Molecular Genetics, Biotechnology
and
Experiment Station Research

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1. What is basic research, and who performs it?

2. Role of a research administrator in allocating funds

   -- basic vs. applied;

   -- maintenance vs. productivity enhancement

3. Setting goals for basic research

4. Identifying the payoffs

5. Choosing specific basic research targets

6. Relating "genetic engineering" to basic research in traditional disciplines

7. Specific applications and realistic perspective

Basic Research: ". . . where you do research just to know what goes on in case you might need it some day."

Hon. J. Whitten
p.335 Hearings,
House Appropriations Committee
March 22, 1984

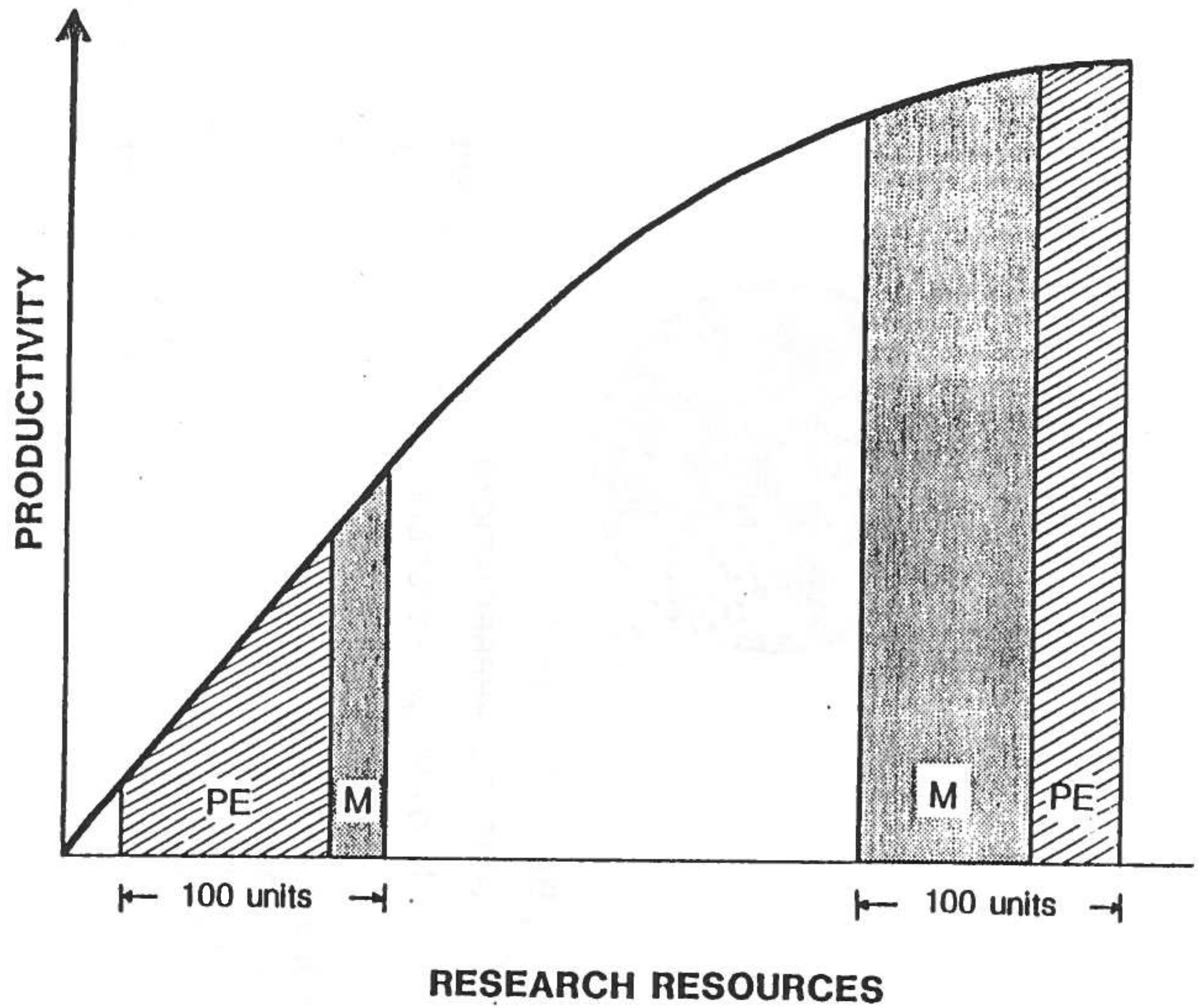
Basic Research: Advances the frontiers of scientific knowledge while addressing mission - or problem-oriented goals.

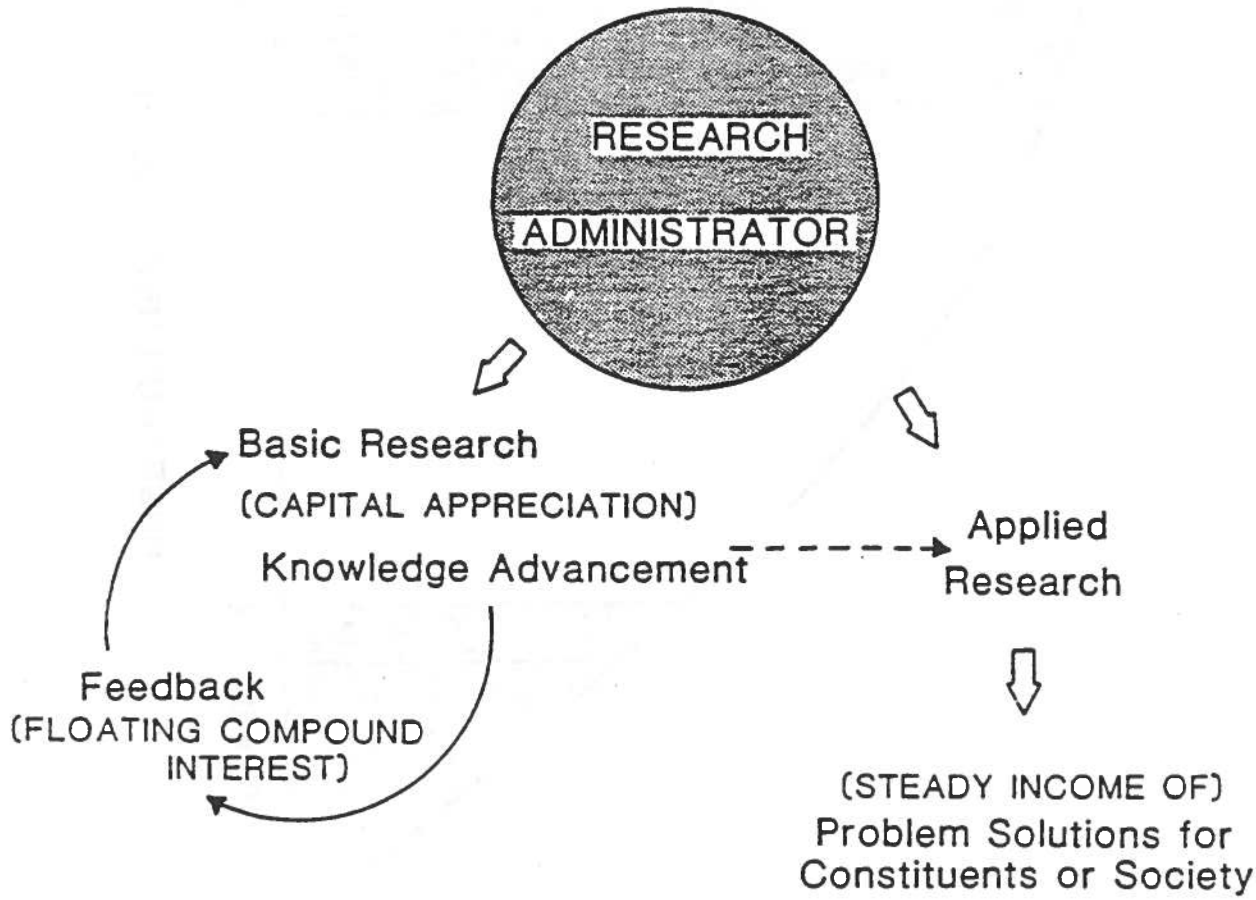
Basic Research: Conducted by the established scientific disciplines, and often amplified by the emerging, cross-cutting biotechnologies called "genetic engineering".

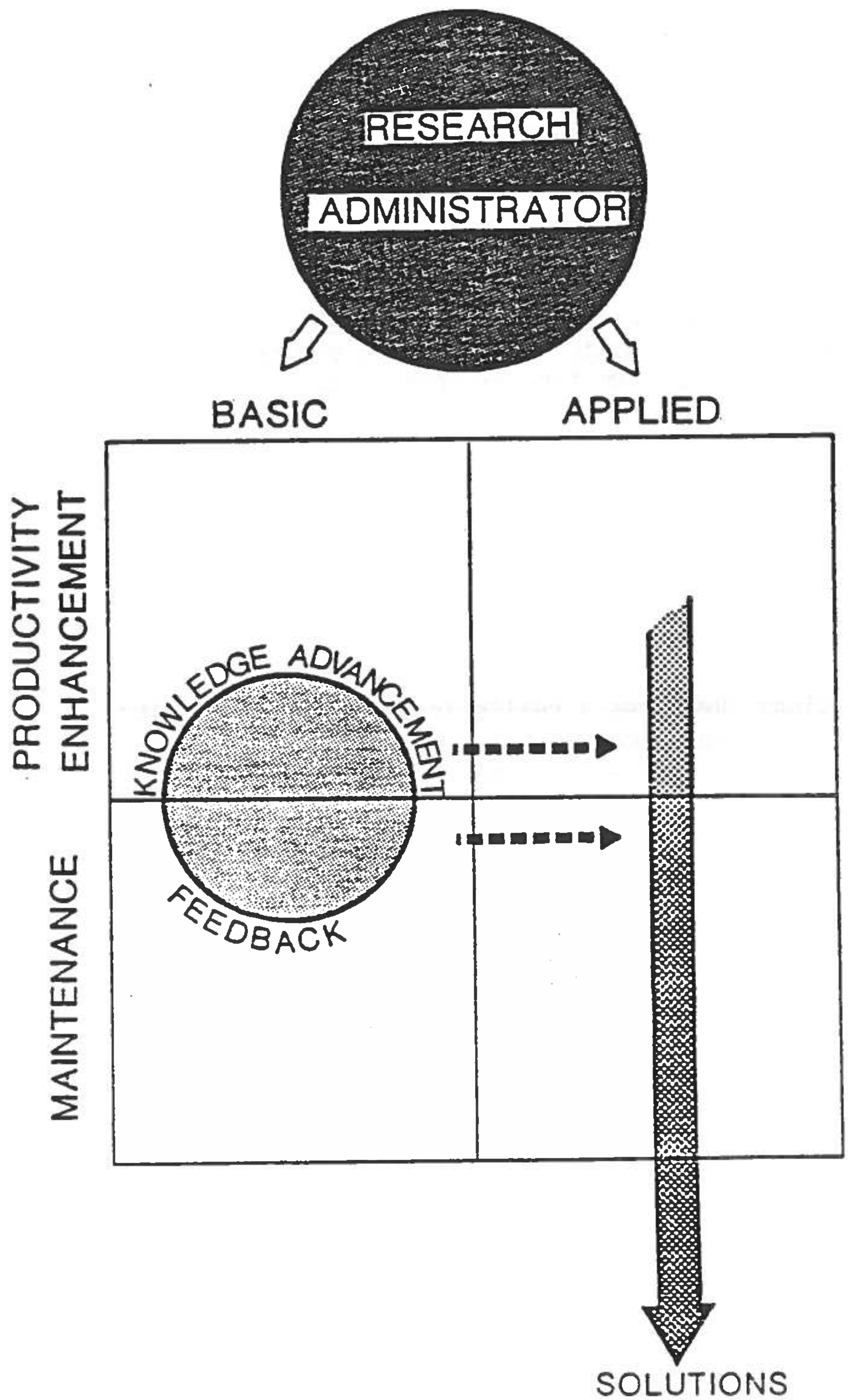
Public Institutions: Agricultural Experiment Stations, Land Grant Colleges and State Universities, USDA-Agricultural Research Service.

RESEARCH INVESTMENT MATRIX

	BASIC	APPLIED
PRODUCTIVITY ENHANCEMENT		
MAINTENANCE		

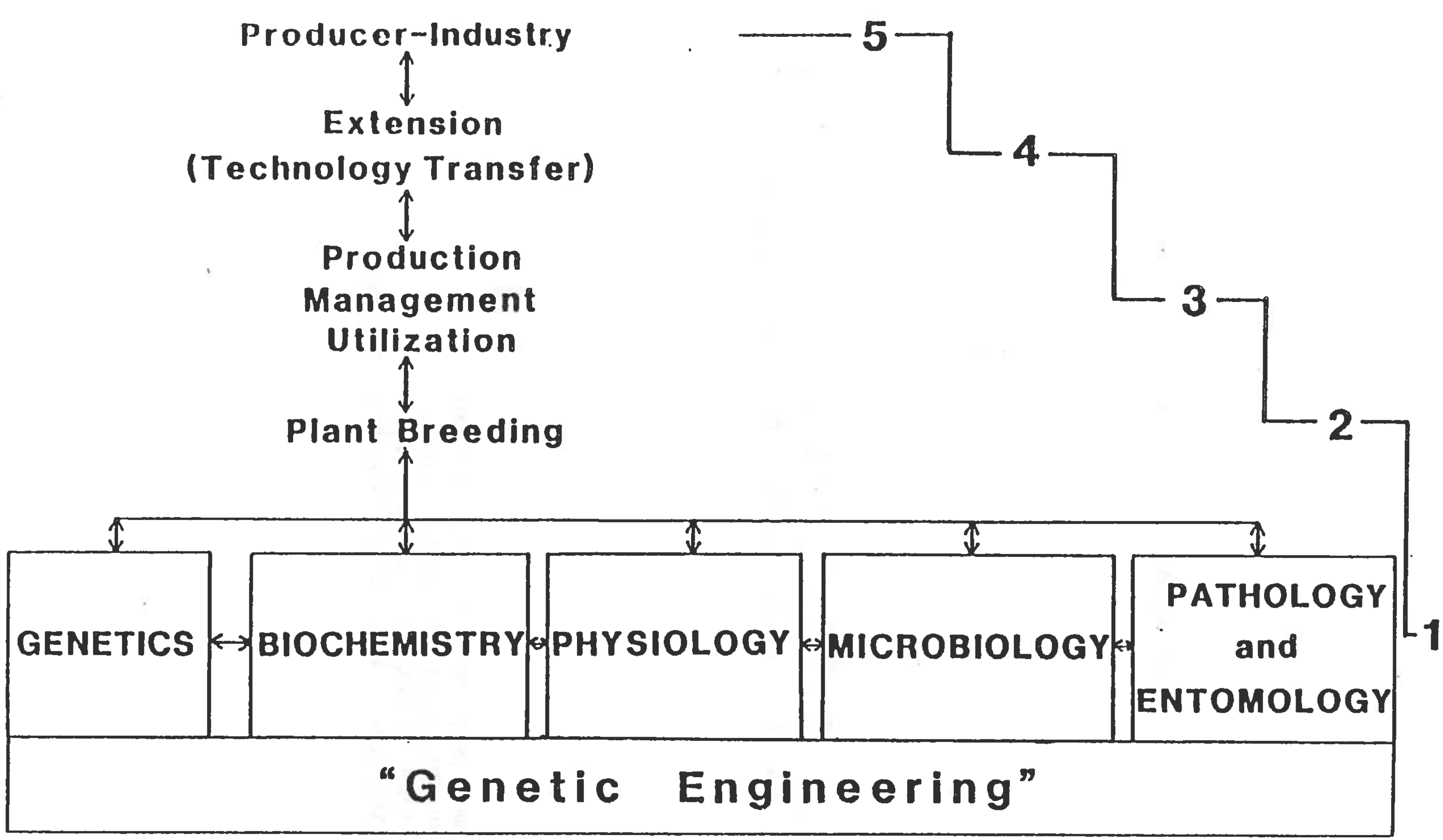

PRODUCTIVITY
PE
M
M
PE
100 units
100 units
RESEARCH RESOURCES


RESEARCH
ADMINISTRATOR
Basic Research
(CAPITAL APPRECIATION)
Knowledge Advancement
Applied
Research
Feedback
(FLOATING COMPOUND
INTEREST)
(STEADY INCOME OF)
Problem Solutions for
Constituents or Society


RESEARCH
ADMINISTRATOR
BASIC
APPLIED
PRODUCTIVITY ENHANCEMENT
MAINTENANCE
KNOWLEDGE ADVANCEMENT
FEEDBACK
SOLUTIONS

Goal Setting for Basic Research:
Requires a realistic understanding of the delivery system for new knowledge.

Goal Setting: Requires a realistic understanding of the intended benefactor of the research (public, industry, other scientists).



Producer-Industry
Extension
(Technology Transfer)
Production
Management
Utilization
Plant Breeding
GENETICS
BIOCHEMISTRY
PHYSIOLOGY
MICROBIOLOGY
PATHOLOGY
and
ENTOMOLOGY
"Genetic Engineering"
1
2
3
4
5

Goal Setting: Requires an accurate specification of the objective or hypothesis, which usually involves repeated iterations of increasingly precise or focused statements.

Goal Setting: Requires familiarity with the interlinkages, the time lags, and the feedback loops between disciplines.

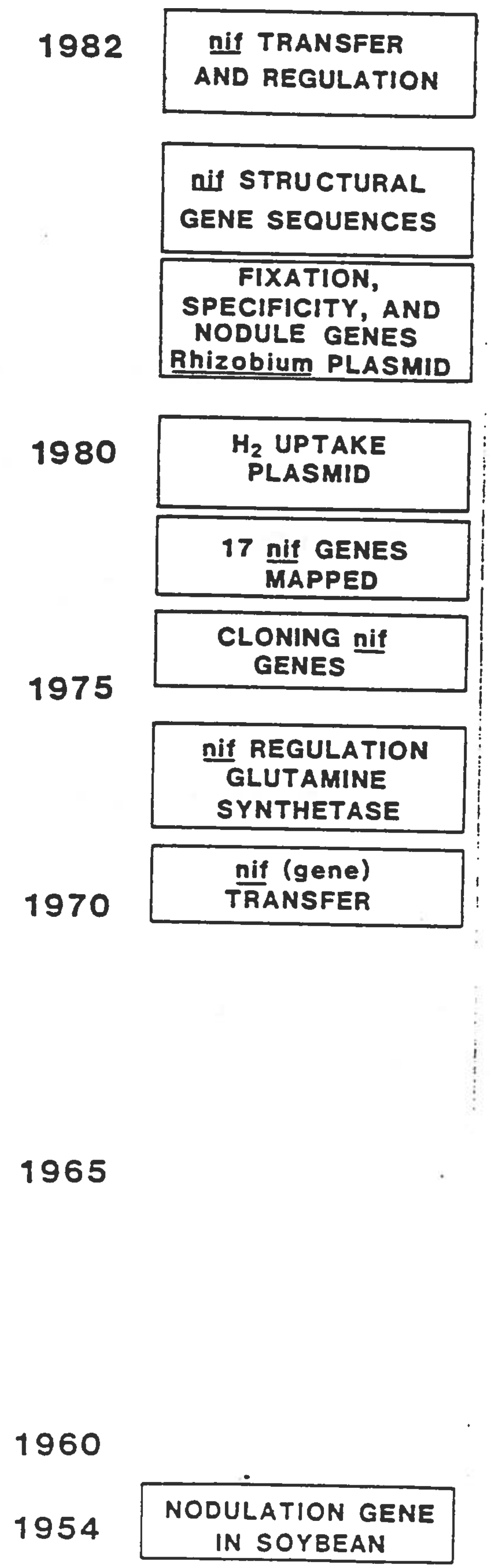
Where Are the Payoffs?

"Dimensions" of Research Programs

Minnesota AES:	370 research projects (CRIS Units)
	240 scientist-years
	24 academic or administrative units
Agricultural Research Service:	2813 research projects (CRIS Units)
	2757 scientist-years
	6 national program objectives

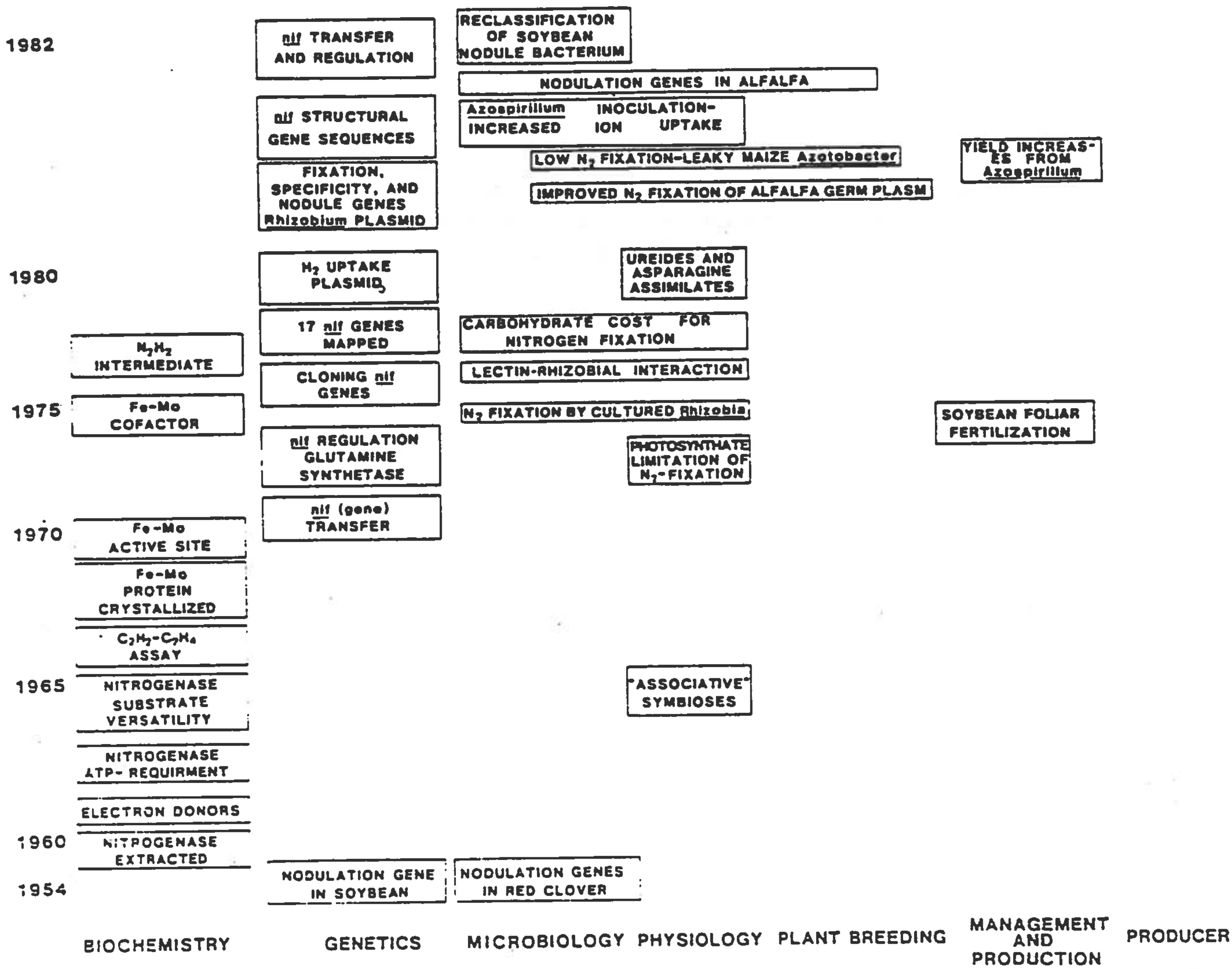
"Tracking" Scientific Advances

Research Administrators: Have limited methods for objectively tracking and predicting scientific advances within a specific discipline.


1982
nif TRANSFER AND REGULATION
nif STRUCTURAL GENE SEQUENCES
FIXATION, SPECIFICITY, AND NODULE GENES Rhizobium PLASMID
1980
H_2 UPTAKE PLASMID
17 nif GENES MAPPED
CLONING nif GENES
1975
nif REGULATION GLUTAMINE SYNTHETASE
nif (gene) TRANSFER
1970
1965
1960
1954
NODULATION GENE IN SOYBEAN

GENETICS

Research Directors: have even more limited methods for objectively tracking and predicting scientific advances <u>across</u> disciplines.



1982
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NODULATION GENE IN SOYBEAN
RECLASSIFICATION OF SOYBEAN NODULE BACTERIUM
NODULATION GENES IN ALFALFA
Azospirillum INOCULATION-INCREASED ION UPTAKE
LOW N_2 FIXATION-LEAKY MAIZE Azotobacter
IMPROVED N_2 FIXATION OF ALFALFA GERM PLASM
YIELD INCREASES FROM Azospirillum
UREIDES AND ASPARAGINE ASSIMILATES
CARBOHYDRATE COST FOR NITROGEN FIXATION
LECTIN-RHIZOBIAL INTERACTION
N_2 FIXATION BY CULTURED Rhizobia
SOYBEAN FOLIAR FERTILIZATION
PHOTOSYNTHATE LIMITATION OF N_2-FIXATION
"ASSOCIATIVE" SYMBIOSES
NODULATION GENES IN RED CLOVER
N_2H_2 INTERMEDIATE
Fe-Mo COFACTOR
Fe-Mo ACTIVE SITE
Fe-Mo PROTEIN CRYSTALLIZED
C_2H_2-C_2H_4 ASSAY
NITROGENASE SUBSTRATE VERSATILITY
NITROGENASE ATP- REQUIRMENT
ELECTRON DONORS
NITROGENASE EXTRACTED
BIOCHEMISTRY
GENETICS
MICROBIOLOGY
PHYSIOLOGY
PLANT BREEDING
MANAGEMENT AND PRODUCTION
PRODUCER

- Scientists - largely comprise the tracking system.
- Peer Review - sharpens the focus of objectives (projects)
- Success - largely measured in historical framework
  (publications, citations, honors, grants)

Research Goals in Public Institutions

- Developed chiefly by the process of consensus among scientists.
- Occasionally mandated by legislative action.
- Occasionally developed in response to negative criticism from clientele or oversight groups.

Reseach Administrators (entrepreneurial role):

invest resources in goals that -

- Promise high probability of new knowledge
- Are potentially applicable across many disciplines
- Will solve problems of clientele

Payoffs in Two Types of Currency

- New knowledge = currency of science
- New solutions = currency of clientele

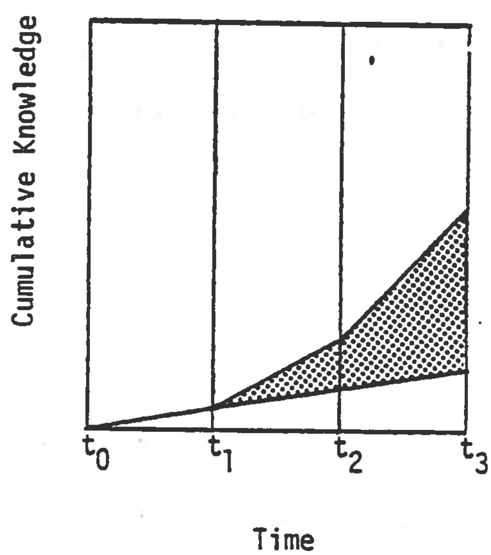
Choice of Goals for Basic Research

- Availability and quality of current human resources
- Effective two-way communication in the "chain of command"

- Ability to focus interdisciplinary expertise on a problem
- Commitment to long term support

- Opportunities to capitalize on "grass roots developments" from scientific staff
- Availability of discretionary funds to foster "opportunism"

- Comparative "exclusivity" of research opportunity (via time or staff expertise) in comparison with other institutions


Cumulative Knowledge
t_0
t_1
t_2
t_3
Time

"Old" Biotechnology - traditional plant and animal breeding; foodstuffs produced by fermentation.

- relies upon genetic selection in natural populations to obtain desired traits or characteristics

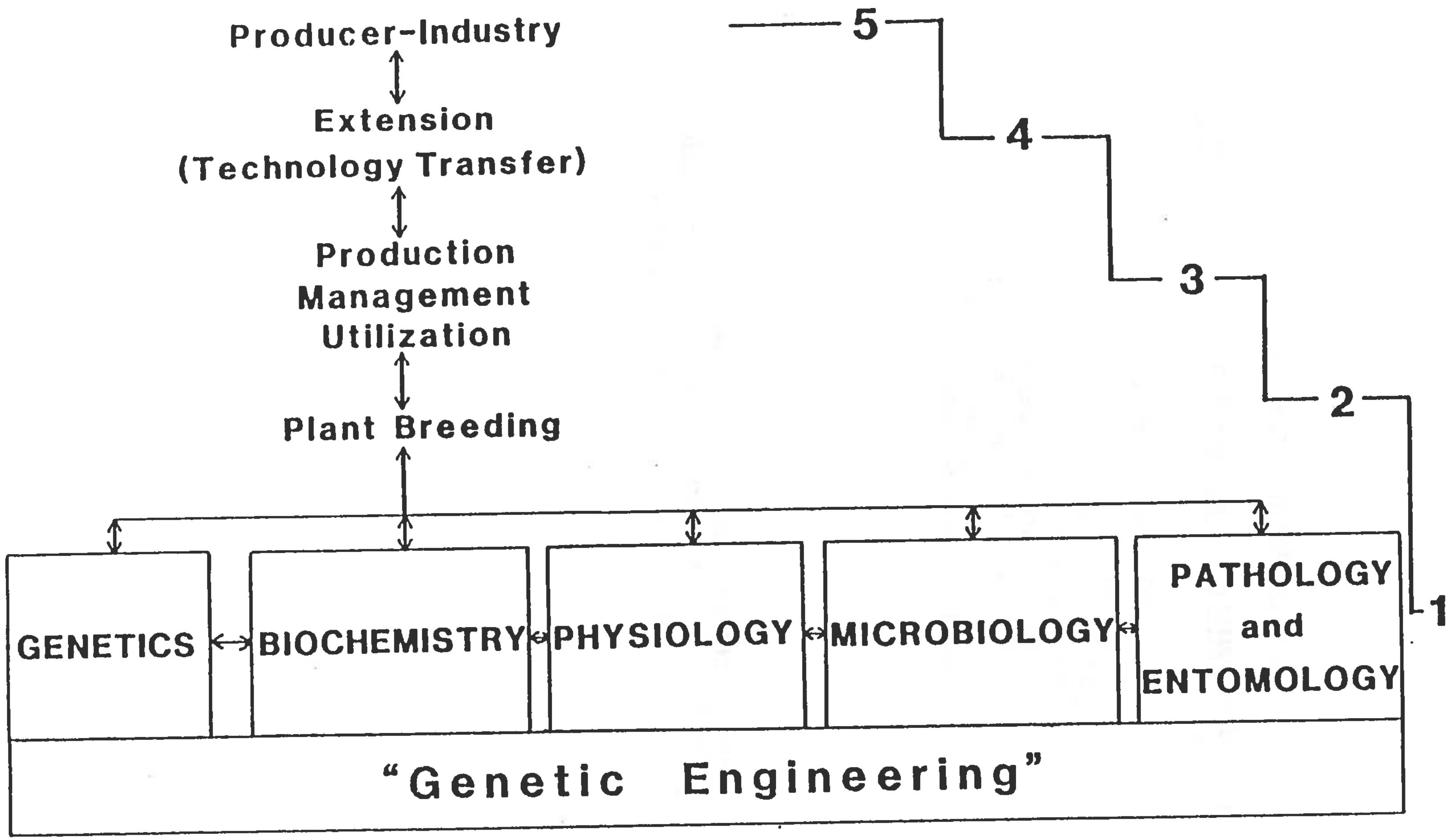
"New" Biotechnology - uses an understanding of the molecular constitution of organisms to achieve alteration at the cellular and molecular level. (Recombinant DNA; Plant Cell and Tissue Culture)

What is "Genetic Engineering"?

An expanding family of new research techniques (tools) including:

- Plant cell and protoplast culture
- Plant regeneration
- Somatic hybridization
- Embryo transfer
- Recombinant DNA approaches (gene identification, characterization, splicing, replication, regulation, and transfer)

"Genetic Engineering" is not a new discipline, since the family of research techniques is applicable to all of the existing biological disciplines



Producer-Industry
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GENETICS
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and
ENTOMOLOGY
"Genetic Engineering"

Biotechnology Research and Funding in SAES

Year	Total Research	Plant Breeding	Animal Breeding	-------Biotechnology--------		
	(FTE)	(FTE)	(FTE)	(FTE)	(Projects)	(K$/FTE)
1982	6853	446(6.5%)	159(2.3%)	251(3.7%)	521	--
1983	6392	460(7.2%)	157(2.5%)	283(4.4%)	579	146.8

Biotechnology Research and Funding in ARS

Year	Total Research	Plant Breeding	Animal Breeding	-------Biotechnology--------		
	(FTE)	(FTE)	(FTE)	(FTE)	(Projects)	(K$/FTE)
1982	2757	--	--	--	--	--
1983	2806	265(9.4%)	29(1.0%)	78(2.7%)	94	177.4

Areas of Research Opportunity for Application of Genetic Engineering Techniques

- Genome structure and gene expression in plants
- Plant developmental biology
- Plant and pest interactions
- Organisms in the rhizosphere
- Photosynthesis (energy and carbon)
- Mineral uptake, deficiencies, and excesses
- Biological nitrogen fixation

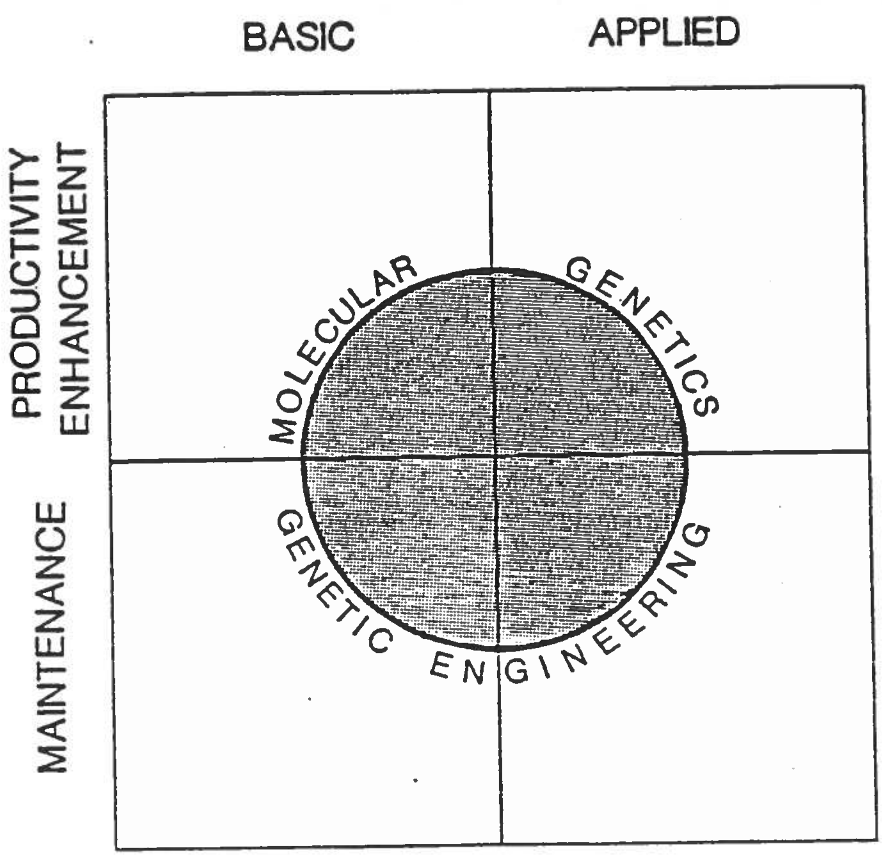
Realistic Assessment of Value

- Most important contribution - better understanding of basic biology of plants
- Practical advantages over conventional breeding techniques unpredictable in near term

- Traits controlled by single gene or small groups of genes will be most easily transferred
- Engineering simple disease resistance traits impractical because of rapid mutation of pathogen

- New sources of desirable genetic variability - by mutant selection or somaclonal (unselected genotypic) variation
- Engineering multigene systems is far in the future - may even be beyond current techniques of genetic engineering

RESEARCH INVESTMENT MATRIX



SUMMARY

Molecular Genetics and Related Biotechnologies

- an emergent group of techniques potentially applicable to all traditional biological disciplines.

- potentially applicable throughout the research portfolio: basic, applied, maintenance, and productivity enhancement research.

- properly directed, will amplify the effectiveness of (but not substitute for) mission - oriented research conducted in traditional disciplines.

- should interface with existing research delivery systems for realization of greatest returns on investment. Where is the "competitive advantage"?

Genetic Engineering and Plant Breeding

My remarks today are to stimulate discussion of courses of action which can support or impede the development of new molecular technologies for the improvement of crop productivity. Plant breeding and agricultural research in general have been affected greatly by biotechnology or genetic engineering to date. The effects are not new products, but instead jarring challenges to our agricultural research traditions and organizations. If biotechnology had no other effect, I feel this jarring of conventional wisdom and the resultant changes that will surely follow as we in research administration respond to the challenges will improve our agricultural research worldwide. The critical factor is to approach the biotechnology research challenge as an opportunity rather than a threat.

Biotechnology research provides us at Pioneer many opportunities. I will describe to you how we have organized our Biotechnology Research Department, how the research is funded, and what we hope to obtain from our research investment. I will highlight how our research activities must be supported by public sector research. I will finally give an outsider's view of the challenges I see before you in public sector research administration.

Pioneer developed its Biotechnology Research Department in 1982. That official beginning was proceeded by an assessment of the emerging technologies for several years culminated by a decision to build a laboratory in December, 1980. A rough staffing plan was made. We wanted to apply molecular and cellular biology research to improve crop productivity. Improved disease resistance is a priority because diseases often do limit yield and resistance can be simply inherited. Transformation and *in vitro* selection will initially be limited to the manipulation of simply inherited traits, *i.e.* single-gene traits. It was obvious from the beginning that a multidisciplinary research effort will be required. We will have to link the laboratory research at the cellular level to the whole-plant research being done by our field pathologists, other whole-plant biologists, and our plant breeders who will ultimately develop the products which incorporate our biotechnology research successes.

We have had a functioning biotechnology research effort for three years. We grew slowly from 1 scientist to eight, with a total of 21 employees today. We are planning for 9 research projects and 30-35 total employees who will work to apply the emerging technologies in molecular and cellular biology for the improvement of corn germplasm or of corn breeding efficiency and effectiveness. We are not a product development department *per se*. Our plant breeders are responsible for products; our biotechnology researchers are to support the corn breeding effort however possible.

A critical decision in our establishing a biotechnology research effort was to develop the Department with new research money. There was no reduced support for plant breeding, or for the other support departments such as the Research Specialists, Data Management, and Germination. The investment was made to increase our research effort in the emerging technologies so that our product development effort could utilize all the tools available. By not establishing competition for fixed research dollars, we have minimized the polarization that often develops between breeders and molecular biologists. I believe our breeders are enthusiastic about our biotechnology research at the best and have a wait-and-see attitude at the worst. I have not seen open hostility from our breeders. Acceptance, or at least tolerance, of our molecular and cellular biologists will be critical if we are to realize the full potential of our research investment.

Acceptance of the Biotechnology Research Department is not adequate, however. Technology transfer requires multidisciplinary research teams that span from the applied to the basic. One of my most critical administrative challenges has been to develop a new Department that is truly a part of the Plant Breeding Division. This is not trivial when you hire 20 new employees who know nothing about Pioneer, our research, or our products. How do you integrate such a group into the Company and how do they establish research programs that are relevant to the problems our plant breeders face in the field? I do not have good answers to those questions, but we have tried a number of different things to insure our Biotechnology Department is an integral part of our Plant Breeding Division. Some ideas worked; others failed.

Our first attempt at integration was a failure. We hired staff before we had any laboratories or offices. I thought the perfect way to build some ties for the future would be to office the biotechnologists in one of the Research Specialists' laboratories. The Research Specialists Department is composed of whole plant biologists, i.e. pathologists, an entomologist, a physiologist, a biochemist, and an agronomist, whose jobs are to support the breeding programs through research in their specialty areas. This forced mixing of biotechnologists and specialists did not build ties for the future. The reasons for the failure were very complex. Biotechnology had center stage at Pioneer at the time. Biotechnology and newly hired biotechnologists received an inordinate amount of attention by corporate officers, corporate information specialists, and Plant Breeding administrators. Much of that attention was indeed justified based on the size of the investment. Biotechnology was Pioneer's first sports car; we had always driven Chevy sedans before. In addition, the new biotechnologists were unable to do science because they had no equipment; they could only talk about what they might do. Their primary work was to spend a few hundred thousand dollars on some shiney, new lab equipment to fill a new 20,000-ft^2 laboratory. In retrospect, it is not unreasonable to suspect that some jealousies may develop. This experiment in integration of the new with the established was a failure we are still overcoming.

Some experiments worked better. Each Biotechnology project manager is to visit a breeding station during their first summer. These meetings on the breeder's turf are quite educational in what a breeder's job is and what traits are important in a saleable seed product. The harsh realities of improving polygenic, agronomic traits that are not characterized genetically emerge from the day's discussions. The breeder also begins thinking about the genetic basis of the traits with which he or she works. If molecular biology is to impact crop productivity, breeders and biotechnologists must begin developing a genetic understanding of complex agronomic traits.

We have also developed project teams in the areas of *in vitro* selection, gene isolation, and transformation. Each project is headed by someone in the Biotechnology Department. The teams include several from Biotechnology plus representatives from the Research Specialists and Corn Breeding. These teams decide on the research priorities and the germplasm to use, and they monitor progress. Plant materials to be grown in the field are the responsibility of a corn breeder or specialist. This concept is working best for the *in vitro* selection project because regenerated plants are now being evaluated in the field. Having plants as a focal point greatly facilitates discussion between the laboratory and field scientists.

Communications and cooperation between the Biotechnology Department and the rest of the Plant Breeding Division remain critical issues for us. We have now added a corn breeding project to our Biotechnology Department to help bridge the gap. The corn breeder has many responsibilities. First, he must learn the jargon of the molecular and cellular biologists. He will be a key translator to facilitate more effective communications among researchers. He will advise us on appropriate germplasm to use and he will develop useful genetic materials for experiments if necessary. He will keep us honest, *i.e.* insure that the problems our research is supposed to solve need solutions. He will evaluate plants coming from the laboratories and identify or develop materials that are ready to enter our product development programs at our breeding stations. He will think about the genetic basis of yield, standability, maturity, polygenic disease resistance, and other complex traits that are critical to improved crop performance, but which are seemingly unapproachable with the technology we know today in molecular and cellular biology. Obviously, I see this corn breeding position as critical to the success of our Biotechnology Department. If we can accomplish half of what I just outlined, I will be pleased.

My comments have focused on organizational challenges that our decision to develop a Biotechnology Department presented. We did not organize the Biotechnology Department for organizational challenges; we organized it because we feel the research will be important to our continued plant breeding success. Data by Don Duvick of Pioneer show corn breeders have improved corn yields at the rate of 1.4

bu/A/yr from 1930-1980. We anticipate that same rate of gain through the year 2000, at least. It is important to recognize, however, that the research investment to maintain that 1.4 bu/A/yr rate of gain has increased dramatically over the 50-year period. We have gone from 1 corn breeder, Raymond Baker, in 1929 to over 400 employees in the Plant Breeding Division in 1985. Assuming linear growth on employee numbers (which is false), every 1.4 bu/A gain in yield took 7.1 additional employees. The point I am making is that yield gains continue to accrue, but we must know more about our crop and we must test new hybrids more thoroughly to identify the superior genotypes for sale. Over 15 years ago, we began supporting our breeders by hiring pathologists and entomologists. In 1974, I was the first physiologist hired. We formed the Data Management Department in 1980, but the computerization of our breeding programs was already well underway. Biotechnology research is a further commitment to support the breeding effort.

I indicated previously that our Biotechnology Department is not a product development group. Our goals are to supply useful germplasm to breeders for use in their breeding programs, to provide a better genetic understanding of our crops' performance so breeders might be more effective or efficient in their selection of superior inbreds and hybrids, and to apply the techniques of molecular genetics or cellular biology to breeding problems to allow for more precise selections. I will give examples for each of those three goals. Providing desirable germplasm for breeders might be achieved through effective _in vitro_ selection of plant cells resistant to a disease toxin such as _Helminthosporium carbonum_ Race I. Selection of resistant callus, _i.e._ directed mutation, using tissue culture is much more desirable to us than using tissue culture to generate random, somaclonal variation. But the potential utility of _in vitro_ selection technology may be limited in the near term. We lack basic information on plant biochemistry as it relates to crop productivity or quality and to host-pathogen interactions. There are too few biochemical targets for selection of desirable mutants to allow broad exploitation of in vitro selection technology today.

We also hope that corn transformation, i.e. direct gene transfers, will provide useful germplasm for breeders. Transformation technology will soon become routine for all plants. Our limitation will not likely be transformation technology for corn, but instead useful genes to transfer. Unfortunately plant breeding and genetics have developed in too much isolation from each other. We do not know of single genes that have significant impact on quantitative traits such as yield or polygenic disease resistance. We do not know what insights the geneticists' mutants may provide physiologists or breeders about agronomic traits. Should we not expect mutants to unravel the mysteries of plant biology in the same manner that mutants revealed the intricacies of microbial genetics? Can we ever engineer improved plants to the extent we can engineer improved bacteria and yeast without expanding our genetic understanding of plant biology manifold? Today, I believe that our goal of providing improved germplasm to breeders is long-term. Furthermore, the significance of such germplasm is questionable today. The short-term prospects for improved germplasm cannot be the major justification for our biotechnology research investment.

The lack of genes for transformation and of well characterized pathways to target in vitro selections is a limitation today, but the technologies being developed also provide us the opportunities to overcome our shortcomings in genetic and biochemical understanding. The information that genetic transformation will provide us about gene expression and regulation will make identification of desirable plant types more likely. Selection schemes for desirable agronomic traits will surely be more effective and efficient if the genetic bases of those traits are known or if some of the most important genes controlling a trait can at least be selected. I have heard it said that plant breeding is analogous to herding cattle in the dark. I doubt plant breeding will ever be like herding cattle in full sunlight, but perhaps our molecular and cellular biology research can at least shed some moonlight on the process. We hope for a clear night and a full moon.

I believe our investment in biotechnology research will help us build a stronger genetic foundation on which to place our plant breeding research. My confidence that such a foundation is being built today and my faith that such a foundation will make our breeders more effective are the primary justifications I see for our biotechnology research investment.

These goals I have discussed to this point are indeed long-term. Much of the work is sufficiently long-term and complex that it will have to be done at public institutions. As a private company, we can afford some long-term research, but it will be applied research. We must continue to apply the basic research results from the public sector to solve problems we face today. Our third research goal focuses our attention on this need. We are seeking ways to apply the techniques of molecular biology to solve today's problems. For example, virus diseases are difficult to diagnose based upon visual symptoms. Infections often include a complex of viruses, thus further confounding the problem. The successful selection for any desirable trait is dependent on an effective characterization of the breeding materials for that trait. Selecting for resistance to virus diseases around the world is extremely confusing. If a single virus or virus complex is prevalent at a breeding location, resistant germplasm can be selected. Resistance will be for that virus only, not all viruses. Thus, if a virus outbreak occurs in Spain, what inbreds or hybrids should be sent there to stabilize production in the presence of the disease? What virus or viruses are involved? We need simple, effective diagnostics for viruses. Antibodies in an ELISA test (enzyme-linked immunosorbent assay) offer the diagnostic capabilities we need. If we accurately identify the organisms causing infection at a given location, we can accumulate accurate disease resistance ratings. Subsequent diagnosis of the same viral problem anywhere in the world should allow us to predict the inbreds and hybrids needed in the infected region quickly and efficiently.

Other techniques such as Southern blotting to identify differences in the DNA code among lines, i.e. RFLP analysis (restriction fragment length polymorphisms), may offer new selection opportunities. For example, if we could find a restriction fragment that is closely linked to a disease resistance gene, we could select for the presence of that specific restriction fragment and be reasonably confident the disease resistance would also be present in the line. Assume a corn virus is prevalent in Mexico, but is not found in Texas. Our breeder in Texas would want to select for virus resistance, but would not be willing to inoculate the breeding nursery with the virus. Instead he or she could select for a restriction fragment known to be linked to virus resistance. We are currently trying to develop a series of DNA probes that will map to every chromosome in the corn genome. We hope they will be useful in establishing linkages with traits that are hard to select directly in the field based upon plant phenotype or appearance. The molecular techniques being developed are very precise. We hope to apply the techniques to problems our breeders face today.

We continue to look to the public institutions for the basic research to draw upon to support our own research. I feel you public research administrators face some significant challenges if you are to meet industry's and society's needs for basic research in plant biology. I want to share my views from outside the public research arena. My desire is to stimulate your thinking; my remarks are thus rather direct.

Multidisciplinary research teams will be needed, and researchers must commit to long-term projects in plant biology. Molecular and cellular research will be important, but those results must be integrated to the whole-plant level. Molecular genetics must relate to whole-plant genetics and geneticists must address the traits of concern to plant breeders. The physiologists, pathologists, and entomologists must be part of this effort.

I see many barriers to such long-term, multi-disciplinary research efforts within our public institutions. First, our granting agencies provide barriers. Independent research is most valued and best supported. Funds are short-term, i.e. 1 to 3 years. There is no funding for a 10-year project, especially if few publications could be anticipated in the first 2 to 3 years. Funds are also quite limited; many good projects cannot be funded. Furthermore, certain research areas are favored. Thus, biotechnology may likely receive funding, but whole-plant physiology and plant breeding may have little chance. In fact, the chances of funding plant breeding research through granting agencies are bleak today. The competition for limited dollars is creating antagonism between research groups at a time when research teams are needed.

Administrators are providing barriers to the formation of multidisciplinary research teams and long-term research projects. Faculty promotion is tied to publications, and publications with one or two authors count much more than those with 4 or 6 or more authors. Collaborative research is penalized. New criteria for evaluating research productivity are needed.

Our university organization provides more barriers. Collaboration across colleges or even departments means sharing funds and recognition. Collaboration outside the agricultural experiment station means sharing Hatch monies. Collaboration with another university where molecular biology is excellent, but where agriculture is absent, is nearly inconceivable. New collaborations must happen if progress is to be made, however.

Seemingly, the move to establish Biotechnology Centers on nearly every campus is an attempt to overcome some of these barriers. I believe they may help, but the fundamental barriers to collaborative research still remain, i.e. priority for independent research, short-term funding, and faculty promotion tied most closely to number of publications as senior author. I am hopeful that Biotechnology Centers are signs of organizational and administrative change within the entire university system that will result in more multidisciplinary research. I feel these should be centers for collaboration. I doubt all will become meccas for international biotechnology research, nor centers for industrial investment.

Finally, I hope research administrators do not get conned by reports in the Wall Street Journal. Biotechnology is not products, it is technology. Technology can be applied to develop products, but product development is an expensive, time-consuming process. The private sector is better prepared to apply technology than are public research institutions. The monetary gains that biotechnology has promised, but not delivered to date, have impeded the free exchange of scientific information. The only way for society to benefit from its investment in science is for the scientific results to be reported and discussed so that the science continues to develop as rapidly as possible. If the science progresses, then new products will follow. Today, too many people are duplicating each others' science because there is not free and full exchange of scientific information. The pace of discovery and application of the science will slow if we allow these trends toward secrecy to proliferate.

To summarize, we at Pioneer are attempting to develop a long-term, multidisciplinary research effort that includes molecular and cellular biologists, whole-plant biologists, and plant breeders. We hope our organization will encourage and support that effort and that impediments to cooperation can be minimized or removed. I feel that public research institutions should also develop multidisciplinary teams. Many barriers exist, however, to collaborative research within the public research community. I have identified barriers I see from the outside. I leave to you the challenge of identifying the real barriers and of removing them so that scientific exchange and progress are maximized.

Nick Frey

Presented at the Agricultural Research Policy Seminar sponsored by the University of Minnesota International Service for National Agricultural Research, April 19, 1985.