a double-beam spectrophotometer. The third responsibility was the provision of knowledge and service and the development of up-to-date methods in electron This service was not a new function, because the microscopy. Electron Microscope Centre was already in place as part of the former Cell Biology Research Institute. Finally, the Technological Unit was set up to handle all routine analyses for other laboratories and was placed under the direction of A. Brossard, with supervision from staff of the Analytical Chemistry Service.

However, in the initial stages of development of the new Analytical Chemistry Service and Instrumentation Centre, only two formal institute sections were established, the General Analytical Chemistry Section staffed by Don Gamble, Milan Ihnat, and Dunc Shearer and the Pesticide Residue Section, staffed by Roy Greenhalgh, Vic Morley, and R. Purkayastha. To meet the administrative needs of the enlarged institute, two new units were established within a newly created Administrative Services Section. These included the Office Services Unit, and the General Services Unit, the latter being responsible for the purchase and maintenance of supplies and equipment and related duties.

Finally, to provide an opportunity for the exchange of ideas, R.M. Hochster inaugurated a weekly series of CBRI seminars to which interested parties in other establishments and universities in the Ottawa area were welcomed. Also, through invitations extended occasionally to researchers in scientific institutions elsewhere, an opportunity was provided to the members of the institute to hear the latest advances in their respective fields in other parts of North America and the world. The seminars proved to be a valuable component of the institute's program.

During the year, R.M.D. Sutton of the Agricultural Microbiology Section left the institute on a transfer to the Environment Secretariate of the National Research Council of Canada in Ottawa. H. Rohleder joined the institute as a visiting scientist for 2 years. The institute acquired two National Research Council of Canada postdoctorate fellows for 1971-1972.

Just as the institute was getting under way, R.M. Hochster died on 16 September 1971. He had great aspirations for the institute, and despite the fact that he had been aware of the seriousness of his illness, he supervised the consolidation of the union and had already carefully drafted, in consultation with his staff, a detailed program for the new institute and set it into motion. At the time of Dr. Hochster's death, Academic Press announced the publication of volumes 3 and 4 of a set of books entitled Metabolic Inhibitors. Dr. Hochster had been instrumental in initiating the series in 1963. Together with J.H. Quastel he edited and contributed to volumes 1 and 2 and undertook the gathering of new material in 1969 for volumes 3 and 4. Morris Kates and J.H. Quastel completed the task of editing the latest volume. Ramesh Sinha and Richard Miller, members of CBRI, had also contributed articles to the series in the areas of inhibitors of plant viruses and mycoplasmas, and of enzymes. In tribute to Dr. Hochster and in recognition of his distinguished career as an agricultural biochemist, as director of the Chemistry and Biology Research Institute, and as senior editor of the Academic Press publications on metabolic inhibitors, volumes 3 and 4 were dedicated to him. With the death of R.M. Hochster, Jim Rouatt became acting director of the new institute and continued to implement the new program and organization set out by Dr. Hochster.

In 1972, after a full year of the institute's operation, Jim Rouatt, the acting director, was replaced by George Fleischmann

who, at the age of 36, became director of the institute. Dr. Fleischmann earned his Ph.D. from the University of Toronto, specializing in plant pathology and genetics. He joined the staff of the Winnipeg Research Station in 1962 and started a productive research program on crown rust of oats, for which he won international recognition. He was responsible for establishing North America's first regional scheme to defeat the rust pathogen by deploying different resistance genes along the south-to-north path of its movement, and he collaborated in developing a rapid contaminant-free method of isolating single-spore cultures of rusts. In 1964-1965, Dr. Fleischmann spent a year in Israel as a C.D. Howe memorial fellow, and in 1971 he visited Czechoslovakia on a National Research Council of Canada senior scientist travel award. Energetic and full of convictions about the course that research should take in the institute, he was certain to leave strong impressions on the institute, although in his first year of directorship he chose not to alter greatly the organization as developed by R.M. Hochster.

Some reorganization did take place, nevertheless. George Fleischmann perceived the need for, and quickly established, a separate Technological Service Unit under D.A. Shearer's supervision to provide the research establishments across Canada with a comprehensive technical service in analytical chemistry that included analyses of a wide spectrum of chemical elements and constituents of agricultural materials and an instrumentation center, with Roy Greenhalgh as adviser to provide the branch with equipment and staff for work in newly developing areas. The Electron Microscope Centre was expanded to include a postal service to be supervised by Joan Bronskill. Also, in line with the rapid developments that were taking place world wide in bioengineering, the Cell Bioengineering Section was created to produce new plant hybrids by use of anther culture, cell fusion, and exogenous incorporation of DNA. Ram Behki and Stan Lesley of the Host-Parasite Relationship Section were transferred to the new section.

During the same year, Scotty Stevenson moved to Alberta to assume the position of associate director of the research station in Lethbridge, and H.V. Morley of the Pesticide Residue Section was seconded to the coordination group as acting coordinator for environmental quality. Dr. Morley eventually became the new director of the London Research Institute. L.R. Barran joined the Agricultural Microbiology Section and J.C. Young, the General Analytical Chemistry Section. The institute experienced a sad loss in the death of R. Purkayastha, an able chemistry research officer with the Pesticide Residue Section. His contributions to the section and his gentle and cheerful disposition were missed by his associates. Two visiting scientists and two National Research Council of Canada postdoctorate fellows worked at CBRI in 1972 and 1973.

The year 1974 saw more substantial changes, with new terms of reference introduced into the program. The institute was enlarged by the addition of the Agrometeorolgy Research and This Service, formerly part of the Plant Research Institute. addition necessitated the transfer of seven scientists, W. Baier, R.L. Desjardins, H.N. Hayhoe, S.N. Edey, C.E. Ouellet, W.K. Sly, and G.D.V. Williams, all of whom continued to work in their original premises. An Environmental Chemistry Section consisting of the former Pesticide Residue and Chemical Methodology sections was created, giving the Research Branch the opportunity to work on environmental problems in agriculture. Also, the chemical services provided by the institute to branch establishments were combined under the Analytical Chemistry Service, which included the Technological Services Unit, the Instrumentation Centre, the Amino Acid Analysis Laboratory, the Microchemical Laboratory, and the Pesticide Residue Laboratory.

Three members of staff, J.G. Saha, W.D. Marshall, and S.U. Khan were added to the institute to strengthen the Environmental Chemistry Section. During the year, George Fleischmann prepared a comprehensive report of the organization, activities, and progress of the institute embodying the new steps to be taken to improve its operation, appropriately entitled the "New Look." The report was a useful appraisal of the state of CBRI 3 years after its founding.

In 1974 the institute again underwent a change in its management with the departure of Dr. Fleischmann when he assumed the position of director general, Policy and Program Development Directorate of the Environmental Management Service, Department of the Environment. Jim Rouatt once again took over supervising the operation of the institute in the capacity of acting director. With his usual patience and quiet understanding, he ensured the continuity of the program of research. The only change introduced was renaming the Cell Bioengineering Section the Biochemistry of Plant Cell Differentiation Section.

In 1975, Jadu Saha was appointed director of the institute. Dr. Saha had obtained a B.Sc. in chemistry from the University of Calcutta in 1953 and an M.Sc. in applied chemistry from the same university in 1956. Between 1956 and 1959, he conducted applied research in the utilization of coal tar in the Central Fuel Research Institute in India. He came to Canada in 1959 and received a Ph.D. in organic chemistry from the University of Saskatchewan in 1962. In 1962-1963 he won a postdoctoral fellowship in the radiation laboratory at Notre Dame University. He returned to the University of Saskatchewan as a post doctoral fellow during 1963-1964. In 1965, he accepted a research post at the Saskatoon Research Station, where he undertook research in pesticide chemistry. In 1973, Dr. Saha moved to CBRI, where he carried out pesticide residue studies in the Environmental Chemistry Section. A skilled organic chemist, he had made many widely recognized contributions to pesticide chemistry. His publication on mercury pollution was a landmark in the literature on environmental hazards.

Dr. Saha was to head the institute for the subsequent 3 years and during that time the institute again experienced major transformations in both organization and orientation in research. At first he invited five senior scientists to provide advice on research direction for the entire institute; section heads were appointed to improve the efficiency and management of the various resources.

At the end of 1975 Jim Rouatt, who had assumed the demanding and responsible duties of acting director on numerous occasions, retired after 36 years of scientific research and supervision. Jas Singh undertook postdoctorate research in the Winterhardiness Section and later joined CBRI's staff.

On 17 February 1975, a special celebration took place in the nitrogen laboratory of the Analytical Chemistry Service to mark the completion of the 300 000th Kjeldahl determination since 1958, when the laboratory was moved to the K.W. Neatby Building.

In 1978, a group of scientists working in soil chemistry and mineralogy were transferred from the Soil Research Institute to the new Soil Chemistry and Biology Section of CBRI. The scientists transferred included Morris Schnitzer, who became head of the section, Don Gamble, Karl Ivarson, H. Kodama, Suku Mathur, Kaz Morita, John Ross, Surinder Singh, and Norm Miles. Accompanying the transfer of these scientists was a reciprocal transfer of the whole of the Agrometeorology Section from CBRI to the Land Resource Research Institute, formerly the Soil Research Institute.

Additional changes made by Dr. Saha in the previous year also signaled a new era. Dr. Saha took stock of the institute since its establishment, as had his predecessors, and in a critical review of its progress, assessed to what degree each section had fulfilled its original mandate. The assessment was made in an attempt to foresee future needs and developments in agriculture and to adapt the institute's program accordingly. Through its various sections, CBRI was already engaged in new research in response to a rapidly broadening spectrum of problems of concern to agriculture.

The goal of the Winterhardiness Section and program was to gain an understanding of the nature and causes of damage due to frost and ice encasement and to increase the tolerance and winter survival of crop plants. The research dealt with studies of the cytological and biochemical processes in the frost hardening of trees, comparative aspects of the growth capacity and efficiency of spring and winter wheats, and environmental factors and respiratory control in mitochondria associated with cold hardening in wheat. Studies were also conducted on the simulation of freezing and osmotic stresses of plant cells by pure lipid membrane systems, the role of changes in polar lipid unsaturation during hardening, the effect of ice encasement on damage to winter wheat, and the application of new methods for rating cereals for winter survival capacity.

The Host-Parasite-Relationship Section and program, which was now grouped with a cell modification program, had the twofold responsibility of directing efforts to reducing plant disease and improving crop plants through genetic modification of cells. Scientists concentrated their research on host-parasite interactions of viruses and mycoplasmas and their transmission vectors; epidemiology of various virus diseases of wheat, clover, barley, and other crop plants; use of tetracycline-related antibiotics for control of aster yellows and clover phyllody disease; use of serological methods for identification of virus; and genetic manipulation of plant cells through the use of isolated protoplasts, anther cultures, and cell transformation using foreign DNA.

The Agricultural Microbiology Section, whose responsibility was to investigate plant-bacteria-fungi relationships with special reference to soil organisms, had made extensive investigations on many aspects of the soil-borne pathogen <u>Fusarium</u> spp. The studies included its biochemistry; cell wall composition and structure; membrane properties; key enzymes involved in its oxidative and membrane processes; and the induction, survival, and ultrastructure of its chlamydospores, on host-specific toxins and on amino acid production by rumen microorganisms.

The Electron Microscope Centre, which was expanded in 1970, hosted a large number of visiting scientists who conducted diverse investigations on plant cells, viruses, mycoplasmas, bacteria, aphids, milk proteins and cheese, and soil structures. These studies, as well as the postal service that the center had inaugurated under the supervision of Joan Bronskill, were helped by the availability of the new scanning microscope equipment, the development of the new freezing and cryofracture techniques, and continued improvement of the electron microscope equipment.

The Environmental Chemistry Section and program, which was created in 1973 by placing the General Analytical Section and the Pesticide Residues and Operation Section under one wing, had developed new analytical methodology for detecting and determining the persistence, degradation, and translocation of a wide range of pesticides as well as inorganic and organic toxic chemicals in soils, crops, and foods. These chemicals included DDT, 2,4-D, paraquat, lindane, diazinon, malathion, maleic hydrazine, selenium, and chromium among others. In addition, methods for identifying and determining insect pheromones were developed.

The Analytical Chemistry Service, which now consisted of the former Technological Services Unit, Micro-Chemical Laboratory, Amino-Acid Analysis Laboratory, Instrumentation Centre, and Pesticide Residue Laboratory, had provided extensive services to other research establishments. These services included facilities for a large variety of chemical analyses ranging from nitrogen and amino acid analyses and fiber determinations to the identification of trace quantities of chemicals by computerized gas chromatography and mass spectrometry.

The Agrometeorology Section, which was transferred to the Land Resource Research Institute, had as part of CBRI provided valuable service to many research establishments and had made progress in several areas of research on the application of crop weather data to agriculture.

With the substantial enlargement of CBRI, Jadu Saha took the opportunity of effecting some major organizational changes. Of particular concern to agriculture was the depletion of the nitrogen resources that was occurring around the world and the rising energy costs of producing synthetic nitrogen fertilizer. In response to this urgency, Dr. Saha decided to initiate a symbiotic nitrogen fixation program, with a view to increasing the efficiency of biological nitrogen used by legume crops through genetic engineering. To construct such a unit he called on a number of scientists in other sections of CBRI: Dick Miller, L. Barran, E.A. Peterson, Ram Behki, Stan Lesley, Fergus Macdowall, and Claude Sirois. Dr. Miller was to head up the new program. Dr. Saha then combined the remaining members of the Host-Parasite Relationships and Agricultural Microbiology sections to form a plant pathology program. In the meantime, the new Soil Chemistry and Biology Section was increased by the addition of two new soil scientists, Caroline Preston and Laura M. Benzing-Purdie. Bill Marshall of the Environmental Chemistry Section left CBRI to take up a position with the chemistry department of Macdonald College of McGill University.

The general pattern of organization of CBRI and its programs for the years to follow until the present was now well defined, and the institute was on its way to becoming a national center of research. On 26 April 1976 Walter Jopkiewiez retired after 17 years of service with Agriculture Canada, during which time he completed approximately 300 000 analyses for scientists and technicians of the Research Branch. Those who knew him could prove, that he could "out-analyze" any known automated equipment. In April 1978, Joan Bronskill of the Electron Microscope Centre died. Plagued by illness, she nevertheless gave of her energy unstintingly to the task of supervising the busy postal service of the Electron Microscope Centre. Her spirit never flagged and her cheerful presence and dedication are still missed.

In 1979, Jadu Saha left CBRI having assumed new duties, first in May as leader of the task force on managerial accountability and later in November as acting director general of Central Region of the Research Branch. W. Baier, who was transferred with the entire Agrometeorology Section to the Land Resource Research Institute, was appointed acting director in May 1979. S.U. Khan of the Environmental Section assumed additional duties in the capacity of assistant director of CBRI in August of the same year. A further change occurred with the splitting off of a group of scientists from the Plant Pathology Section to form a separate Virus and Mycoplasma Section. The scientists of the new section included Ramesh Sinha, Lloyd Chiykowski, and Yogesh Paliwal.

A publication describing details of the Analytical Chemistry Service of CBRI was published in 1979 and widely distributed across the country. A reference guide entitled <u>Mass Spectra of</u> <u>Insecticides, Herbicides, Fungicides and Metabolites</u>, prepared by Stu Skinner and Roy Greenhalgh, was also published in 1979.

Three visiting scientists and a graduate student conducted research at CBRI that year. The institute lost the dedicated services of Allan Van Dusen, who died in August 1979.

In June 1979 Jacques Langevin, a valuable assistant to Laura Benzing-Purdie, was honored by becoming the 11th recipient of the Julien-Daoust trophy, awarded to the citizen of the year in the National Capital area. This prestigious award was granted to him for the many years he dedicated to volunteer work in sports, summer festivals, and programs for the mentally retarded.

In May 1980 W. Baier returned to the Land Resource Research Institute to resume his former duties as head of the Agrometeorology Section, and A.I. de la Roche became director of CBRI, a position he still holds. Ian de la Roche, was born in Montreal, Que., and obtained a B.Sc. in horticulture from Macdonald College in 1963, followed by an M.Sc. in plant breeding from the University of Massachusetts. In 1970 he was granted a Ph.D. in genetics from the University of Illinois and subsequently spent a year as a postdoctoral fellow with the Department of Chemistry at the University of Ottawa. He joined

the research team of the Ottawa Research Station in 1971 and was later named head of the Grain Quality Laboratory and chief of the Cytogenetics Section. Over the years, he has made a significant contribution to the genetics and biochemistry of grain quality and has attained international recognition for his studies of membrane lipids of fungi and plants and their relation to freezing tolerance in the latter. In 1979, Dr. de la Roche was appointed research coordinator of crops, Planning and Evaluation Directorate. His responsibilities were to advise the department of the planning and evaluation of all aspects of research and development in crops. He also worked closely with various federal and provincial agencies, agri-business, and several international organizations. With his background, Dr. de la Roche brought to CBRI special qualifications, not the least of which was his experience as a "bench" scientist actively collaborating with many other scientists in the institute and aware of their interests and concerns.

G.T. Spurr was seconded in 1980 to Research Branch headquarters. During the year, Dunc Shearer retired. He was long-established and valued member and leader of CBRI's programs in analytical and environmental chemistry. Y.K. Chan joined the Soil Chemistry and Biology Program. Five visiting scientists and one graduate student conducted research at CBRI that year.

The death of Bobby Anderson, a dedicated and skillful technician in the Analytical Chemistry Service, was a sad loss to the institute.

In 1981, Ian de la Roche set out to realign CBRI sections to meet the new demands and challenges in agriculture. Sections were termed programs, and an important step was the addition of a new program on fungal mycotoxins in response to potential hazards in the agri-food industry. Research was to be centered on the development of analytical procedures for mycotoxin detection and on methodology for the production of large quantities of vomitoxins for animal feed trials. Roy Greenhalgh and Chris Young, of the Environmental Chemistry Program, and H. Morita, of the Soil Chemistry Program, were chosen to staff the new Mycotoxin Program. Later on, J.D. Miller and Barbara Blackwell were added to the team.

The biotechnology and other components of the Nitrogen Fixation Program were strengthened by the addition of a new scientist, R.J. (Bob) Watson and by the transfer of Les Barran from the Plant Pathology Program to the Nitrogen Fixation Program. R.B. Pringle retired, C. Madhosingh was seconded to Research Branch headquarters, and E.F. Schneider was moved to the Winterhardiness Program. Also, new studies were initiated on the consequences of acid rain on soils, and CBRI cooperated with members of the Land Resource Research Institute to prepare and publish a publication on acid rain. M. Ihnat, a member of the Soil Chemistry and Biology Program, was also appointed head of the Analytical Chemistry Service, replacing S.U. Khan, who still remained head of the Environmental Chemistry Program. G.S. Gamble of the Soil Chemistry and Biology Program was transferred to the Environmental Chemistry Program. P. Tremblay joined the institute as a new postdoctoral fellow.

In 1981, there were a number of retirements and departures, including E.A. (Pete) Peterson of the Nitrogen Fixation Program and David Siminovitch of the Winterhardiness Program. Dr. Siminovitch did not leave CBRI altogether, but was made an honorary research associate of the institute and was allotted space and facilities to allow him to write on winterhardiness. Yves Cloutier was recruited to replace Dr. Siminovitch on the Winterhardiness Program. S.P. Mathur was transferred from the Soil Chemistry and Biology Program to the Land Resource Research Institute.

The Chemistry and Biology Research Institute was now placing particular emphasis on the transformation and management of soil nitrogen and organic matter, mineralogy, and the impact of acid rain on agricultural soils; mechanisms of winterhardiness and the development of legumes and cereals more resistant to environmental stresses such as frost and ice encasement; enhancement of inoculants and the improvement of host response to biologically fixed nitrogen; environmental impact of pesticide residues; virus and mycoplasma diseases of crop plants and the development of rapid diagnostic methodology such as serology; and toxic fungal metabolites. The Electron Microscope Centre, Analytical Chemistry Service, and Mineralogical Analytical Service maintained by CBRI continued to provide advice and instrumentation analyses contributing to research of Branch scientists throughout Canada in support of branch objectives.

In 1982, in an awareness of the multiplicity of climatic stresses in a country as large as Canada, the Winterhardiness Program was changed to become the Stress Physiology Program. This change allowed the program to respond more directly to a broader range of climatic problems, including drought, icing conditions, and salinity, as well as low freezing temperatures. During the year, the Mineral Analysis Service was created to provide a facility for mineral analyses, and was headed by H. Kodama of the Soil Chemistry and Biology Program. Also, the name of the Virus and Mycoplasma Program reverted to Plant Pathology Program. C. Madhosingh, after serving in Research Branch headquarters, moved to the London Research Centre. The large number of visiting scientists reflected the growing recognition the institute was receiving world-wide. Nine new visiting scientists were now working at CBRI. E.S.P. Bromfield was appointed to the Nitrogen Fixation Program, and Anna Picman joined the Environmental Chemistry Program.

In 1983 new strategies that were being developed reflected CBRI's awareness of the shifting priorities and requirements in agriculture and in environmental stress. The institute was now carrying out research and development in areas of national significance to agriculture, such as food safety, conservation of natural resources, energy, biotechnology, and environmental pollution. Multidisciplinary teams working on mycotoxins and nitrogen fixation responded to health hazards, to the economy of fungal metabolites in food and feeds, and to the need of increasing the nitrogen-fixing capacity of forage legumes through conventional technology and biotechnology. Emphasis in the stress physiology program shifted toward combining genetic engineering technology with the existing knowledge of mechanisms of stress injury and resistance in order to develop crops resistant to low temperature, drought, and salinity. The Plant Pathology Program was developing new immunological methods for rapid diagnosis of virus and mycoplasma diseases. The institute was able to expand its efforts in biotechnology and to place more emphasis on plant and fungal genetics and the application of recombinant DNA techniques to crop production, protection, and nitrogen fixation. The functional capacity of the Electron Microscope Centre, the Analytical Chemistry Service, and the Mineral Analyses Service was enhanced by the acquisition of new instruments and by modification of existing equipment. Although basic research remained the top priority of CBRI's activities, technology transfer to industry and to other public research organizations assumed an ever-increasing emphasis. During the year, CBRI initiated collaborative projects with eight Canadian industries. The institute was therefore achieving the objectives it had set and was becoming widely recognized as a truly national center of research serving Canadian agriculture. This recognition was acknowledged by the honors many of the scientists received from various scientific bodies as well as by the invitations extended to them to organize, chair, or participate in national and international conferences and symposia. More than 1000 research papers have been published by the scientists of CBRI since its founding. Behind these achievements there is a solid core of skilled and dedicated technicians who provide the support required for such an enterprise. The office staff was as efficient in fulfilling the administrative functions of the institute.

The death of Claude Sirois in 1983 was a great loss to CBRI. Dr. Sirois had contributed significantly in the field of plant growth regulators and nitrogen fixation and had retired in March 1983. J.C. Sirois is sorely missed by his colleagues and friends. The foregoing chapters traced the development of CBRI from its origin to the present. In the chapter that follows we describe in more detail the evolution of the institute in the light of its scientific achievements. We are first highlighting the research accomplishments of the programs established at the time CBRI was formed, followed by highlights of the development of new research programs established in response to the needs of Canada's agriculture and food sector.

#### Stress Physiology Program

Objectives: Scientists in the Stress Physiology Program conduct investigations on the mechanisms of cold adaptation and freezing injury in plants and on the role of the environment on overwintering damage to winter cereals under field conditions. Studies on fundamental mechanisms are focused on biochemical and structural changes in cells and cell membranes during cold acclimation freezing stress and ice encasement. Protoplasts and isolated cells that reflect whole-plant responses are used extensively in these investigations. Laboratory screening tests of genetically different materials are conducted to assist plant breeders in the selection of more stress-tolerant winter cereal cultivars. Field trials are being conducted on the cultivars that have been selected for use in eastern and central Ontario. The role of fungal and virus pathogens in overwintering damage to winter cereals is under investigation.

The research highlights of the Stress Physiology Program fall into two main categories: applied field and controlled environmental studies and studies on the basic mechanisms of freezing injury and tolerance. One of the first applied projects was an intensive cooperative program with private industry to develop a foam insulative cover to protect plants from freezing damage. The tests performed demonstrated the feasibility of using modified fire-fighting foams for the protection of sensitive vegetable and strawberry plants against freezing damage and aroused world-wide interest. More recently, field studies were conducted over the past decade on the role of environmental stresses, such as low freezing temperatures, ice encasement, flooding, and snow molds on overwintering potential of winter cereals at the Central Experimental Farm and at various locations in eastern and central Ontario. It is now clear that all four stresses may contribute to winter damage, and furthermore that the genetic elements underlying the inherited tolerance of freezing temperatures are not the same as those affecting tolerance of ice encasement or flooding. Thus, breeding of winter cereals for use in Ontario is now taking into full account all these heritable factors in selecting the best possible varieties for production in various areas. Based on these evaluations, field trials are being conducted to determine suitable cultivars and cultural practices as well as optimum seeding dates for winter wheat in eastern and central Ontario.

The mounting body of evidence pointing to both ice encasement and flooding as two of the major factors in overwintering damage to winter cereals in Ontario has led to investigations concerning the mechanism of injury inflicted by

these stresses. The circumstances surrounding the incidence of the damage have caused the investigations to focus on the whole question of the physiology of anaerobiosis-derived injury in plants and plant tissues. At least three products of anaerobic metabolism have been identified as responsible agents in this These results have led in turn to new research on injury. metabolism of carbohydrates, which also appear to be implicated in the whole complex of factors affecting winter survival. In addition, the widespread incidence of virus infections in winter cereals in Ontario has had an impact on winter damage. Studies have shown that these infections are also determining factors in winter survival. The increasing recognition of the extent to which the snow mold pathogen can exert significant damage to cereals in winters when snow conditions promote growth of the fungus is resulting in research being conducted on the physiology and growth habits of the mold. Based on these studies, methods are being developed to effectively screen large populations of winter cereals for resistance to snow mold diseases.

The initial research at the Central Experimental Farm on the fundamental mechanisms of cold acclimation and freezing injury in plants was directed at seasonal synthetic processes occurring in the cells of living bark of black locust tree during their period of acclimation in fall to winter freezing temperatures. The cumulative results of these investigations strongly suggested that the increase of protoplasm and cell membrane constituents was an integral part of the acclimation process and was probably instrumental in the mechanism of hardening. These observations, coupled with early investigations on dehydration injury caused by plasmolysis or intercellular freezing, added conviction to the hypothesis that the sites of freezing injury and resistance to freezing injury in plant cells were located in the surface membranes of these cells.

Analytical and physical studies on cells of tree bark showed that although the amount of polar lipids in cold-hardened cells is greater than in unhardened cells, the degree of unsaturation of their lipids is not, thereby dispelling the previously held view that an increase in unsaturation of membrane lipids of plant cells is an important element in their freezing tolerance.

The growing conviction that the plasma membrane is the site both of the adaptive changes in hardening and of freezing injury prompted studies of the behavior during freezing of artificial membrane systems known as liposomes. It was found that the freezing behavior of natural cells could be simulated by these microscopic spheres of membrane lipids, thereby further reinforcing the hypothesis that the plasma membrane of living cells is the site of freezing injury. Amino acid and protein analysis revealed important differences between the major membrane proteins of the chloroplasts of cold-hardened and unhardened winter wheat plants. These studies showed that although the amino-acid composition of the proteins of cold-hardened and unhardened plants was identical, the number of exposed sulfhydryl groups in the proteins of unhardened plants was double that of hardened plants, and furthermore that the unfolding of these groups in the presence of certain detergents was more rapid in the unhardened plants.

The successful development of methods for the isolation of free protoplasts, first from cells of cereals and later from cells of tree bark, enabled studies to be made of the freezing behavior of the living protoplasts of these cells free of the complication of the cell walls. Protoplasts of hardened cells were found to retain the same capacity for tolerating freezing as their whole-cell counterparts, thus affording a new system for investigating the cytology and biochemistry of hardening processes. New methods of separation and isolation of whole cells from plant tissues as well as the use of electron spin resonance have provided new opportunities for exploring cytologically the membrane phenomena associated with freezing and cold acclimation.

Electron microscope studies of frozen winter cereal cells showing expulsion and loss of lipids from the surface membranes added new support to the theory of membrane-localized sources of freezing injury and new insight into the nature of this injury. Electron spin resonance studies of changes in fluidity and rigidity of cells of hardened and unhardened cereals during exposure to freezing temperature provided more knowledge of molecular mechanisms involved in freezing injury and of the differences between the cells of hardened and unhardened plants. Nuclear magnetic resonance investigations bolstered the ultrastructural evidence that freezing and other dehydrative stresses resulted in loss of membrane bilayers from cell membranes during freezing.

The discovery that the epicotyls of young seedlings of unhardened winter cereals subjected to desiccation for only 24 hours at room temperature could be conditioned to tolerate freezing has altered the views on the environmental factors that control hardening. The observation that temporary dehydration of plant tissues, even at room temperature, can induce frost hardening was a revelation in the field of physiology of plant freezing resistance. On the basis of these observations, methods of rapid screening of cereals for winterhardiness without use of low-temperature facilities have become possible.

Radioactive tracer techniques have been used to determine the effect of environmental stress on the physical and biochemical processes of winter hardening and injury in isolated winter wheat cells. These studies have revealed that the ion transport system of the cells is an early and primary site of freezing injury in winter wheat. Since this system is associated with the plasma membrane, these observations provide further evidence of a central role for the plasma membrane in cold acclimation and low-temperature injury.

The emerging technology of genetic engineering holds great promise for advances in the development of new varieties of plants more tolerant of adverse winter conditions. The Stress Physiology Program, embracing these new techniques, which include tissue and cell culture, is embarking on new ventures to improve the potential of crop plants for winter survival. Also, the program has been broadened to include research on drought and salinity injury and tolerance.

#### Plant Pathology Program

Objectives: Scientists in the Plant Pathology Program conduct research on the diseases (caused by viruses and mycoplasmas) of cereals, forage crops, and certain stone fruits, in order to develop knowledge and technology for improved disease-control measures. The manner in which such diseases are contracted and spread by various insect vectors--aphids, leafhoppers, mites, and beetles--is investigated on a national basis. Biological and chemical properties of viruses and mycoplasmas are determined, and serological methods for rapid disease diagnosis are developed. How viruses and mycoplasmas damage their plant hosts is investigated by electron microscopy. Various virus strains that infect crops each year, their overwintering hosts, and their capacity to infect various cultivars are studied to help plant breeders develop resistant varieties. The effects of viral infections on winter survival of cereals and on symbiotic nitrogen fixation in leguminous plants are also being investigated.

Progress and research achievements: Most virus diseases are spread by vector insects, but very little was known in the late 1960s about the relationships of viruses with their vectors, and techniques for rapid identification of viruses affecting Canadian crops had not been developed. Such information was essential for formulating control measures. However, there was no specific group in the Research Branch at that time working in these areas. This problem was recognized at the 1966 virus work planning meeting held in Vancouver, and the group in Ottawa took up the task of determining the relationships of selected viruses to their insect vectors and of developing serological methods for rapidly identifying the diseases they caused.

In 1967, the work published from Japan suggested that certain plant diseases may be caused by mycoplasma-like organisms and not by viruses, as had been assumed for several years. Scientists working in Ottawa soon demonstrated that several diseases of the yellows type, which caused economic losses to a variety of crops in Canada, are caused by nonhelical and nonculturable mycoplasmas. These mycoplasmas were shown to have a wide range of hosts and were capable of infecting forages, cereals, vegetables, small fruit plants, and fruit trees. Several newly recognized mycoplasma diseases and their vectors were described. It was demonstrated that mycoplasmas not only multiply in host plants, but do so in their leafhopper vectors. Detailed work on transmission characteristics of mycoplasmas described the mechanism of vector transmission and specificity. For the first time, methods were developed to purify mycoplasmas from diseased plants, and their structural characteristics and chemical composition were determined. Scanning electron microscope techniques were developed that allowed the visualization of mycoplasmas in three-dimensional form in plants. Specific antisera against mycoplasmas were prepared and immunological methods were developed for rapid diagnosis of diseases caused by these microbes, such as aster yellows, clover phyllody, and peach-X. The screening of a number of inhibitors and their uptake and persistence in variety of plants revealed that tetracycline antibiotics can be used effectively to provide protection against mycoplasmas and to cause remission.

The research on viruses was similarly conducted. Wheat striate mosaic virus was shown to multiply in its leafhopper vector, its mechanism of transmission was determined, and the factors affecting its spread were identified. The virus was purified, specific antiserum was prepared, and serological methods were developed for disease diagnosis. The transmission mechanism by mite vectors of wheat streak virus, another virus affecting cereals, was described and was found to be distinct from the mechanisms known in other virus-vector systems. Similar work was carried out with tomato spotted wilt virus, which is transmitted by thrips. Late in the 1970s, an epidemic of barley yellow dwarf virus occurred in eastern Canada, and scientists of the Plant Pathology Program started working on this virus. During an exhaustive 4-year study, it was found that the virus occurs in four strains, is specifically transmitted by various aphid vector species, and is responsible for infection in oat and barley crops in eastern Ontario and western Quebec. It was further demonstrated that the so called "resistant" varieties of oats were in fact only "tolerant" of the virus because they had symptomless infection and could act as virus reservoirs from which the infection could spread to other susceptible hosts. Plant breeders were advised that yield comparisons, and not symptoms, should be used to assess the

cereal varieties being developed for tolerance of the virus. Methods were then developed for purification of the virus, specific antiserum was prepared, and serodiagnostic techniques were applied for rapid disease identification in small samples of plants. It was demonstrated that the virus in plants is phloem-specific, causing a vascular disease different from other known viral diseases. Persistent transmission of the virus by aphid vectors was shown to be due to efficient preservation and circulation of the virus, and not to its multiplication in the aphid body. It was further demonstrated that the virus infection in winter cereals greatly reduced cold hardiness of oats and barley. Detailed studies were also carried out on the virus diseases of forage legumes. White clover mosaic virus infection in red clovers caused a significant reduction in the plant growth, nodulation, and nitrogenase activity in nodules produced by Rhizobium trifolii, and it was demonstrated that the nitrogen-fixing capacity of clovers is reduced significantly because of infection by this virus. Recently, the newly recognized lucerne transient streak virus, which attacks alfalfa in Canada, was described. Biochemical studies on this virus revealed that it contains a large linear RNA and a circular viroidlike RNA in the virions--a rare RNA complement for viruses. It was shown that the replication of the viroidlike RNA of the virus can be directed by the genome of southern bean mosaic virus (SBMV), and that the RNA is encapsidated in the The SBMV virions that contain the RNA induced a SBMV virions. disease of increased severity in plants showing the pathogenicity-modifying property of the RNA.

In summary, the most significant contributions on plant viruses and mycoplasmas have been in the following research areas: specificity and mechanisms of transmission by different types of vectors, biochemical properties of pathogens, development of immunological methods for rapid disease diagnosis, and identification of disease agents and their vectors new to Canadian agriculture.

#### Environmental Chemistry Program

Objectives: The Environmental Chemistry Program is concerned with the effectiveness and environmental consequences of pesticides that are being used in Canadian agriculture. Research is undertaken on the chemistry, distribution, persistence, bioavailability, and fate of these pesticides and their residues in plants, animals, soils, and water. Bound pesticide residues in soils are examined as potential sources of crop contamination and, when present in food crops, with reference to their toxicological significance to mammals. Prediction models are developed to forecast variations in pesticide persistence caused by climatic and soil conditions.

Scientists in the Environmental Chemistry Program collaborate with other scientists in conducting research on a broad range of problems in support of departmental goals and the Canadian agricultural research effort. An essential prerequisite in achieving these goals was the development of analytical methodology for the detection of minute quantities (micrograms per gram or nanograms per gram) of a large number of organic or inorganic pesticide residues present in agricultural commodities and of some unusual biologically active organic compounds related to agricutural production. The methods rely heavily on gas chromatography (GC), high pressure liquid chromatography (HPLC), and mass spectrophotometry (MS). Consequently, the evaluation of the most sensitive detectors was an important component of the program. Among the detectors that were tested and used were electron capture, thermionic flame photometer, Hall and Goulson electric conductivity detectors for gas chromatography, and ultraviolet and fluorescence detectors for liquid chromatography.

All these methods were used to study the fate, persistence, binding metabolism, translocation, and chemical and biological degradation of pesticides in the biosphere. In the course of these investigations, a large number of established and newly synthesized insecticides, fungicides, herbicides, and growth regulators present in animals, plants, soil, and water were investigated.

Studies of the metabolism and fate of one of the most persistent and widely used organic chlorine compound, chlordane, using radioactive carbon derivatives as a tracer, showed that residues are readily absorbed from soils by carrots but are not degraded into other products in soils or in carrots. Similar studies performed on the metabolism and degradation of lindane in chickens, farm animals, soils, and stored grain of wheat showed that this compound also is not easily degraded. Examination of the levels of methoxychlor and products of degradation resulting from the use of methoxychlor instead of DDT for control of Dutch elm disease provided information on its stability on the bark of trees after spraying. The efficacy of methoxychlor was found to exceed that of DDT in controlling horn flies on cattle, but methoxychlor was also shown to be as toxic to trout fingerlings as was DDT. The determination of levels of the organophosphorous compound dimethoate in wheat and soil in connection with a grasshopper control program revealed the disappearance of most of the dimethoate, after 7 days. Decay curves over a 2-week period on another organophosphorous compound, diazinon, applied to sheep for the control of the blow fly showed diazinon to concentrate in wool but not in blood, kidney, or liver fat. A study of the uptake, fate metabolism, and transport through seed coats of the organophosphorous compound fenitriothion, used to control spruce budworm, was also undertaken. Gas chromatography and electron capture were used

to determine simultaneously 2,4-D and 2,4,5-T in soil and water. After sensitive analytical procedures were devised for the nonselective herbicides paraquat, diquat, and linuron, as well as dyfonate and carbofuran, they were applied to the study of the persistence and movement of these pesticides in various soils and their transport in vegetable crops.

Some other typical investigations included methodology for determinating levels of mesurol, a bird repellent, on blueberries; of etherel on apples and methods of washing it off; of malathion and etherel residues on lettuce; of atrazine in eggs after being fed to chickens; of dithiocarbamate fungicide residues on field-treated tomatoes; of fensulfothion and its derivatives and other phorate compounds that showed rapid uptake and high residue levels of these compounds in tips of various vegetables and crop plants; of simazine and prometryn in various types of soils; of prometryn on animals dip-treated for the control of blow fly; and of glyphosate taken up by oats after treatment of soils. Apart from these pesticides, scientists in the Environmental Chemistry Program conducted research on the development of analytical procedures for essential and toxic compounds in agricultural products. One of these projects was the methodology for estimating minute quantities (nanograms per gram) of N-nitrosamines in processed-meat products where there had been a concern about the carcinogenicity of these compounds. Another growing concern was the possible hazard associated with consumption of ergot-alkaloid-contaminated feeds by domestic animals. Methods were thus developed for determining the alkaloid content of ergot sclerotia and were applied to samples collected from rye and triticale. The potential dangers of these and other toxic compounds in foods eventually led to the recognition of the need for a separate program in CBRI. The Environmental Chemistry Program was also engaged in research on pheromonal communication and feeding attraction in the honey bee. Identification and isolation of these pheromones could ultimately serve to improve hive management by chemical means.

In the area of inorganic chemistry, the environmental chemistry program undertook the identification and estimation of trace elements in soil, plants, and water. The elements, which are either required by or toxic to animals, included selenium, chromium, silicon, magnesium, calcium, phosphorus, manganese, iron, zinc, copper, cadmium, and lead--all of which are suspecting of having or have been found to have adverse or protective effects on animals. Appropriate methods have been developed for the accurate determination of trace or ultratrace levels of these elements in biological samples. Detailed investigations of the analytical chemistry of selenium, together with an interlaboratory study under the auspices of the Association of Official Analytical Chemists (AOAC) led to the development of an official fluorometric method for the determination of this important element in foods.

#### Soil Chemistry and Soil Mineralogy programs

Objectives: Scientists in the Soil Chemistry and Soil Mineralogy programs conduct research on organic and inorganic soil components in order to develop knowledge and technology for maintaining optimum agricultural productivity in soils. Studies are undertaken on the chemical and physical characteristics of the soil humic and fulvic acid fractions and their interactions with metals and clay minerals. Nitrogen investigations are concerned with soil tranformations and losses of this important plant nutrient. Research is also aimed at the characterization of the large nitrogen reservoirs in soil that are immobilized in stable organic forms.

Mineralogical studies of various Canadian soils are carried out in order to understand soil formative processes. Improved methodologies are developed for the identification and quantification of crystalline and noncrystalline inorganic soil components. The reactions of these components in acid environments are investigated to evaluate their effect on the capacity of soils to adsorb, store, and release water, nutrients, and toxic elements to plants and microbes.

Progress and research achievements: The transfer of the soil chemistry and mineralogy group in 1978 completed the final stages of CBRI's evolution.

The scientists in the soil chemistry and mineralogy programs are equipped with sophisticated X-ray, infrared, and Mossbauer spectroscopic equipment required for basic investigations of the mineralogy and chemistry of soils and for a mineralogical analytical service to soil scientists.

The incorporation of new methodology following acquisition of new X-ray diffraction equipment in 1981 allowed for further expansion of these facilities and services. During 1977 and 1978, the Soil Mineralogy Section carried out the mineral composition analyses of 100 Canadian soil profiles for the soil tours that were organized in connection with the eleventh congress of the International Society of Soil Science held in Edmonton in 1978.

The compilation of all of the available mineralogical data on Canadian soils published from 1937 to 1977 showed that clay minerals in subsoils were inherited mainly from bedrock. The genetics of soils could not be characterized simply by specific

assemblages of the clay mineral content. The data also showed that Podzolic soils exhibited the most prominent mineral transformations of all Canadian genetic soil types. Studies had shown that the reactions and properties of clay material could be used effectively as criteria to distinguish common soil clay materials. It was also demonstrated that clay content, clay mineral composition, and specific surface area have a major influence on the contraction and expansion of soils. Aluminum hydrous oxides were found to affect the stability and exchange characteristics of interlayered clays. X-ray diffraction and Mossbauer techniques and procedures were proved to be especially useful for the characterization and quantification of inorganic soil components, including crystalline and noncrystalline The studies on the aqueous and solid phases of components. aluminum showed that the presence of the type of anions associated in acid conditions play a significant role in controlling the forms of soluble aluminum and solid-phase The formation and coexistence at room temperature of aluminum. four crystalline aluminum components in solid phases as products of aluminum sulfate hydrolysis were demonstrated. The fulvic acid at high concentrations was found to inhibit crystallization of aluminum hydroxides. The occurrence of lepidocrocite in well-drained soils, a mineral not previously reported to be contained in these soils, and identification of the mineral halloysite in the clay fraction of Canadian soils were new findings.

With new instrumentation and modern physical techniques, scientists succeeded in making significant inroads into the problem of the composition of humic and fulvic acids as well as discovering the nature of the "unknown" immobilized nitrogen fraction of soils. With the use of a new procedure developed for obtaining a fraction rich in unknown nitrogen, the important discovery was made that a large part (up to 25%) of the unknown nitrogen could be accounted for in terms of purine and pyrimidine bases. Biodegradation experiments with soil microbes showed that these nucleic acid bases were ready sources of available nitrogen when absorbed on clays but not when absorbed on iron and aluminum oxides. New hydrolytic procedures were devised to isolate the fraction that contained almost 99% of the elusive unknown nitrogen. Modifications of these hydrolytic procedures applied to some of these fractions rich in unknown nitrogen, and characterization by mass spectrometry revealed that the major fractions contained hydroxy and oxyindoles, and a number of benzylamines and nitriles.

Turning to the chemistry of humic and fulvic acids, studies of these compounds extracted from soils of widely differing organic matters showed that all humic substances have essentially similar structures and characteristics. Major humic building blocks were in all instances complex phenolic and benzene carboxylic structures. Studies have demonstrated the need and potential of nuclear magnetic resonance (NMR) spectroscopy to resolve the complex problem of the chemistry of humic and fulvic acids. Solid state <sup>13</sup>C-NMR of humic and fulvic acids, humins, and hydrolysis residues of four soils showed that solid-state <sup>13</sup>C-NMR (a nondestructive technique requiring no sample preparation) showed distinct chemical differences between fractions and provided useful structural information on these organic soil components. It was demonstrated that the enzyme pronase was even better than acid hydrolysis in preserving the integrity of humic acid materials for chemical studies.

It was shown, for the first time, that all acidic carboxyl groups in these materials are attached to aromatic structures. This finding is of particular importance to interactions of metal with organic matter, where the strength of metal binding of organic ligands is strongly affected by the type of structure to which the functional group is attached. Studies also confirmed that phenolic hydroxyl groups are important functional groups in humic materials.

The work of soil microbiologists on microbial interactions with iron compounds and other soil minerals in relation to acid sulfate soils and to clogging of agricultural tiles has produced results of immediate consequence to farmers. It was also shown that clogging and sealing of farmers' fields by iron oxides is very extensive, with soil microbes playing a major role in the clogging.

A finding of considerable interest by the soil microbiologists was that the fungus <u>Scytalidium acidophilum</u> can grow in acid hydrolysates (pH 1.0) of waste paper and can convert 97% of the sugars in the hydrolysate to a fungal biomass containing high amounts (45%) of protein.

Efforts to devise an economic and safe method of mitigating organic soil loss are being pursued. Studies have shown that both the rate of carbon loss through aerobic soil microbial respiration and the level of an indicator enzyme are correlated with copper in the soils. It has now been well established that climatic conditions and intensive agricultural practices, such as fertilization, irrigation, improved drainage, and no-tillage cultivation, all contribute to soil acidification. As a result, aluminum becomes an important exchangeable cation in these soils. Acid rain (precipitation) derived from industrial plants is now becoming a growing factor in the problem of acidity. In an effort to alleviate this problem it was found that  $SO^{2-}$  ions could reduce the amount of Al<sup>3+</sup> by complexing with it. Thus, application of gypsum to acid soils before liming should be effective in reducing aluminum toxicity and increasing base exchange capacity.

Work is also continuing on the nature and availability of soil nitrogen. Plant uptake, acid hydrolysis, and incubation were used to assess the relative availability of residual fertilizer <sup>15</sup>N immobilized in the soil in either clay-fixed ammonium or in organic forms. The results confirmed recent reports that fertilizer-derived clay-fixed ammonium is a relatively dynamic fraction, more available for plant uptake than residual nitrogen fertilizer in organic forms.

#### Nitrogen Fixation Program

Objectives: The major objective of the Nitrogen Fixation Program is to enhance the contribution of symbiotically fixed nitrogen to the nitrogen economy of major crops. Because of the complexity of this naturally occurring symbiotic interaction, the biochemical, physiological, and genetic aspects of both the bacterial and plant partners are being studied. Methods are being developed for the rapid identification of symbiotic nitrogen-fixing microorganisms to screen field isolates and genetically modified Rhizobium strains for competitiveness and survival under controlled and field conditions. Genetic engineering techniques are used to manipulate the bacterial genes in order to improve the competitive and nitrogen-fixing traits required for effective inoculants. Studies are also under way on the energy requirements and efficiency of the nitrogen fixation process. A breeding program aimed at producing alfalfa varieties with improved host compatibility and growth response to biologically fixed nitrogen has been initiated. Various growth parameters are investigated in relation to the photosynthetic capacity of alfalfa plants. These plants are used to select varieties that have more active nitrogenase systems and earlier response to symbiotically fixed nitrogen.

Progress and research achievement: The Nitrogen Fixation Program at CBRI began officially in 1978. Following discussions with scientists from the Research Branch, the National Research Council of Canada, and the University of Wisconsin, an interdisciplinary approach was adopted. The forage legume system, with alfalfa as host and <u>Rhizobium meliloti</u> as microsymbiont, was selected as the focus of the program.

In the early days, some encouraging studies were carried out on the use of bacteriophages and intrinsic antibiotic resistance as potential techniques for identification of the multitudinous strains of the indigenous <u>R. meliloti</u>. At the same time, a method based on immunological techniques for tackling the identification problem was developed. The importance of having identification methods for <u>Rhizobium</u> isolates obtained from intact nodules became apparent when it was demonstrated unequivocally that many field isolates were not effective in reducing atmospheric nitrogen; in fact, some were pathogenic, in the sense that although they readily formed root nodules on some alfalfa cultivars, they actually caused a decrease in the rate of growth of young alfalfa seedlings. Other studies established quantitative differences in the growth rate of alfalfa in response to symbiotic nitrogen as opposed to chemical fertilizers. Existing cultivars were developed for their response to chemical fertilizers, as might be expected from their breeding history, because symbiotic growth had not heretofore been a breeding criterion.

Cooperative studies with Carleton University were initiated on the introduction of genetic markers into <u>R</u>. <u>meliloti</u> strains, on the examination of the plasmids of <u>R</u>. <u>meliloti</u>, and on the development of vectors for modifying the <u>Rhizobium</u> genome. Studies of the multiple role of adenosine triphosphate (ATP) in driving the energetically expensive nitrogenase system were undertaken. This knowledge was later applied to a more general study of the intranodule physiological requirements of symbiotic fixation. A plasmid coding for an uptake hydrogenase enzyme system was transferred successfully and fully between various <u>Rhizobium</u> species, but unfortunately the gene was not expressed to a significant extent in alfalfa nodules infected with the transconjugant.

The three Rhizobium identification methods were evaluated, and the bacteriophage technique proved to be the most widely applicable. However, if the antibiotic method were applied carefully, it could be used in the laboratory to assess competitive abilities of strains, in spite of genetic instability of intrinsic antibiotic resistance. In 1982 a program was initiated on lectin binding to carbohydrates with the use of nuclear magnetic resonance spectroscopy. The significance of this reaction lies in the fact that lectins are thought to be involved in the initial binding of Rhizobium cells to plant root hairs. Diversification of this program could include studies on nitrogen fixation in free-living soil organisms; on the transfer of nitrogenase nutrients between plant species in the field; and on symbiotic interaction at the cellular level in plant tissue culture systems.

The early research achievements of the group were largely concerned with definition of the limitations on symbiotic nitrogen fixation in contributing to the nitrogen economy of legume crops. Many constraints were identified, including those of photosynthate and oxygen supply to root nodules. Many naturally occurring strains of <u>R. meliloti</u> were not effective enough in fixing nitrogen, and host-microsymbiont interactions were shown to play a major role in determining both the competitive ability and the effectiveness of inoculant strains under field conditions. The process of differentiation that the bacterial partner must undergo within nodules in order to express nitrogen-fixing capability was investigated. The way in which the energy embodied in adenosine triphosphate (ATP) supports the conversion of atmospheric nitrogen to nutrient forms was elucidated further. The effects of root aeration, CO<sub>2</sub> fertilization, light intensity, root temperature, divalent cation, nutrients, and other aspects of symbiotic nitrogen fixation in alfalfa were determined, and optimum conditions were defined in the laboratory. The possibility of extrapolation to field conditions is now being examined intensively.

On the genetic side, cloning vectors for modification of the <u>Rhizobium</u> genome have been developed, and genes for certain traits, normally lacking but potentially useful to the symbiosis, have been introduced into <u>R</u>. <u>meliloti</u> by transconjugation. Mutants of <u>R</u>. <u>meliloti</u> have been obtained that allow further mapping of the genes required for establishment and maintenance of more effective and less costly (to the host) symbiotic associations. The area of genetic engineering of host and symbiont holds the greatest promise for enhancement of biological nitrogen fixation, and consequently, much of the research effort is directed to this area.

#### Mycotoxin Program

Objectives: The Mycotoxin Program is concerned with the potential hazards of fungal toxins in food and feed on human and animal health, and the economic losses brought about by reduced grain quality. Analytical procedures are being developed for the detection of toxins or their metabolites in grain as well as biosynthetic studies, and for the rapid screening of mycotoxins in field crops and stored grain. Research is concerned with studying the metabolic pathways of toxin formation by fungi in crops in order to develop control measures. Large-scale production of fungal toxins is being carried out to produce sufficient quantities of trichothecenes for feeding trials in order to establish safe levels for registration and to study their toxicology. Other areas of research are concerned with the decontamination of grain and the chemotaxonomy of fungi that produce toxins.

Progress and research achievements: The Mycotoxin Program launched in 1981 was the result of a growing awareness of the hazards to both humans and animals of certain natural toxins present in cereal foods. The program was a logical offshoot of the existing program in environmental chemistry, and its creation as a separate entity was a recognition of the threat to health that these toxins present. The program has addressed itself to two major sources of food and feedstuff toxicity: the products and secondary metabolites of the fungus <u>Fusarium</u> <u>graminearum</u> (such as zearalenone, deoxynivalenol (DON), and the trichothecenes), which infect grain and grain products; and the ergot alkaloid produced in sclerotia on barley and triticale, which can enter the food chain in processing and milling.

Culture methods for fungi were refined in order to examine the effects of these toxins on the quality and safety of food and feedstuff and to explore ways of eliminating or minimizing these effects. Procedures were also developed for large-scale production of fungal toxins, from <u>Fusarium roseum</u> and <u>F</u>. <u>culmonum</u>, in large fermentation tanks in order to produce sufficient quantities of toxins for feeding trials and to develop accurate methods for detection and routine analysis. The techniques of gas chromatography and high performance liquid chromatography were found to be very effective for such analysis, especially when combined with mass spectrometry.

Milling of mycotoxin-contaminated wheat led to the fractionation of levels of toxins that were higher in bran and lower in the inner flour portions. The effect of baking on nonyeast products resulted in further reductions of toxins. The results of these studies, together with the existing toxicological data, enabled Health and Welfare Canada to increase the allowable amount of toxins in contaminated wheat to 2 ppm for human consumption.

A major finding of the many animal feeding trials carried out in collaboration with the Animal Research Centre with naturally contaminated wheat and clean wheat to which equivalent amounts of toxins were added, is that the naturally contaminated wheat is usually more toxic. These data suggest that other secondary metabolites of <u>F</u>. <u>graminearum</u> may be the causative factors of contamination or that synergistic effects do occur. The large-scale production of deoxynivalenol has allowed the isolation of some 16 minor metabolites, which were characterized by nuclear magnetic resonance spectroscopy. In addition to the isolation of several new trichothecenes, some compounds with a modified trichothecene structure have also been identified as metabolites of <u>F</u>. <u>roseum</u> and <u>F</u>. <u>culmorum</u>. The potential toxicity of these compounds is being investigated.

The genotypic response of <u>F. culmorum</u> and <u>F. graminearum</u> isolates has been determined in liquid cultures using mass spectral analysis of the secondary metabolites produced. Information on the biosynthetic pathways involved in the formation of these metabolites has also been studied with the use of stable isotopes and analysis by nuclear magnetic resonance spectroscopy. These results allow specific nutrient limitations to be identified, and physical and cellular parameters that regulate the biochemical pathways during mycotoxin formation to be determined. It has been established that resistant strains of wheat, rather than susceptible strains, can degrade trichothecene mycotoxins. This information will increase our understanding of mycotoxin formation and its eventual control in the field.

Similar approaches are being made in research on the ergot alkaloids. Analytical procedures were developed for these alkaloids that enable the determination of the variability in total and individual alkaloid content in sclerotia of triticale and barley varieties in various locations throughout Canada. Feeding trials were also conducted on growing swine, and it was shown that after 6 weeks of feeding trials, 0.1% dietary wheat or rye ergot reduced their average daily weight gain. Methods of decontaminating were also explored, and it was found that chlorine and heat were effective in achieving a 90% reduction in alkaloid content of treated ergot sclerotia. With the progress already made, there is much hope that ways of alleviating the threat of these and other food toxins will be identified.

#### Electron Microscope Centre

Objectives: The Electron Microscope Centre provides a research and service facility for transmission and scanning electron microscopy, electron diffraction, and X-ray spectrometry. It is a continuing task of the unit to develop or modify methodology and analytical procedures to meet the specialized requirements of branch scientists for solving agricultural problems.

Progress and research achievements: In the late fifties and early sixties there was a growing awareness of a need for electron microscopy facilities for scientists in Ottawa. In 1964 a Siemens 1A transmission electron microscope (TEM) was installed, and the Electron Microscope Centre was set up. Early concerns over the new style of operation, in which instrumentation and ancillary facilities were available to everyone, quickly dissipated and the number of trained users increased dramatically. By 1969 the demand for electron microscopy facilities led to a marked expansion of the center. The physical size was doubled, and a Cambridge scanning electron microscope (SEM) was added. In 1971 the Siemens microscope was replaced by a Philips 300 transmission microscope. In 1972 a postal service was instituted. This new service permitted branch establishments across the country that did not have electron microscope facilities to use the services of the Electron Microscope Centre in support of their programs. A second scanning microscope (AMR 1000A) was added in 1977 and a

X-ray spectrometer interfaced with the Cambridge SEM to facilitate investigations of soil scientists. By 1978 the Electron Microscope Centre was playing a very prominent role in many branch programs. The Ottawa campus had as many as 65 regular users, and the center was contributing to 40-60 scientific publications per year. Early studies on the ultrastructure of cells and on taxonomy were extended to the examination of structure in relation to function (dormancy, stress, disease). Ancillary studies using X-ray diffraction and spectrometry contributed to mineral classification and the examination of soil structure. The Food Research Institute pioneered the world-renowned studies on the ultrastructure of dairy products. An atlas of pollen structure was produced by the Biosystematics Research Institute. Scanning microscopy was proving to be a major tool in studies of phylogeny and classification of plants, insects, and arachnids -- a boon to the Research Branch, the custodian of the national collections. Special methodologies were also developed for the preparation of diverse biological specimens. Of special note are the classical freeze-fracture methods, which reduce freezing damage in biological specimens to be viewed by scanning electron microscope or transmission electron microscope.

The facilities of the Electron Microscope Centre were now widely recognized, and consequently the center was frequently asked to provide support and training for Ottawa universities, community colleges, other government departments, hospitals, and the Canadian Red Cross. Visiting scientists from Canada and around the world have made use of the facilities over the years.

In the early 1980s, microscopy was trying to keep pace with the advancements in bioengineering. Needs for high-resolution microscopy were increasing rapidly, and the new preparatory techniques required more sophisticated microscopes and auxiliary instrumentation. In 1983, a Balzer's (BAT-400T) freeze-etch device was added to facilitate specimen preparation for scanning electron microscope and transmission electron microscope. In 1984 the technical capability of the center was further increased by the addition of a high-resolution scanning microscope (ISI-DS-130), an X-ray spectrometer (T-N 5500), and an image analyzer system (Kontron SEM-IPS). The interfacing of these pieces of equipment permits the morphologic and geometric quantification of biological and physical specimens, as well as elemental identification and distribution. The Electron Microscope Centre also provides glass-blowing services to all the research programs at CBRI as well as to other branch establishments.

Today, Agriculture Canada scientists have access to one of the best equipped electron microscopy centers in North America. The provision of in-house support is a major attraction for researchers who are exploring electron microscopy for the first time or who are branching out to more sophisticated techniques.

#### **Research Services**

Objectives: Research Services provide support to branch establishments that lack personnel and equipment for carrying out their own analyses. The services include the Mass Spectrometry-Nuclear Magnetic Resonance Spectrometry (MS-NMR) Unit, the Mineralogical Analyses Service, and the Analytical Chemistry Service. The MS-NMR Unit provides research and service facilities for the identification or confirmation of chemical compounds. The Mineralogical Analyses Service contributes to the identification and characterization of minerals in soil. The Analytical Chemistry Service offers a wide range of chemical analyses of soils, plants, animal tissues, and other agricultural products.

Progress and achievements: The Mass Spectrometry-Nuclear Magnetic Resonance (MS-NMR) Unit is used to identify unknown compounds isolated in conjunction with many projects in the branch. In 1973 mass spectral analysis was limited to sampling by direct probe and interfacing with a gas chromatograph. In subsequent years, spectral facilities were greatly expanded with the installation in 1974 of a Finnegan 1015 gas chromatographmass spectrometry (GC-MS) data acquisition instrument. From 1973 to 1978, the number of spectra produced for the identification and confirmation of organic compounds more than tripled. These spectra were prepared in collaboration with a number of branch establishments. Scientists working on pesticide residues took advantage of the spectral analyses provided by the new instrument in the unit. These spectral analyses provided confirmation of pesticide residues and their metabolites. The Dupont mass spectrometer was adapted so that 15N analyses could be carried out routinely. Data banks of mass, nuclear magnetic resonance, ultraviolet, and infrared spectra have been developed over the years and have proved invaluable in supporting various research programs. The data bank of mass spectra of insecticides and their metabolites was published by Agricultural Canada and has had worldwide circulation. In 1979 the unit adopted capillary column GC-MS as a routine analytical technique for the separation of components in several complex biological samples. In 1982 the unit acquired a high-resolution MAT 319 mass spectrometer to provide confirmation of mycotoxin residues in grain in support of Canadian exports.

The Mineralogical Analyses Service provides support to several professional and technical staff from research

institutes, research stations, and outside agencies. X-ray diffraction, infrared absorption, and Mossbauer spectrometry as well as thermogravimetric analyses are being carried out. Over the years the Service has produced thousands of X-ray diffractograms and infrared spectra, and has contributed supportive data to dozens of papers and reports on mineral characterization of Canadian soils and mineral weathering. The recent addition of a fully automated X-ray diffractometer has increased the production of diffractograms by 50% and has improved the quality of diffraction data.

Over the years the Analytical Chemistry Service has provided CBRI and other branch establishments with analyses in support of research projects in soil management and protection; land use, energy production and conservation; environmental quality; improvement of dairy cattle and poultry as well as cereals, oilseeds, forages, and horticultural and field crops; food processing; and new product development.

During 1972-1973, over 30000 samples for various types of chemical constituents submitted by the research establishments were analyzed. With the purchase in 1977 of a Coleman carbon and nitrogen analyzer, the number of analyses for these elements increased sixfold between 1977 and 1980. In 1979 two analytical methods were developed: the microdetermination of total sulfur and inorganic sulfate in biological material and the microdetermination of molybdenum in plant tissues and blood plasma. In that year more than 78 000 analyses were performed by the Analytical Chemistry Service. In order to handle the work, a computerized system for recording and processing analytical data was adopted. In addition, two computer Datacom 400 terminals were acquired to improve the calculation, compilation, and storing of data. In 1980 a method was developed to rapidly estimate sulfide in rumen and blood with the use of sulfide-specific electrode. In 1983 over 70 000 determinations were provided to 130 scientists and technical staff from 20 research establishments. Priority analyses of amino acids were conducted for the Mycotoxin Program. The acquisition of an automated atomic absorption spectrometer was instrumental in increasing the laboratory's capacity to measure metallic elements. Over the years, the Analytical Chemistry Service provided CBRI and other branch establishments with analyses on a wide range of research materials including dietary fiber, fat, cellulose, lignin, ash, macroelements, trace elements, nitrogen, proteins, amino acids, carbohydrates, and organic functional groups. Technique-dedicated microcomputers have been introduced into several laboratories to speed up data acquisition and processing.

Evaluation of automated spectrophotometric and manual titrimetric methods for total and reducing sugars was completed,

with the former procedure adopted for rapid routine application. The nitrogen laboratory was renovated with the bulk of the nitrogen determinations transferred to the Technicon autoanalyzer. Acquisition and calculation of amino acid data were facilitated by interfacing a microcomputer with the amino acid analyzer. Protocols were devised for the assessment of sample homogeneity and for the quantification of elemental concentrations in agricultural reference materials. Maize reference materials recently produced were analyzed by cooperating laboratories with the use of independent methodologies to establish levels of macro- and micronutrient elements, as well as elements of toxicological and environmental interest. These materials will be available to departmental laboratories as to well as the international analytical community for analytical data quality control.

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In the preparation of the history of CBRI, I tried to mention as many people as possible. I apologize for any inadvertant omissions. Furthermore, the limitations of space preclude the mention of everyone who was directly or indirectly associated with the institute.

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ADMINISTRATION	STRESS PHYSIOLOGY PROGRAM	PLANT PATHOLOGY PROGRAM	ENVIRONMENTAL CHEMISTRY PROGRAM	MYCOTOXIN PROGRAM	NITROGEN FIXATION PROGRAM	SOIL CHEMISTRY PROGRAM	SOIL MINERALOGY PROGRAM	ELECTRON MICROSCOPE CENTRE
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