Proceedings

# THIRD NATIONAL PEANUT RESEARCH CONFERENCE

July 9-10, 1964

Held At

AUBURN UNIVERSITY Auburn, Alabama

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PAPERS AND ADDRESSES

## Third National Peanut Research Conference

AUBURN UNIVERSITY Auburn, Alabama

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Sponsored by PEANUT IMPROVEMENT WORKING GROUP in cooperation with AUBURN UNIVERSITY

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## RESEARCH IN THE USDA OF CONCERN TO THE PEANUT INDUSTRY

NYLE C. BRADY, Director of Science and Education U. S. Department of Agriculture

It is a special pleasure for mc to talk to this Third National Peanut Research Conference. We share many common interests and problems in this intensely complicated business of agriculture.

You want a profitable industry and a good product that will satisfy your consumers. We in the Department of Agriculture want exactly the same thingfor you and for every other segment of American agriculture.

Your industry, by the way, has long been of particular interest to me, dating back to the time when I was a graduate student at North Carolina State College. I was working on my doctoral thesis on the effect of cations on the quality of the peanut fruit. In 1947, the National Peanut Council was kind enough to present my colleagues and me with an award based on the research we had carried out.

Today, some 17 years and many experiences later, I am still interested in peanuts and talking about them.

I would like to consider with you in broad outline some of the work the Department of Agriculture is doing that is of special interest to you as scientists, growers, and processors in the peanut industry . . . and as consumers interested in the welfare and future of agriculture.

To fully appreciate agricultural research, however, it's first necessary to understand one of its great sources of power—the long-standing cooperation between State and Federal agencies that dates back for nearly a hundred years. This unique cooperative way of life has contributed vastly to the extraordinary effectiveness of American agriculture.

Today, however, the problems that we face are proliferating so rapidly and the requirements for research knowledge are so exacting, that scientific cooperation has become a matter of agricultural survival—even of national survival.

The peanut industry has felt the pressures of this increasing complexity and the ever-growing need for close cooperation in research. This is reflected by the various agencies represented here today. State, industry, and Federal people along with growers and processors are bringing their ideas . . . their special points of view . . . and their experiences to bear upon the increasingly difficult problems of your industry.

As you know, most of the research on peanuts is cooperative with the peanutproducing States. Although the work is limited, it has been unusually effective and has brought about some highly significant and far-reaching improvements.

Look at the ingenious State-Federal study a few years back for controlling stem rot.

Scientists evolved a simple, inexpensive procedure for controlling this disease by deep burial of surface trash in land preparation, and by cultivating the plants so that no soil is thrown around the base at any stage of cultivation. On top of that, there has been a consistent striking reduction in weeds, yields have increased, and market quality has improved.

The soundness of this work has been demonstrated by the fact that all major peanut-growing States are recommending these procedures.

In another cooperative disease study, scientists discovered that two species of fungi, *Pythium* and *Rhizotonia*, may cause pod rot—a disease that has puzzled growers and scientists for some time and can cause losses as high as 30 percent in the northern part of the peanut belt.

Experimentally, at least, pod rot has been controlled by deep plowing to bury surface organic matter below the fruiting zone, combined with applications of land plaster at the rate of 1,000 ponnds an acre. Work is continuing to develop more satisfactory methods of control. Breeding resistant varieties is ultimately the most satisfactory way to control the diseases that take an estimated 28 percent of the peanut crop each year. But we've had very little luck in identifying any nsable genetic resistance to any major disease of peanuts. Three improved varieties developed at the Florida Station, however, may prove to be exceptions. These varieties have measurable resistance, although the nature of this resistance is nnknown.

We urgently need to find factors for resistance to diseases and insects, to determine the nature of that resistance, and learn how it is inherited.

In one effort to develop improved varieties, State and Federal geneticists have discovered an excellent tool for determining the extent of natural crossing in peanuts. This is a genetic marker in the form of a cnrled leaf, which provides early visual evidence of the cross-pollination. This genetic marker is being need to speed up work on breeding.

In weed control, too, cooperative work has produced two unusually effective herbicides mixtures that have successfully controlled annual weeds such as crabgrass, pigweed, lambsquarters, and broadleaved plants. The use of these herbicide mixtures has had some startling and unexpected results. About 90 percent of the hand-hoeing involved in peanut production has been eliminated and from 50 to 80 percent of the mechanical cultivation. More than half the total acreage of this year's crop has been treated with these herbicide mixtures.

Weed control has always been one of the most difficult and expensive operations in raising peanuts, and will become an increasingly urgent problem in the years ahead. Research efforts to find fully effective and dependable means of controlling the yearly loss of some 15 percent of the crop will assume greater importance.

Work by State and Federal agricultural engineers and machinery manufacturers has caused a virtual revolution in mechanizing the production, harvesting, and curing of peanuts in the last 15 or 20 years. This joint effort has been extremely effective in helping growers hold down costs of production during periods of steadily increasing costs.

And so the farm research on peanuts moves ahead . . . spread thinly at times in certain areas . . . but productive far beyond the money and manpower we have invested.

Utilization research is also producing information of value to the peanut industry—much of it from the standpoint of developing improved products that will appeal to consumers. There's one area, by the way, where most of the work is being done by the Department of Agriculture. I see no reason why the States cannot do more utilization research than they have in the past. We would all benefit—the States, most of all.

An especially interesting pioneering research study on seed protein—conducted mostly on peanuts—may have far-reaching implications some day should it ever become necessary to replace animal protein with plant protein as a source of food. Dr. Aaron M. Altschul, who long ago recognized the importance of oilseeds as a source of protein, directs this study. He and his colleagues have already isolated pure peanut proteins and developed techniques for studying them. They have determined the location of protein in the subcellular particles and determined their relationship to the other biochemical properties of the seed.

Just this year, Dr. Altschul received an award from the National Peanut Council for his remarkably imaginative efforts in this uncharted field of study.

The challenges in production and utilization research have been tremendous over the past few decades. Just as great a challenge is the work in marketing. Efficiency in marketing is a must for growers who operate on a relatively narrow margin of profit, as you do. A great deal of cooperative effort has gone into the development of better ways to handle, process, store, grade, and sample peanuts, and in controlling insects in storage. In the inspection and grading of stock peanuts, for example, the pneumatic samplers for use in sampling trucks have already been installed at 165 places in the Southeast. An improved cleaner will reduce the time needed to hand-clean the samples to determine foreign material present, and will speed up the mechanization of the grading process.

In our cooperative efforts to maintain high quality, we have found that offflavors develop in peanuts when they are cured in the absence of oxygen . . . and that time of harvest as well as curing treatment affects the flavor of peanut butter.

In work on storage pests, we are experimenting with controlled atmospheric storage—by replacing oxygen with mitrogen or carbon dioxide—in an effort to control insects and leave no residues.

Just recently, we have run into the problem of resistance to malathion—the most effective and economical treatment yet developed to control stored pests. At present, we are checking closely to determine exactly how much tolerance insects develop, and if it is enough to prevent proper control.

These few examples in the production, utilization, and marketing of peanuts and peanut products give some indication of the cooperative efforts underway. We've made some real progress despite many limitations, and we've reason to be proud.

This progress is only part of the overall advances we've made in agriculture. The result is one we all know—a great nutpouring of abundance and an efficient mechanized farming system that has released 93 percent of the U. S. labor force to produce the other goods and services that we all enjoy. In many ways, agriculture is the most progressive section of nur economy. It has added more to the health, comfort, and well-being of the American people than any single human endeavor.

Yet the picture of agriculture in the mind of the average person is narrow. It benefits only the dwindling number of farmers, so goes the reasoning, and has little or nothing to do with me, the consumer. Research serves only to increase production, and the public gets no return for its investment.

Let's look at the research of the Department. About one-quarter of our effort is devoted to utilization studies to find new industrial uses for farm products and to develop attractive new food products, as well as to help farm products compete against new synthetics. Consumers benefit mostly from this type of research. There's no thought of increasing production here—we are working to increase opportunities for farmers and to widen the choices for consumers.

Marketing research comprises over 12 percent of our effort. And this should be of real interest to consumers since about 60 percent of the price they pay for food products is for services performed after the crop is produced.

Resource conservation—which develops the information we must have to make intelligent, long-range use of our country's natural resources—takes np about 18 percent of our research effort. Every American gains from this effort to protect our soil, water, forest, and air resources.

About 22 percent of our research is on resource protection, which is concerned with safeguarding our crops, livestock, and forests against such natural enemies as fire, insects, diseases, parasites, weeds, and nematodes. Here, too, our aim is to protect our agriculture from losing ground in the continual struggle with nature.

Nearly 3 percent of our work is on human nutrition and consumer use, in which we study the needs of people for food, clothing, and shelter and how agriculture can best meet these needs.

And, finally, about 20 percent of the Department rescarch is on improved quality and efficiency. This includes the scientific improvement of crops and livestock through better breeding, feeding, cultural practices, management, fertilizer technology, equipment, and land nse.

So, as you can see, the true picture of agriculture is quite different from the

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commonly-held public image. We are deeply concerned about consumer welfare and protection, and feel that research is important in providing them.

Why, you might ask, if the Department was created to help farmers, do we place so much emphasis on consumer services? Because, by serving the consumer, we best serve the farmer. The farmer can best maintain his market of family and industrial consumers by providing them with better quality products that are safe and wholesome, at prices that are reasonable and competitive.

That fact is well known to everyone concerned with the peanut industry. When you lose consumer confidence in a product—when that confidence is shaken due to real or potential danger—then growers and processors and everyone else associated with the industry are in trouble.

At the present time all concerned with agriculture in its broadest sense are especially sensitive to the effects of our modern foods on human and animal health. Two possible sources of difficulty have been identified in recent years. These are (1) naturally occurring toxic substances (mycotoxins) formed by molds growing on certain of our agricultural products and (2) residues of pesticides applied to reduce the damage of pests to our crops and stored foods.

The USDA and its partners in the States have taken vigorous action on each of these problems. With adequate support we expect to take even more vigorous action.

For example, we have directed the formation of a working group of scientists to study the problem of toxic molds in food and feeds, and to develop a Department-wide research program aimed at eliminating the molds in all agricultural products. The first consideration of this group is to develop a crash research program on toxins formed by molds to enable ns to find the answers to several very important questions.

How, for example, do you keep molds from thriving on agricultural products? How do you detect the presence of mold? How do you salvage the agricultural products where mold is found?

We can expect that scientists at the State Experiment Stations can make substantial contributions in this study particularly in the field of microbiology. Experiment Station personnel were recently given a briefing on the Department's progress and plans. This will make possible better coordination of our total effort.

The Department has also established close working relationships with the Department of Health, Education, and Welfare to get the benefit of the widest possible thinking on mycotoxins, for the greatest public protection.

We have stepped up our present research effort to prevent or minimize mycotoxins. Under this intensified program, scientists are looking for a fast, simple, and highly sensitive method for determining the presence of the toxins... determining the environmental conditions under which the mold grows and produces them . . . finding which strains produce them . . . examining samples of various commodities for molds as the products go through marketing channels . . . conducting pilot-plant studies of a method for removing the mycotoxins during processing of the agricultural products . . . and conducting long-term feeding experiments to determine the physiological effects on rats fed diets containing feed or food contaminated with the mycotoxius.

The Department is also reminding growers and processors of the importance of maintaining good farm and marketing practices in fighting toxic producing molds. Growers are being advised to follow practices which prevent the growth of molds. For grain and oilseed crops this means harvest near maturity, test the crop for moisture, dry it promptly, clean it thoroughly, keep it cool and dry, clean the storage area, control storage insects, and check the stored products frequently.

USDA is doing everything it can to keep products coming in clean and free of molds. And, in the meantime, the Department of Health, Education, and

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Welfare and the Department of Agriculture want to assure consumers that the agricultural products on the market are absolutely safe for human consumption.

We want to insure that public confidence is maintained in all our food products.

That's why we are so deeply concerned about pesticides, which are so widely used on many food crops. In fact, some 57,000 pesticides products, containing more than 600 different chemicals, have been registered for use by the Department of Agriculture. If not *properly used* every one of them is a possible source of trouble, and can be harmful to man, animals, plants, wildlife, and beneficial insects.

The Department recognizes these dangers, but it also knows that we are utterly dependent upon pesticides in order to continue producing our supply of food and fiber . . . if we are to live as comfortable and well as we do now.

The only solutions to this dilemma can come through a great expansion of pestcontrol research, coupled with a strengthening of pesticide regulations, and intensified education on proper use of these materials.

Secretary Freeman has called for a crash program of research, regulation, and education to be conducted by the Department of Agriculture in cooperation with the State agricultural experiment stations. In my opinion, this is one of the most important tasks facing agriculture, and I am in complete agreement with the Secretary on the need for such a far-reaching program.

This project would place great emphasis on every major problem of pest control on farms and ranches, in homes, forests, and in marketing channels. Newer methods of controlling pests would be stressed more than they are now. Basic studies would be increased. New facilities would have to be constructed to carry on the additional work and old facilities would have to be improved.

The Secretary's proposal would result in tightened controls over the sale and nse of pesticides, and would coordinate Federal activities more closely. It would provide for a more critical review of registration applications, and for stricter enforcement activities to make sure products are properly formulated and distributed. It calls for developing and improving pesticide detection methods, and for closer coordination of Federal activities related to nse of chemicals. The proposal also calls for more monitoring of pesticide use in cooperative pest control programs.

The Secretary has called for a greatly expanded effort to educate people to use pesticides with the utmost care. This means the producers; home gardeners; householders; distributors, and retailers of food products; and public agencies that conduct spraying programs. Specially trained Extension agents would be needed to work with these people to insure that pesticides are used as directed.

This is not to say, however, that we have been moving slowly in pest control research, regulation, and education. Much has been accomplished in the last several years—revolutionary, non-chemical concepts of pest control, new and improved chemicals, greatly refined techniques for testing for residues, stricter registration and labelling procedures for chemicals, and greatly increased cooperation among the Government agencies concerned with pesticides.

Currently, we are spending in the neighborhood of \$35 million for research and education on pest-control techniques. This includes the work the States are doing under their Federal-grant funds. The figure is very impressive, but we are going to have to spend even more if we are ever going to resolve a problem of this magnitude.

So—in the field of pesticides or peannts, livestock or economics—we advance only as we apply science to the solution of our problems.

Your meetings here today and tomorrow can help us decide the directions that science should be taking in your field. I regard these sessions as a vital path of communication, a way to reduce difficulties to workable proportions, so we can all get on with the job of helping the peanut industry for the greater good of all our people.

## NATIONAL PEANUT COUNCIL RESEARCH COMMITTEE REPORT

#### GEORGE F. HARTNETT, Chairman George F. Harinett and Company, Chicago, Ill.

The last time I can recall facing such an impressive group of talented people was back in 1956 when I was taking oral examinations at the completion of my final year in Law School. Naturally there were not as many seated in front of me as there are today, but the three terrifyingly serious professors who tested me posed such a sufficient number of questions, that there might as well have been a much larger group in the room. Of course on that particular day the situation was the reverse of this meeting today—they wanted me to furnish all the answers. Today I am the one, speaking for the peanut industry asking you to help furnish the answers. There is one other difference. Those learned gentlemen knew in advance the answer to each question which they put before me. I, too, know the questions we are all facing today, but I do not know many of the answers. You gentlemen must provide your peanut industry with some answers and some help.

Let me start at the beginning in order to aid your understanding of how the National Peanut Council Research Committee came into being.

My first contact with the word Aflatoxin was on March 29, 1963. Dr. B. F. Daubert, Director of Nutrition at General Foods Corporation addressed the 23rd Annual Convention of the National Peanut Council in Boca Raton, Florida. His address was concerned with food additives, and he opened a PANDORA'S BOX to my complacent world with these words: "The second development of major importance to the peanut industry that I wish to discuss concerns the findings of the British Scientists that a relatively common mold found in peanut meal could produce toxic materials." Eight words later I heard a new word: AFLATOXIN. Dr. Daubert then discussed the loss of large numbers of turkey poults in England in 1960 due to their diet consisting of contaminated peanut meal that was extracted from moldy Brazilian peanuts.

The next time this subject confronted me was at the Southeastern Peanut Convention in June of 1963. About 40 of us were asked to attend an unscheduled meeting where Steve Pace, the late Ed Young, Pete Donaldson, Drs. Salmon, Diener, and Coyt Wilson of Auburn University and certain Southeastern peanut shellers and growers were advised of Dr. Salmon's work with domestic peanuts, peanut meal and Aflatoxins. As a result of that meeting, four members of the group were elected to initiate industry study of the problem and to take whatever steps were necessary to begin industry action as soon as it was deemed advisable.

I was named Chairman of this group because I was then Chairman of the Board of the National Peanut Council and, being a broker, served as an interested neutral party that was not prejudiced toward any one segment of the peanut industry.

I called an unpublicized meeting of approximately 16 industry members in July of last year. The group represented all segments of the peanut family and included about six members who were research scientists for large peanut product manufacturers. Their attendance was imperative because they had been accomulating knowledge of this subject and thus could speak intelligently and practically to the group. They layed the known facts on the table and from that meeting a seven man technical advisory committee was named. It included three technical men, one grower, one sheller, one legal counsel, and myself as Chairman. This group became the nucleus of our present research committee, which now numbers fifteen members, representative of the entire peanut industry.

It was the immediate feeling of the Committee of Seven that the situation was extremely serious and that we should do all in our power to initiate research. With this goal in mind we set about making contact with the U.S.D.A. in order to combine forces so that all possible facilities and energies might be brought to bear on the problem.

Our Committee was aware that we were dealing with a mold infection that attacked many commodities throughout the world. We knew that with the proper combination of moisture and temperature, mold must be considered inevitable on wheat, rice, cottonseed, barley, corn, soybeans, oats and many other items. It was, then, a total Agricultural problem that touched on many of the commodities and products that are eaten by all mankind. It was a problem that must be solved in order to preserve foods as we know them, and in order to safeguard the economies of many nations and areas of the world that were dependent upon agricultural commodities, not only to feed their people but also as export items representing large percentages of their nation's total revenues. We knew that there were nations in Africa, for instance, that could be reduced to poverty and bankruptcy if molds could not be controlled.

I might digress here for just a moment to tell of the tremendous impact this discovery has had on the world scientific community. It was believed that there was absolutely nothing new about molds. Man had encountered them since the beginning of time, and you can bet that the apple that Adam ate was a bost to millions of mold spores. Over the centuries we have been bothered by molds; annoyed by them, really, because they weaken our houses, damage our clothing, spoil our foods, and give off unpleasant odors and sights that are not aesthetically pleasing to us. Most civilized people have known from childhood that molds made food less tasty and they naturally took normal precautions to prevent mold actually enhanced the flavors of Roquefort and Camembert Cheese. These favorites are over 100 years old.

Furthermore, I recall my Junior Year in High School when I wrote my Junior Theme on a new wonder drug. I remember how intrigued I was with the knowledge that Sir Alexander Fleming, in a London Hospital in 1929 noticed that mold growing on culture dishes inbibited the growth of bacteria. Ten years later a substance was isolated from the liquid in which this mold was grown and it was named Penicillin.

I realize this is not "news" to you, but I emphasize it to bring into sharper focus the stunning realization in the past few years that mostly annoying and occasionally beneficial molds are capable of producing toxic substances. This has created a great impact in scientific circles and has brought the study of mycology to international prominence. Only last March the Massachusetts Institute of Technology sponsored a Conference on this subject attended by scientists and research people from all parts of the world.

The total effect of this attention to molds is of great importance because it will stimulate people such as yourselves to work on and eventually solve the problem. But all this attention has also placed a spotlight on the peanut industry, for as I mentioned before, the first significant dollar loss that was traced to Aflatoxin was those turkey poults in England in 1960 that were fed peanut meal from moldy Brazilian peanuts.

Thus the industry research committee realized it must emphasize that this is a mold problem, not simply a peannt problem. To this day it has consistently stressed this absolute truth that is often overlooked. But because peanuts *are* affected, our industry must shoulder the responsibility of doing all it can to prevent mold from contaminating peanuts. This will be a monumental task, and it will be up to research people like yourselves to discover the answers to many many questions.

For instance, when do the mold spores attach to the peannt or peanut hull? Is it in the ground, during harvest, during curing or later on? If it occurs at all of the aforementioned stages, when is it most likely to occur? How can we protect the seed, prior to, during and after planting? If mold attaches to a peanut, where is it going to be found? On the skin, or iu it or under it? Does it permeate the meat or only stay ou the surface? When we discover it, how can we quickly and effectively learn whether or not it is the type of mold which will produce Aflatoxin? If it does produce Aflatoxin how may we remove it? By abrasion, by roasting, by washing, by grinding?

The list of potential and important questions is endless. Some of the answers are known in a general way, but most are not. For instance, we are quite certain that damaged, discolored and moldy peanuts are most apt to be the hosts of Aflatoxin. So we, as an industry, will wear sack cloth and ashes and remove these peanuts from edible channels. This will be very costly, to all parties concerned. But it will be done because it must be done. Uncertainties do exist, however. When is a peanut discolored? Obviously if it is black, it is discolored, and if it is clear white, it is not. But what of the dozens of shades in the middle? The same is true if the peanut has not been blanched. While pink skin is acceptable, what about dark pink, or skins that are mottled pink and red?

So the problem creates many questions, and they all need answers. Sooner or later our cooperative research will provide those answers. At the moment we must dedicate ourselves to channeling our activities toward the creation of the means necessary to providing these answers.

I have already mentioned that the National Peanut Couucil Research Committee continues to emphasize this as a total agricultural problem even as it devotes itself to peanut industry efforts to master it. In this regard the Committee met, continues to meet, and will meet ofteu in the future with members of the Department of Agriculture. Dr. Brady has spoken of the department's research program and we are proud to be playing a part in its development. After many months of hard work we believe an effective program will be operational for the 1964 Crop of peanuts. We hope it will provide much material and many answers.

Our Committee has met with members of the State Extension Services and State Experiment Stations from the peanut producing states in an effort to stimulate research at the state level. This is absolutely essential to the continued prosperity of the peauut industry. We are keenly aware of the need to transmit all possible information to the many growers of peanuts and have therefore worked with the Extension Services and grower representatives to help educate the grower to the safest and most practical methods of harvesting aud curing peanuts in order to eliminate mold growth.

Our Committee has worked closely with Jim Thigpen of the Department of Agriculture in attempting to analyze representative samples of 1963 Crop peanuts owned in cold storage by the C.C.C. This was an essential first step toward the eventual determination of what types and grades of peanuts were most affected by mold. A number of the samples were analyzed by Dr. Leo Goldblatt at the Southern Utilization Laboratory in New Orleans. Because of the pressing need for all available information, five of our technical members offered the services of their company laboratories in analyzing samples drawn by U.S.D.A. I might add that this was a most generous offer since the analytical methods at this date are both expensive and time consuming.

The results of all of this testing confirmed what the Committee had felt would be the case: Damaged peanuts almost invariably carry traces of Aflatoxin, and small immature peanuts that do not grade as splits or #1 Grade Peanuts or better have a marked tendency to be hosts for aflatoxin. On the basis of these results, Jim Thigpen and the peanut shellers have spent many hours working out a contract for 1964 Crop peanuts that will be signed by C.C.C. and, I ardently hope, all of the peanut shellers in the country. And, let me add here that I publicly applaud the tireless efforts of the shelling industry to impose self-restrictions to improve quality in peanuts. Their earnest cooperation has been truly inspiring. This contract should be in final form within a few days. It will, I believe, be an effective means of preventing undesirable peanuts from reaching any peanut product that is purchased by the public. There must be total cooperation and attention to the constant picking of damaged peanuts by the manufacturer, however. The sheller cannot deliver a finished product, and so the processor must strive to continue picking peanuts before and even after the roast, if possible.

The Committee realizes one of its major responsibilities is communicating with the peanut industry. This means that we will publish information that is pertinent and helpful, but will not publish anything just to hear ourselves speak. We are thoroughly cognizant of the dangers involved with a subject of this nature. We realize that anything we say might reappear in print in an article that could be misinterpreted by the public and bring resultant harm to the industry. We have made several statements on this problem, however, and expect to make more in the future. Our previous statements have been issued in order to give the peannt industry a complete picture of the situation, and to prevent unnecessary fears, while at the same time stressing the absolute importance of industry knowledge and vigilance. Several weeks ago, we sent a letter to every known American food processor who uses peanuts in his edible products—421 firms in all. None were returned for remailing the second time. The letters stressed Pure Food and Drug and U.S.D.A. concern over the problem and advised the recipient to review his processing procedures so as to remove all damaged, discolored aud moldy peannts from entering his finished products. We believe most manufacturers are now trying diligently to cooperate by applying increased attention to their purchasing and manufacturing policies.

In the future, we hope to offer suggestions for food processors to improve their picking operations, handling and storage practices, transportation methods, final disposition of pickouts, etc. We will only be offering suggestions, of course, but we feel confident that they will be wise and beneficial and accepted as such by the processors.

Perhaps the most pressing problem facing the Committee has been the determination of an efficient, effective, rapid and inexpensive method of testing peannts for the presence of contamination. This was immediately necessary because unfess we could locate and analyze the peanut mold, we could not eliminate it. Since this required a crash program, and since none of the Committee Members were able to devote full time to it, we hired the firm of Arthur D. Little, Inc. of Cambridge, Massachusetts, to help us. This is a large scientific organization with au impressive history of aiding businesses. It is world wide in scope and entertains an excellent reputation for successful work. We were fortunate in securing the services and personal attention of Senior Vice President, Dr. Charles J. Kensler, who I hope you will all meet at this Conference.

Specifically, Arthur D. Little is engaged to assist the Committee as follows: 1) to assist in the standardization of methodology, both chemical and biological, appropriate for the measurement of aflatoxins; 2) to optimize sampling procedures for quality control purposes; 3) to assist in the preparation of voluntary codes of good practice for all handlers of peannts; 4) to analyze public health disease data and collate the information on species differences, dose-response relationship and thresholds on such materials as the Aflatoxins; and 5) to assist in any other manner requested by the Committee.

We are pleased with the work done to date by Dr. Kensler and his Associates, and appreciate his coordination and study of the hundreds of facets to this problem. He has been particularly active in trying to establish a standard test for Aflatoxin and is working closely with Pure Food and Drug in the United States and in Canada, U.S.D.A., and all companies, facilities and individuals who can contribute knowledge in this area. Retention of this firm offers every person and organization in the peanut industry an opportunity to have confidential contact with a scientific organization of international reputation. A report of this nature is often frustrating. If I only describe a few general areas of our work, it sounds like the Committee is loafing, and yet if I fill it full of the innumerable tasks that the Committee and its individual members have performed, the report would be much too long. I have, therefore, tried only to highlight some of our attitudes, goals, and activities in order to give you an overall picture of our challenging task. I hope you have a better idea of what your industry committee is doing, and I hope each individual in the peanut industry will dedicate himself to coutributing toward some final solutions. No one person or organization can go it alone—everyone must do his share even thongh it hurts, and I can assure you it will hurt, at least for a while.

But there is a certain feeling of pride that you will all feel when the problem is licked, and this great industry advances another giant stride in its victory. There is no thrill like meeting adversity and triumphing over it. The harder you work, and the more you accomplish the greater will be your personal and financial rewards.

I remain supremely confident that the American peanut industry, in cooperation with the American government, will again prove to the world that we have the finest agricultural system known to man. We accept the challenge handed to us by nature and will turn it into another opportunity to demonstrate that American peanuts and peanut products continue to be the cleanest, purest, freshest, tastiest and safest food that can be purchased in the world today.

## FACTORS AFFECTING THE SALE OF PEANUTS IN FOREIGN MARKETS

#### H. W. CURTIS

#### Canada Packers Limited, Toronto, Canada

As a Northerner, you can imagine my dismay at the prospect of visiting Alabama in the heat of July. However, the warmth of the climate is exceeded only by the warmth of the Southern hospitality—that is, the warmth up to now by the time I am finished, I hope that we will still need the air-conditioning and that the atmosphere hasn't become too frigid.

I took my instructions at their basic meaning and in the understanding that this is a sincere effort to improve an industry and that there is a sincere interest in the title, "Factors Affecting the Sale of Peanuts in Foreign Markets."

I felt that, in fairness to you and to give yon the broadest picture that I could, that rather than merely reflect the views of Canada Packers, I should bring you as broad a view of the Canadian market as I could. I therefore contacted the following people who represent 60% of the Canadian peanut consumption in my estimation:

Mr. G. Caldwell	Standard Brands Ltd.
Mr. W. Schwartz	
Mr. A. Vickers	G. E. Barbour Co. Ltd.
Mr. H. Humpert	
Mr. M. Blanch	Loblaw Groceterias Co. Ltd.
Mr. H. G. Kneeshaw	

My secretary suggested that I was doing this because I was hoping they would write my speech for me and, in essence, I was, and in truth they did.

My remarks therefore reflect the views of these gentlemen and their companies and of the company I represent. I hope they are constructive, useful and accepted in the spirit they are delivered.

In order to place my views in perspective, let me quote the Canadian market as it stands:

Total Canadian Imports of peanuts for the past two calendar years have been: 1963 — 83.292,000 lbs.

1962 — 95,606,000 lbs.

Of these figures, imports from the U.S. have been as follows for the past two years:--

> 1963 — 30,967,000 lbs. 1962 — 15,797,133 lbs.

As you can see there was a sizeable increase in the percentage of U.S. and almost double the volume of U.S. peanuts used in Canada in 1963.

This was primarily due to certain world markets being over the U.S. prices for a period in 1963. The smaller importation figure does not represent a decline in consumption, but rather the trading conditions, as when U.S. peanuts are purchased in this quantity, inventory positions are shortened.

We are not a peanut growing country as you know and therefore must import all the peanuts we use. Imports into Canada are subject to inspection by the Food & Drug Department of the Department of Agriculture. We have available to us peanuts from all world markets and of course, purchase American peanuts under the C.C.C. programme.

It is the unanimous opinion of the group of people who I contacted that American peanuts are only of interest to Canadian manufacturers on a price basis. Given equal or even slightly higher prices from other origins, there is no interest in U.S. peanuts. I realize this is rather a harsh statement, however, it is somewhat modified from some of the comments which I received which I will quote later in the paper. The basic reason for this lack of interest at equal prices is the standard of cleaning and grading provided by American sources. It is also the general consensus of opinion that flavour and other characteristics can not be compared to the advantage of American peanuts. Let us break this down by the two peanut types.

#### SPANISH

Canadian industry rates American Spanish as third in its preference of the world peanuts available to us. The standard of excellence in Spanish type peanuts is South African Natals. These peanuts are 99.5% clean. They are evenly graded in the two sizes, 60/70 and 70/80 kernels per cunce, and are highly desirable in texture and flavour. Loblaws, for example, will not use American Spanish peanuts as salting stock because of the excellence of the Natal stock. Indian Javas rate number two and Brazilian Redskins are not considered above the average of American Spanish in cleanliness or grading; they are considered by some of the Canadian trade preferable in flavour.

Some of the basic problems in the American Spanish that were reported are as follows:

#### Cleaning

A "good delivery" permits, in the U.S. No. 1 grade kernels, up to:

3% Damaged, Unshelled and Minor Defects

4% Split or Broken

1/4 % Foreign Material

2% Other Varieties

and the industry generally reports that it is not uncommon to receive deliveries analyzing very close to these maximum tolerances.

Two of our associates indicate that it is uncommon to receive anything better. It is the consensus of opinion that due to the labour costs and loss of weight incurred in recleaning, the basic price of U.S. peannts is increased 5 to 10% by conditions at the time of delivery.

In view of the interest in Aflatoxin, which will be dealt with in great detail tomorrow, we are most concerned with the amount of damage that is allowed as a potential contributor to this condition.

#### Grading

The American specification is for screen size  $(15/16 \times \%$  inch openings) only. Tolerance of 2 to 5% passing through such prescribed screen is permitted. This results in a mass of kernels very irregular in size, with counts ranging as widely as 55/85 per ounce. For some uses, such as candy bar manufacture, regrading is necessary. This, of course, results in increased costs to the confectiouer.

#### General

Spanish peanuts grown in the Sonthwest States are hard, dry and with little taste. These are unsuitable for confectioners generally, and to most salters. Southeast origin rate fair, but not more, in texture and flavour.

There is no reason that the Canadian customers know of for the use of catch weights in peanut shipping. We just do not know and cannot understand why standard weights cannot be used by the industry. The U.S. is the only country with which Canada trades that is on this basis. I would certainly recommend the adoption of such a programme.

With these comments in mind, it is easy to see why the Canadian industry is willing to pay a premium for Spanish type peanuts from a source other than the U.S.

#### VIRGINIAS

The key note of the comments ou Virginia peanuts was a request from Mr. Blanch at Loblaws that I quote him accurately as follows, "If extra large are the best they can do, they will have to do a lot to convince me to buy again. I would be willing to pay a *substantial* premium for Chinese peanuts in view of our experience on damaged and splits." As Chinese peanuts are being offered again, here at least is one customer who has lost interest in American Virginia peanuts.

While I am certainly not advocating waving the wrong flag or trading with the wrong country, we in Canada generally acknowledge the fact that China delivers the finest Virginia-type peanuts available. China contracts and delivers peanuts with a maximum of 1% imperfections and guarantees 99% perfect kernels.

Their size grading is perfect in the fine calibrations, 28/30, 30/32, 34/36, 38/40 and 45/50 per ounce. It is generally an accepted fact in Canada that there will be no extra costs for cleaning, regrading, pick-outs, etc., and peanut traders value the Chinese peaunts at, at least one half cent per pound above any other origin on this basis alone.

The second choice of Canadian users of Virginia type peanuts is Mexicau, on the basis of better grading only, in that Mexican peanuts are generally about equal to American in other respects. Their grading is considered superior in the sizes 28/32, 32/36, 37/45 and 46/55 per ounce.

What are the objections to U.S. Virginia peanuts? The tolerances permitted in cleanliness closely parallel the Spanish tolerances although they are a little better, however, considerable hand picking and recleaning is required. The size grading is basis kernels per pound, which is, as yon are aware:

U.S.	Extra Large	-	512	per	lb.	=	32	per	oz.
U.S.	Mediums	_	640	рег	lЪ.	=	40	per	oz.
U.S.	No. 1's	_	800	Der	Ъ.	-	50	рег	oz.

Your tolerances permit 2 to 3%. As a result, we end np with quite uneven grading which gives rise to a higher percentage of splits in the blanched product. There is little point in dwelling in detail on your Runner type of peanut as Indian Bolds are the only near comparable nut. Since they are both used almost eutirely in Peanut Butter manufacture, we will assume that there is little, if any, advantage of one over the other.

These are the quality factors affecting the sale of peanuts in Canada but there are a number of other factors which I thought would be of interest to you. All of the comments which I received referred to the concern of the continuity of supply. Most of the peanut traders in Canada work to specific coverages within the framework of their company's directives. One, for example, mentioned that his company only allows a three month coverage and any additional coverage must receive the Board of Directors' approval. Receutly, he was allowed three weeks to take a six months' position, not an easy task. Our Atlantic Provinces, because of their location, feel that they must be completely informed as to availability and price in the late Fall, so they can make their purchases, in order for the green peanuts to arrive before the end of April.

In Montreal and Toronto the situation is quite different in that here the traders really have two deadlines, one in late August or early September and the other in the spring.

If, for example C.C.C. or American peanuts are not going to be available to cover the complete period between the close of navigation in the Fall (geuerally November) through to the opening of navigatiou in the Spring (late May or early June) our traders must take a position on foreign peanuts in September to ensure their receipt before navigation closes. While the peanuts may be available later, the necessity of bringing them into a winter port and then shipping by rail adds considerably to the cost and is generally avoided when possible.

Again in late December or early January the traders must have some ideas as to the summer availability in order to buy their requirements for the summer, for arrival at the opening of navigation, if U.S. peanuts are not going to be available. Undoubtedly incomplete market information, fluctuating prices caused by trading has caused Canadian buyers to purchase off-shore when a clearer market picture and programme would have resulted in the U.S. doing the business.

Contrast the current conditions as outlined with purchasing from Natal where once the price has been established it is firm and the buyer is protected against decline.

We realize that selling peanuts under the C.C.C. programme makes foreign trading difficult, not only in Canada, but to the European markets. We suspect at times that speculation on the part of U.S. processors is also a block to sales.

Another factor of great concern to Cauadian manufacturers is the fact that it is possible to obtain complete insurance coverage against losses from mould on all peanuts of off-shore origin and it is impossible to obtain similar coverage for peanuts from the U.S.A. This, of course, has recently been flagged by the emphasis being placed on Aflatoxin. While it is possible to explain this Aflatoxin situation by suggesting that peanuts free from infection when shipped are unlikely to develop mould in the brief transit time to destinations in Canada, this is not realistic as the method of sampling is such that it is quite possible for peanuts to pass U.S. inspection and be rejected by the Canadian inspection.

As it has been the practice of at least some of the Canadian purchasers to store their peanuts in the States, they are even to a greater degree in the dark as to where they actually stand, than in the past. It is conceivable a buyer could take a position, store his later forward requirements and then have all or a major part rejected at the time of receipt. As yet we are not clear where the financial responsibility lies and should it be at a time when other peanuts are not available the loss could be of much greater cousequence than the value of the peanuts and freight.

Surely within the framework and attitude of friendly co-operation between our governments and our nations it is possible at some time to set up an inspection that will be mutually satisfactory and that will eliminate the costly shipping of peanuts that will or are likely to be rejected. The acceptance of mutual standards of quality and inspection should not really be difficult in that I cannot imagine that either country, its manufacturers or consumers want less than standard for either itself or its neighbours.

While we have no growing industry, this is the only phase of the peanut business we are not concerned with. We recognize that we have a responsibility to it and are quite prepared to accept it. I honestly feel that we may have been remiss in not seeking an opportunity to be heard in the past as everyone I contacted was most anxious to have a chance to contribute.

I hope you will accept these remarks within the framework I have established, confident in the knowledge that they are meant to be constructive and helpful. Any of the gentlemen I referred to will be delighted to do anything or expand

any comments that I have made that will be of assistance. I wish to thank you for inviting me to this conference and I hope that we will

I wish to thank you for inviting me to this conference and I hope that we will continue to work together for the betterment of *our* industry.

## INDUSTRY'S RESPONSIBILITIES

#### A. S. YOHALEM Corn Products Corporation New York, N.Y.

The program says I can take 25 minutes to cover the subject of onr peanut industry's responsibilities. Actually I could probably do it in 25 words or less. And I'm confident that every one of you could do it, too—because no one can claim a patent on enunciating our responsibilities.

In fact, those of yon who heard me at this conference two years ago will be hearing an echo of what I said then. Some of yon may have heard a talk I gave at the first Peanut Research Couference seven years ago. I think I said essentially the same thing back then.

What is the message that I think is worth dressing up and parading ont whenever you give me a platform? A simple credo—the credo of *total responsibility to the consumer*—to give her peannts, peanut bntter, peanuts in any form—even those forms we haven't thought of yet... to give her ever more tasty, appealing, nutritious, and wholesome peanut products ... to give her quality—and never to compromise quality.

Simple? Sure! Demanding? Absolutely!

This concept of responsibility demands that every one of us must be continually alert for opportunities to offer the public a better product than yesterday's —and a better one than anything available today.

Let me put it another way. The central purpose of each of us in the peanut industry—no matter what our individual job—no matter what link our activities provide in the chain to the consumer—our central purpose must be to satisfy consumers and increase that satisfactiou.

If we don't, we should realize that there are waiting in the wiugs: people . . . companies . . . iudustries that *will* address themselves to consumer satisfaction, and thereby replace any laggards among us.

Our challenge is to constantly enhance and improve the eye appeal of peanut products—to make their taste ever more irresistible—to give them greater usefulness, improved freshuess, more convenience, flexibility, satisfaction iu every shape and form—in short, quality in its fullest sense. Here is a challenge that is never fully met. It is always there to stimulate creative, truly enterprising people.

Not only people in research—but those in agriculture, marketing, purchasing, processing, those who snpply equipment and materials, and certainly those in

management—everyone associated with peanuts must be alive to the combination of problems and opportunities confronting us.

Needless to say, research has always been a key element in this industry's progress. Without the dedicatiou and unity of purpose of people such as yon, this iudustry would be stagnant and possibly dying. Any thoughtful observer must conclude that the industry's greatest strength lies in research—in you people and your colleagues. This, in fact, is one thing there is absolute industry agreement about—some people have goue so far as to say it is the only thing this highly competitive industry has ever agreed on.

There is full recognition that research holds the key to the potential of the peanut—not only as it applies to this country, but as it cau contribute to man's well-being and happiness throughout the world.

We in the business of marketing peanut products compete tooth and nail to attract the consumer to our particular brands. In the same way, growers in different areas struggle among themselves to move their particular crops to the market place. All of us must recognize our dependence on the solid base you in research provide for us. And we draw a sense of security from the fact that you are here to exchange information and sharpen the effectiveness of your research programs. This proves the vitality of peanut research today.

I might say at this point how much satisfaction it has given us to see the ihdustry's Golden Peanut Award go again this year to one of you who have contributed so much to peanut research. We share your pride in this recognition given to another from your ranks.

Well, I warned you that I was going to talk about responsibility in the same quality terms I used back seven years ago—in the days before we recognized the word aflatoxin. Does this mean I am trying to walk away from the aflatoxin situation? Far from it! You who have brought scientific training and inquiry to this subject, you who have studied it, certainly have an understanding that cannot be matched by a layman. But I—and other laymen in the industry have devoted many hours to trying to acquire more understanding of this subject and many hours more to hard thought about its implications. So I assure you that I'd be the last to pretend aflatoxin wasn't there. And I can assure you that the industry is not doing any pretending either.

I can think of no subject more on the minds of people in the peanut industry. George Hartnett has already this morning discussed the steps being taken by the industry to come to grips with the aflatoxin problem. You have heard how the resources of Arthur D. Little, Inc. have been employed so that even the smallest mannfacturer can draw on scientifically trained, knowledgeable people for help and guidance in setting the practices that will maintain the purity and wholesomeness of our products.

You have also heard Dr. Brady of the Department of Agriculture talk about the Government's research program—research which the industry is cooperating with and supplementing. And, of course, at the conference sessions scheduled you will hear much more about the technical aspects of mold and aflatoxin.

With so many experts speaking on this important subject what can I add? Obviously, very little. But let me use the advantage of coming early on the program to say this: Aflatoxin is not a U.S. problem alone—nor is it a peanut problem alone! No—it is truly a total agricultural problem of international scope with more serious implications in many other nations than ours. Nonetheless, we in the peanut industry in the U.S. must seize this opportunity for leadership, an opportunity, for example, to help those peoples who need peanuts as a basic protein source and a staple of their economies. Eyes are turned to us from many distaut areas and from many international organizations. They look to American research for the resourcefulness and effectiveness for which it has a world-wide reputation. Here is a challenge that cannot be denied—a challenge to serve very basic needs of mankind. In emphasizing the world-wide importance of the aflatoxin problem I think I can fairly say that this doesn't require the slightest modification in my statement of the U.S. peanut industry's total responsibility to the consumer. Certainly if the consuming public is going to buy, they must have confidence in the purity of any food product. Therefore, every member of the peanut industry must recognize a responsibility to participate in the efforts to explore this situation more fully and help dispel the faintest suspicions that have attached themselves to the products we offer the public.

Some people have asked the question: what about those processors whose long-established practices give every indication of being a safeguard against aflatoxin—do they share this industry responsibility? I don't think there can be any doubt about the answer. Maybe a couple of short questions will put that answer in sharp focus. Doesn't every business connected with peanuts gain from a positive public acceptance of the industry's products? Doesn't every one of those businesses stand to lose if even a single peanut product made by a single, isolated manufacturer breaks faith with the consumer's right to purity?

I say our responsibility is total . . . absolute . . . clear and inescapable. It is total in that it applies to everyone in the industry. It is total in that we must get to the very root of aflatoxin. These efforts bear the endorsement not only of our industry, but of the Government and agriculture as a whole.

As I look around this room I am very much encouraged by the brains and talent that can be applied to this task. And I think I can assure you that your work has strong support. I have had meetings on this subject with growers, shellers, brokers, end-users, as well as with Government people and I am impressed by the widespread dedication and will to pursue aflatoxin until we achieve full understanding of it.

I hope that I am registering my feelings about the aflatoxin situation in an accurate way. I don't want to give any impression of gloom or desperation, because I feel neither way. What I have learned about this subject convinces me that it gives the industry as much a challenge as a problem. Therefore I feel proud of the way the industry is responding—by recognizing that this scientific challenge—with its new and strange terminology—is our business.

I also suspect that this response will earn us rewards that no one today can foresee. There are enough examples of industries that benefited from the spur of challenges to lift my optimism to something more than pollyanna moralizing. Perhaps my outlook is influenced by the wisdom passed on by a most practical man, a former great leader in the business of bringing a better peanut product to the consumer. He used to say—and firmly believe—that adversity creates opportunity for progress. He put it in a nautical frame by pointing out that the alert yachtsman can make his greatest speed in a storm. I think there is every reason to believe that the aflatoxin storm gives the peanut industry an opportunity for even greater research progress than would have occurred if the waters had remained calm.

Certainly the research effort that aflatoxin has stimulated may well produce desirable knowledge in other areas. Serendipity—the happy faculty for finding good things you weren't looking for—belonged to three oriental princes in ancient fable. In our modern world the research scientist seems to have the clearest title to this fine property.

Right now aflatoxin is on Track 1, as it should be. When we start moving along at top speed down this track, let's not forget that there are other tracks too. We dare not, for instance, forget that there are other forms of damage we must work to prevent. We don't want to lose our momentum on that track.

And, of course, "quality" should also be kept right on track in our thinking and our efforts. Can our aflatoxin programs derail our quality progress? Here's an example of what concerns me: I—and others—have spoken about the desirability of cultivating more flavorful varieties of peanut, rather than focusing too closely on yield per acre. I think the importance of quality as well as quantity is becoming established. Now I worry that it may become a casualty if all of us become infected with "aflatoxin fever" to the exclusion of all other activities.

And there are, of course, other pitfalls. So much for them! Obstacles should never deter a program designed to reach worthwhile objectives. And seldom has there been such agreement that here is an objective worth our while.

But let us also recall that even before the aflatoxin emergence in Great Britain, the Peanut Improvement Working Group had its sights on the right stars:

-improvement of quality, and

-increasing the consumption of peanut products.

And, in my book, these two are practically synonymous, because increasing consumption is completely dependent on quality. But however we define our long-range course, it seems fully compatible with our pursuit of more aflatoxin answers.

This is our 3rd National Peauut Research Conference. It is the first one at which we have had to consider aflatoxiu—perhaps there will be another conference sometime—in the near future, hopefully—when it will have been put to rest. When that happens, the conferences will still go on.

Even then we still will energetically and resourcefully strive after the twin goals of quality and added consumption. Or to put it back in the terms I started with: we'll be hard at work finding new ways of serving and satisfying consumers.

You can justify this concept of industry's responsibility ou ethical grounds. But you can make just as good a case for purely practical reasous.

Isn't this the only way our industry can continue our growth? I don't know of anyone who feels the industry should rest on its laurels. It has come so far that we have an ingrained expectation of growth. As you koow, in 1920 per capita consumption of peanut products was about 2½ pounds—today it is almost twice that much. This is no place to stop. The only limitation we face is not aflatoxin—it is human resourcefulness. Our industry will continue to grow so long as we accept our responsibility for quality.

This responsibility falls on everyone who has any direct or indirect involvement with peanuts. All of us—the research people in university, experiment station, government and industry laboratories—the extension service representatives the growers and their suppliers—the shellers, warehousemen, transporters, and brokers—the end-users, whether salters, peanut butter processors, confectioners all of us draw our livelihood quite literally at the pleasure of the consumer. We all exist to make the peauut a more gratifying food for people to eat. It must *smell* good, *look* good, *taste* good, and *be* good.

If anyone of us fails, all of us fail. And any failure will cause market place votes to switch to other candidates.

While there is vigorous competition at the market level for the right to serve the consumer, there must be one thing ou which there is full and complete agreement, cooperation, and coordination from the top to the bottom of the industry: We must all be dedicated to getting rid of the one bad nut that can repel a customer. That's all it takes to lose a customer.

At the same time, we can gain new adherents. For it is equally true that our successes will benefit us all, just as surely as failures hurt us all. I am very optimistic about this industry. The fact of our being gathered here with this important agenda before us is evidence of the strength and purpose of a vigorous industry. The fine course you in the Peanut Improvement Working Group are traveling is a further sign of strength—a strength that will bear continuing fruit in the future. I would say too that the positive industry response to the aflatoxin situatiou is another good omen, along with the progress we see in other areas affecting quality. What do you get when you add up these elements? You have a peanut industry that knows where it wants to go and is taking positive steps to get there.

That, to me, is a mark of high responsibility.

And it should give each of us a sense of pride and purpose that will help us get on with the job.

## RECENT DEVELOPMENTS IN RESEARCH ON PEANUT POD ROT

KENNETH H. GARBEN

Crops Research Division, A.R.S., U.S.D.A., Holland, Va. In Cooperation with Tidewater Research Station, Va. Expt. Station

#### **FOREWORD**<sup>1</sup>

Even with allowances for poetic license, there is more truth than poetry in the foreword. In the early stage of pod development, growth is very rapid and this makes for young peanut fruits which are succulent, i.e. tender and almost juicy. The locale of this succulent development is the soil, an environment teeming with organisms involved in breaking down all sorts of organic matter.

In 1961 and 1962 I made rather large samplings throughout the pod maturation season from replicated experimental plots in Virginia and thus I obtained actual data on importance of in-soil rot of peanut pods. For example, five weeks before normal digging time in 1961 au average of 32%, count basis, of the pods in untreated plots were rotting. At normal digging time the rotting-pod-count was down to 21%. Obviously this decrease in pod-rot-count represents pods which had completely disintegrated before digging time.

So much for the background and justification for this study of peanut pod rot. My observations and research with developing peanut pods strougly suggest



<sup>1</sup> The drawing of developing pods used in the foreword is adapted from B. W. Smith, "Arachis hypogaea. Aerial flower and subterranean fruit." Amer. Jour. Bot. 37: 802-815.



FIG. 1. Pods of Va. Bunch 46-2 peanuts from different fields at Holland, Va. in 1963 showing (left) a general breakdown type of pod rot, and (right) a dry, broken-lesion rot. Pods enlarged about 3X.

5 hypotheses. 1. In the soil, an exclusively unique environment for fruit development, peanut fruits are in intimate contact with actively growing microorganisms. 2. The mechanics of peanut fruit growth is such that the fruits cau be invaded by a succession of organisms. 3. Nevertheless, most fruits mature to sound pods. 4. But, under some conditions the microbial successions changed, "rotcausers" become dominant, and many pods rot. 5. Repeated nse of "good peanut fields" has resulted in deficiencies in the soil of these fields and these deficiencies have weakened pod tissue or otherwise promoted growth of semi-parasitic organisms and thns, by natural selection, strains of good pod rotters have originated in these fields. 6. Good bets for 2 conditions favoring pod rot organisms are low available calcium and high organic matter.

In ontlining observations, reasoning, and investigations on which these conclusions are based, I will stress a *modus operandi* for pod rot.

The first step in study of a plant disease should be to attempt to determine its cause. Plant pathologists have a ritual known as Koch's postulates. This ritual, which dates from abont 1875, is used to determine which organisms or pathogens cause which pathogenic disease. I have had very little success with Koch's postulates and peannt pod rot, bnt I have other evidence pointing to 2 suspects for pathogens of peanut pod rot in Virginia.

There are 2 different types of "unidentified" peanut pod rot in Virginia—the more common is a general breakdown, and a rarer form is a dry, brown-lesion rot in which the interior of the pod is consumed by dry rot (Fig. 1).



FIG. 2. Percentages of pods of Va. Bunch 46-2 peanuts rotted at harvest in untreated check plots or in plots treated with the Rhizoctonia-specific fungicide PCNB at 75 lbs. active/acre or the Pythium-specific fungicide DBSS at 100 lbs. active/acre and 800 lbs./acre landplaster applied to all plots in early July in 2 fields at Holland, Va. in 1961-63.

Pythium spp. cause general breakdown types of rots of aerial fruits, and I have isolated much Pythium myriotylum Dresch. from rotting peanut pods. I labeled P. myriotylum "pod rot pathogen suspect 1." Rhizoctonia solani Kühn sometimes causes a watery rot of tomato fruits and potato tubers but it is better known as the cause of a dry rot of succulent sterus and roots. I labelled R. solani "suspect 2."

There are now several highly specific fungicides. DBSS is one which is specific for *Pythia*, and PCNB is specific for *R. solani*. I always found a lot of *P. myriotylum* in field 1 and a high rate of DBSS, the Pythium-specific, greatly reduced pod rot in field 1 (Fig. 2). I never found *R. solani* in field 1, and a high rate of PCNB, the Rhizoctonia-specific, increased pod rot in field 1 (Fig. 2). On the other hand, *R. solani* predominated in field 2 in 1962 and the Rhizoctonia-specific PCNB reduced pod rot somewhat in field 2 in 1962. *P. myriotylum* predominated in field 2 in 1963, and the Pythium-specific DBSS greatly reduced pod rot in field 2 in 1963 (Fig. 2).

These results seem good leads as to causes of peanut pod rot. Costs of these fungicides prohibit their use at anything approaching these rates for pod rot control.

The change or succession of plant life on a field abandoned from cultivation or "turned back to nature" is well known. First annual weeds, then broom sedge, then pines, then mixed forest, and finally hardwoods. Our forester friends interfere with this succession to favor pines.

Similar successions of unseen rot organisms, or microbes, take place in logs in the forest and in crop debris in cultivated fields. Peanuts are evolutionary freaks in that their fruits develop in the soil which, to say the least, is an "unusual" environment for fruit development. It is logical to suspect a succession of microbes in fruits which develop in this unnsual and microbe rich underground environment. The result of "normal" or primary succession in this case must be sound mature pods, some of which coutain microorganisms in a quiescent state. But sometimes something interferes and an "abnormal" or secondary succession leads to rotted pods.



FIG. 3. Microorganisms of indicated broad groups found in sound and ratted peanut pods in 2 fields at Holland, Va. in 1963. Bars for techniques 1 and 2 show microorganisms found in rotted pod pieces incubated on non-nutrient tapwater agar (technique 1) or on tap-water agar with 100 p.p.m. streptomycin (technique 2).

A large number of variations in technique seemed "a must" in attempting to determine microorganisms in developing peanut pods. Some of the variations I used were very slight variations. For example, techniques 1 and 2 of Fig. 3 differed only in that I had no antibiotic in the non-nutrient medium on which microorganisms were cultured from rotting pods, while 2 had 100 parts per million streptomycin in the medium. If I had used only technique 1, I would have missed P. myriotylum, my prime pod rot pathogen suspect. If I had not any technique 2, I might have decided that molds are not associated with rotting peanut pods.

Even a great variety of techniques revealed no microorganisms in 35% of sound pods, but some fining were found in all rotted pods (Fig. 4). The 2 pod rot suspects were not found in sound pods, and *R. solani* was not found in field 1 (Fig. 4).

NOTH	ING		PYTHIUM (SUSPECT I)			OLDS
BACT	ERIA		RHIZOCTO (SUSPECT 2)	NIA		LL OTHERS
ALL TECHNIQUES	10%					
ROTTED PODS		-	<i></i>	UIITEU		DININGALIAN
ROTTED PODS						

FIG. 4. Microorganisms of indicated broad groups found in sound and rotted pods in 2 fields at Holland, Va. in 1963. Bars based on results with several variations in technique such as several different media and several different incubation temperatures.



FIG. 5. Schematic diagrams projecting succession of fungi in sound and rotted peanut pods at Holland, Va. in 1963 based on percentages of isolates obtained from all pods examined by all procedural variations on six dates (August 10 through October 15).

The chart of Fig. 5 projects succession of fungi in sound and rotting peds for the pod matnration scason of 1963 at Holland, Va. It is based on results from all techniques used. As an example of how it should be interpreted, a little less than 20% of the rotted pods examined on Sept. 1 had some mold growing in the still living tissne.

The percentage of organism-free pods decreased slightly as the season went on. Suspect 1, *P. myriotylum*, was prominent early in the season but became obscure somewhat abruptly in mid-season.

Molds in or on peanuts are much in our thoughts now. I found molds in sound pods all through the season. But these results give molds something of a role of invaders of rotting pods. Fig. 5 suggests that suspect 1 (*P. myriotylum*) inhibits mold growth when it is actively rotting pods.

Most peanut growers knowingly use landplaster to prevent a high percentage of "pops" or poorly filled pods. Drought and deficiency of calcium were suggested as the primary causes of "black pod" of peanuts in Southeastern United States.<sup>1</sup> Young peauut pods in Virginia sometimes have gelatinous, poorly differentiated seed, some with small spots of rot in the seed cavity. I suspected that this abnormality was caused by an imbalance of calcium.

Landplaster was applied at peak flowering and at 800 and 8,000 lbs. per acre in an 8-replicate test in field 1 in 1961. This procedure was repeated in field 2 in 1962. It was repeated in both fields in 1963. Pod rot was always reduced when available calcium in the soil was increased greatly (Fig. 6). This convinces me that the level of available calcium is a factor in peanut pod rot.

<sup>&</sup>lt;sup>1</sup>See "Black Pod," page 31, In Beattie, J. H., F. W. Poos and B. B. Higgins, Growing Peanuts, U.S.D.A. Farmers' Bulletin No. 2063, 1954.



FIG. 6. Percentages of Va. Bunch 46-2 peanut pods rotted at digging time and pod yields for 800 and 8,000 lbs. landplaster per acre, over 3 years and in 2 fields at Holland, Va. Seed bed preparation was by deep covering of organic trash and weed control was by non-dirting cultivation.

A 1963 experiment checked effects of land preparation methods and landplaster rates on peanut yields and pod rot. High spots of the results, which may be readily ascertained from a study of Fig. 7, are: 1, Yields increased with in-



FIG. 7. Percentages of Va. Bunch 46-2 peanut pods rotted at digging time and pod yields for conventional seed bed preparation and deep covering seed bed preparation with no landplaster, and increased rates of landplaster for one field in 1963 at Holland, Va.



FIG. 8. Percentages of Va. Bunch 46-2 peanuts rotted at harvest in one field at Holland, Va. in 1962-63 in (left) plots treated with the Rhizoctonia-specific fungicide PCNB at indicated rates in pounds active/acre and 800 lbs./acre landplaster applied to all plots in early July or (right) plots treated with PCNB and landplaster (LP) at indicated rates.

creased landplaster with optimum return from landplaster at 2,000 lbs. per acre. 2. Yield was higher for deep covering than for conventional laud preparation. 3. Pod rot decreased with increased landplaster up to the same 2,000 lbs. optimum. 4. We know conventional plowing does not equal deep covering in keeping raw organic matter out of the fruiting zone, thus we give low organic matter credit for the fact that pod rot was lower for deep covering than for conventional plowing.

Now, let us look at the future of pod rot control as shown in experimental results presented in Fig. 8.

Land preparation here was by deep covering. In 1962 with 800 lbs, landplaster there was a marked reduction in pod rot when the fungicide PCNB was used at a moderate rate, but at a high rate of PCNB there was only a slight reduction. In 1963 without landplaster a moderate rate of PCNB increased the amount of pod rot. A high rate of PCNB with slightly increased landplaster had little effect on pod rot, but with landplaster slightly increased this moderate rate of PCNB reduced pod rot very significantly. Biologists call an obvious interaction of this type a synergism. These and other results not mentioned suggest that control of pod rot will require a synergism even more complex than this.

#### SUMMARY

In their nnique soil locale peanut fruits are in intimate contact with a balanced soil microflora. This, and the mechanism of pod growth, combine to insure that peanut pods are invaded by a succession of soil organisms, but with enough calcium, with low organic matter, and with the physical environment "right", the result is predominantly sound pods with quiescent fungi.

When calcium is low and/or organic matter is high, the balance is disturbed and *Pythium* sp. and/or *Rhizoctonia* sp. rot some to many pods. We can have little influence over the physical environment but we may try to control pod rot (reduce loss from it) by increasing the available calcium or by decreasing the organic matter in the fruiting zone. A several prouged attack seems onr best bet. This summary is "summarized" in the following chart:

PHYSICAL ENVIRONMENT

HIGH

CALCIUM .

CONTROL

LOW

BALANCED SOIL MICROFLDRA MOLDS BACTERIA

DECAYERS

FUSARIA

PYTHIUM

RHIZOCTONIA

ETC. 4



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Severe infestations of the southern stem rot fungus, Sclerotium rolfsii, drastically reduce peanut production. Although recent developments in sonthern stem rot control are very promising, estimated losses of over 50% have been reported for individual farms (Loden and Hildebrand 1950) and losses of 15 to 25% have been common. Accurate estimates of yield losses associated with stem and root rot diseases of any crop, however, are difficult to determine. This difficulty is inherently compounded in a crop like peanuts where the harvested product is produced in the soil. Estimates generally are a comparison of an assumed production without the disease with the harvested yield. Accuracy of these estimates is dependent upon a thorough knowledge of the crop, disease severity and the

SOUND

OULET"

ROTTED PODS

LOW

ORGANIC

MATTER

HIGH

PODS

FUNGI

productivity of the fields under prevailing conditions. These estimates have been supplemented by gleaning sample areas of the field to determine weight of pods left in the field after harvest. This is not entirely satisfactory as pods infected early usually are destroyed completely by the time of harvest and other peg decays and mechanical breakage also contribute to the number of pods left in the soil at harvest. Thus an accurate means of estimating southern stem rot losses in growers' plantings is needed.

The general relationship between the stem rot phase of the disease with yields has been shown. Reyes (1937), Cooper (1953, 1956a), Boyle and Hammons (1956) and Garren (1959, 1964) record disease severity as the number or percentage of diseased or dead stems or plants and the yield of sound pods associated with the various variables under study. This relationship is frequently expressed as the correlatiou coefficient (Garren 1959, 1964) between disease level and yield. Although these correlations are valid comparisons between treatments uuder study, they do not always specifically indicate disease loss. Introduced variables, such as cultural and chemical control, varieties, etc., may influence production per se. For example, Boyle and Hammons (1956) and Garren aud Duke (1958) have indicated that deep land preparation (deep burial of crop debris) and flat cultivation (non-dirting) may influence peanut yields iudependent of that associated with disease control. The same limitations hold for disease severity and yield comparisons in chemical coutrol studies where diseases other than southern stem rot may also be influenced by the fungicide (Harrison 1961). In spite of these limitations this relationship between the stem rot severity and yield probably is the best approach to estimating southern stem rot losses.

Disease severity and yields were determined in tests in North Carolina of various aspects of the southern stem rot problem by using rather uniform plot size, inoculation rate and procedure. In early summer a measured quantity of steamed oat-S. rolfsii-mycelium inoculum was scattered through the plants to the soil surface. The rows were then lightly ridged with a flat sweep operated at a high speed. Six to 8 weeks later the number of dead stems (branches), counted as if they originated at soil surface, was determined. At maturity the plants were harvested and the sound mature pods were picked and weighed. The specific objective of these studies on isolate pathogenicity, chemical control, varietal resistance has been reported (Cooper 1953, 1956a, 1956b); however, where the data represent an unbiased relationship between the number of dead stems and yields, the regression values of yield upou uumber of dead stems have been determined and are shown in Tables 1 aud 2. For comparison hetween years and tests, regression values have been transformed to show the relationship between the number of dead stems per 100 ft. of row, counted as if they originated at the soil surface, and the yield to pounds per acre and to percentage of the adjusted yield for southern stem rot free peanuts.

The low regression values for the 1950 pathogenicity test probably results from two factors: 1) excessive ridging which resulted in additional branches in contact with the newly formed soil surface with each branch subject to decay,

TABLE	1.	CORR	ELA	TION	COEFI	FICIE	NTS	AND	RECRESS	ION	<b>OF</b>	THE	ì	IELD	ON	THE
	Nt	MBER	OF	DEA	d Stei	MS I	чP	THO	GENICITY	TES	TS	OF :	S.	rolfsi	i	
		]	SOL.	ATES	ON N	C 4	PEA	NUTS	-1950.	195	1. ]	1952				

Vear	No.	Dead stems/	Yields-lbs/A		Correlation	Regression	coefficient
Teal	isolates	100 ft. row	Avg.	Adj. ck.	<sup>-</sup> coefficient	lb/A	%
$1950 \\ 1951 \\ 1952$	$40 \\ 49 \\ 15$	659 470 421	$1360 \\ 636 \\ 1156$	2205 1437 1561	0.694** 0.702** 0.976**	844 698 805	038 049 052

and 2) the increase in soil depth which may have reduced the pod and peg rot phase of the disease in relation to the stem rot phase.

Variety NC 4 in Table 2 is directly comparable to data in Table 1. The regression of .069 is somewhat greater than those for 1951 and 1952 in the pathogenicity studies. A comparison of within-variety regressions when expressed as percentage reduction instead of pounds per acre, entries B 33 and C 42 become equal to NC 4. These differences reflect the adjusted yields and indicate a problem of varietal comparison of disease losses—should one consider the average reduction per unit of infection as pounds per acre or as the percentage of potential yield?

The regression values, as percentages, of the pathogenicity of S. *rolfsii* isolates tests, and the NC 4 entry in the varietal test and their meaus are plotted in Fig. 1. Since the regression lines do not indicate yields as great as disease-free yields it appears that these estimates are too low at low disease levels. A curviliuear regression would probably correct the deficiency.



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FIG. 1. The regression of peanut yields, expressed as the per cent of the yield of disease-free plants, on the average number of branches killed per 100 feet of row by southern stem rot.

Entry st	Dead stems/	Yield	s lbs/A	Correlation	Regression	coefficient
Entry	100 ft row	Avg.	Adj. ck.	⊂ coe⊞cient ~	Ib/A	%
B 33	178	1554	2039	8935	-1.309	064
C 42	252	$1170_{997}$	1979	6387	-1.332	067
C 12	348	1110	1731	9588	-0.823 -1.233	071
C 87	878	650	1217	9367	-0.950	078
C 18	414	732	1401	9202	-1.102	079

TABLE 2. THE WITHIN-VARIETY CORRELATION COEFFICIENT AND REGRESSION
OF YIELD ON THE NUMBER OF DEAD STEMS OF PEANUT SELECTIONS
INOCULATED WITH S. rolfsii Isolates-1952.
(Cooper and Gregory, unpublished)

The average line or regression values for these 4 comparisons are: -.819 lb/A or -.052% of the potential yield. By multiplying the average number of dead stems (counted at the soil surface) per 100 foot of row by the appropriate regression value indicates the disease loss as Ibs/acre or percentage of the potential yield for a particular field. The relationship between severity of the stem rot phase of southern stem rot of peanuts and yield reduction is such that the yield losses may be estimated by evaluating the severity of the stem rot phase of the disease.

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### CHEMICAL CONTROL OF Meloidogyne arenaria ON SPANISH PEANUTS IN TEXAS

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Infestations of the peanut root-knot nematode, Meloidogyne arenaria have beeu found in Eastland, Erath and Comanche counties in North Central Texas. Limited surveys of peanut fields indicate the infestations occur in small irregular

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areas of a few tenths of an acre scattered across several acres of a field. Heavy infestations drastically reduce yield and quality of peanuts. *Sclerotium rolfsii* and Cercospora leaf spot cause considerable damage in some years, also in irregular areas of fields. Moisture is often in critical supply and not many growers use irrigation. Rotations with grain sorghum are considered beneficial. Some growers abandon infested fields and plant them to Coastal bermudagrass. Control of damage by soil fumigation is not being used.

Soil fumigation trials have been conducted on a heavily infested field in Comanche Connty since 1959 in order to gain some knowledge of the economics of chemical control under the conditions outlined above. The results of trials conducted in 1961, 1962 and 1963 are reported here.

Methods: Nematocides were applied with a Fab Metals Applicator in-the-row 34 to 42 days prior to plauting—at a depth of 8 inches in beds 6 to 8 inches high. Plots were rebedded but not rolled after the treatment. Soil moisture and temperature were good at time of treatment in all three years. The soil texture is a loamy sand. Treatments were replicated 6 to 8 times in plots of two rows, 50 feet long. Peanuts were harvested from the middle 15 feet of each row and dried on the vine under shelter to 8 percent moisture in the kernels. Pods were removed from the vine, cleaned, and weighed. A 250 gram aliquot was shelled and graded for each plot. Value per ton was determined based on the price support schedule for the appropriate year. Gross value per acre was then determined for each plot and the per acre cost of fumigant deducted to arrive at net value per acre. No other production costs were considered.

Results: The 1961 trial received considerable moisture early and adequate rainfall for the rest of the year. Damage caused by Sclerotium rolfsii and Cercospora leaf spot were very low. Table 1 shows the results of the grading for that trial. Column 1 gives the treatment in gallons of formulations per acre, one gallon per acre corresponding to 0.28 milliliters per linear foot at this row spacing. Column 2 lists percent of pod weight due to kernels, the shells accounting for the other 25 per cent or so. These kernels were then placed on a 15/64 inch

Treatment	Total kernels	Kernels not retained	Retained on 15/64	Damaged kernels	Souud kernels
Check	74.3	7.9	66.4	8.0	63.4
Telone 14 gal	76.3	5.7	70.6	2.7	67.9
D-D 14 gal	76.2	5.3	70.9	2.2	68.7
Fum 70-E 2 gal	76.4	5.4	71.0	2.0	69.0
Fum 70-E 3 gal	76.3	5.5	70.8	2.0	68.8
Fum 70-E 4 gal	76.4	5.2	71.2	1.5	69.7
Rp 1% (p=2)	1.40	1.95	2.99	NSD	3.53
2-1	1.90	2.20	4.2		4.5

TABLE	1.	RESULTS	OF GRADE	EVALUATIO	N, 1961 TRIAL.	PERCENT
		OF POD	WEIGHT.	MEANS OF	8 REPLICATES	

TABLE	2.	YIELD	AND	VALUE	OF	PEANUTS,	1961	TRIAL
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Treatments	Pods, lb per plot	Pods, lb per acre	Kernels, lb per acre	Value per ton	Net value per acre
Check	2.47	1133	854	\$204.75	\$120.55
Telone 14 gal	4.27	1958	1502	217.25	193.95
D-D 14 gal	4,39	2013	1536	220.55	208.74
Fum 70-E 2 gal	4.46	2121	1628	222.12	211.01
Fum 70-E 3 gal	4.63	2045	1565	222.02	195.49
Fum 70-E 4 gal	4.69	2151	1651	225.89	198.88
Rp 1%	1.28		463.1	14.18	67.75
	1.80		646	12.50	73.40

Treatments	Pods, lb	Pods, lb	Kernels, lb	Value	Net value
	per plot	per acre	per acre	per ton	per acre
Penphene 3 gal Fum 70-E 3 gal D-D 14 gal Fum 70-E 1 gal Telone 14 gal D-D 10 gal Telone 10 gal Check	8.40 a 2.76 a b 2.72 a b 2.48 b 2.45 b 2.27 b 2.04 c 1.42 c	$1480 \\ 1200 \\ 1186 \\ 1082 \\ 1068 \\ 987 \\ 887 \\ 620$	1086 a 855 b 836 h 770 bc 757 bc 693 bc 611 cd 422 d	\$192.34 178.57 166.87 153.27 172.98 166.07 135.34 167.25 NSD	\$113.61 78.08 83.92 101.90 74.93 70.37 74.27 51.48 NSD

TABLE 3. YIELD AND VALUE OF PEANUTS, 1962 TRIAL

TABLE 4. YIELD AND VALUE OF PEANUTS, 1963 TRIAL

Treatments	Pods, lb per plot	Pods, lb per acre	Kernels, lb per acre	Value, per ton	Net value per acre
Vidden-D 10 gal	3.30	1135	809	\$163.99	\$105.48
Fum 70-E 1.5 gal	2.98	1025	731	151.54	98.86
Vidden-D 15 gal	2.98	1023	729	183.94	73.69
Fum 70-E 0.5 gal	2.88	991	722	196.50	93.03
Fum 70-E 1.0 gal	2.84	977	697	1S1.63	59.03
Telone 15 gal	2.80	961	698	184.60	68.37
Telone 10 gal	2.78	957	678	153.00	73.93
Penphene 1.0 gal	2.76	949	680	159.20	80.96
Penphene 0.5 gal	2.60	895	633	142.18	58.79
Check	2.52	866	614	160.97	74.27
Penphene 1.5 gal	2.21	759	534	136.98	61.79
		NSD, Trea	tments		

grading screen and shaken, allowing small shriveled kernels to go through. Damaged kernels were picked off the screen and weighed, as were the remaining sound mature kernels. Checks were significantly different from all treatments. There was no significant difference between fumigants.

Table 2 presents the yield and value obtained. The lowest return for treatment was \$73.40 more than for check. There was no significant difference between fumigants.

In 1962 rainfall was not adequate for good growth and in addition *Sclerotium* rolfsii was heavy in some plots. There was no significant difference in grading factors for the treatments of this year. Total kernel weight was much lower than for 1961 and percent damaged kernels about double. Table 3 gives the yield and quality obtained in this trial. Yields were significantly higher for most treatments than for untreated but quality was variable and net value per acre not significantly different for any individual treatment. Comparison of untreated versus mean of treated is significant at the 5 per cent level.

In 1963 moisture was available early and a heavy set of pods resulted, but drouth conditions developed before the pods filled and continued until harvest. Large irregular areas in the trial were defoliated and *Sclerotium rolfsii* was abundant. Yields from the different replicates were variable as was quality and there were no significant differences between any of the treatments.

*Conclusions*: Soil furnigation results in a demonstrable improvement of both yield and quality when growing conditions are favorable and infestations of *Meloidogyne arenaria* constitute the principal problem. But in years when moisture is limited and *Sclerotium rolfsii* and Cercospora leaf spot damage heavy, there is no economic return from furnigation.

## PHYTOTOXICITY OF HERBICIDES TO SPANISH PEANUTS

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Investigations on the use of chemicals for weed control in peanuts have been conducted in Texas since 1952, and these investigations were intensified during the past four years. Many materials were tested to determine their effectiveness for controlling weeds commonly present in peanut fields and at the same time to determine the effect of these materials npon the peanut plant. In the early stages of these investigations it was apparent that many materials were not promising as herbicides in peanuts because of poor weed control performance or phytotoxicity to the peanut plant. Some materials were found to be effective herbicides under some conditions but gave poor or erratic weed control under other conditions. None of the herbicides approved by FDA (Food and Drug Administration) for use on peanuts prior to 1963 were recommended for general nse in Texas because of damage to the crop under some conditions or unsatisfactory weed control.

Some growers in various parts of the State have had unfortunate experiences with the nse of certain herbicides on peanuts. Research in Texas has indicated that several herbicides, which are recommended and proven to be acceptable in other states do not possess the necessary safety margin for use on peanuts in Texas.

The pnrpose of this paper is to summarize results obtained with 2,4-DEP (tris(2,4-dichlorophenoxyethyl) phosphite), 2,4-DES (2,4-dichlorophenoxyethyl sulphate), and NPA (sodium salt of N-1-napthyl phthalamic acid) on peanuts, characterize the phytotoxicity which has been observed and list some conditions which emphasize it.

Results of investigations with herbicides iu peanuts have been published from various states during the past 15 years. Various degrees of phytotoxicity to peanuts have been reported ranging from only slight stunting to severe stunting and reduction in stand. Most of these publications have not reported any loss in yield from herbicidal treatments except where excessive rates have been applied. Georgia research workers have reported that plants from seedlings showing a high degree of formative reaction to 2,4-DES were more likely to become diseased. Researchers in certain states recommend phenoxy type herbicides for use on peanuts which have caused considerable injury to peannts in Texas.

#### MATERIALS AND METHODS

Experiments reported were conducted at one or more of five locations in Texas in each of three years.

1. At the Plant Disease Laboratory near Yoakum on either Tabor fine sand or Wilson fine sandy loam.

2. At the West Cross Timbers Experiment Station near Stephenville on Winthorst fine sandy loam.

3. At the O.D. Sadberry farm near Comanche on Nimrod fine sand.

4. At the Prairie View Station, Prairie View, on Hockley fine sand.

5. At the Frio County Peanut Growers' Association Research Farm near Pearsall on Daval fine sandy loam soil.

The peanuts were planted with a runner opener planter equipped with wings or blades to level the top of the bed so that a two-to-four-inch-high flat-top bed remained after planting. The herbicides were applied in 14-inch bands with a tractor mounted small plot diaphragm sprayer equipped with quick-cut-off solenoid valves. The chemicals were applied at the rate of 50-gallons per acre on a broadcast basis. Yield data were obtained for the individual plots and grade analyses were determined on the peanuts harvested from each replicated treatment.

#### **RESULTS AND DISCUSSION**

In a number of tests where phytotoxicity was severe, rainfall had occurred between application of the herbicide and emergence of the peanuts. In the 1963 test at the West Cross Timbers Experiment Station at Stephenville this injury was very pronounced. In this test 1.7-inches of rainfall had occurred on the third day after planting and applying the treatments. In the 2,4-DEP and 2,4-DES treated plots at 2 and 3 lbs per acre (active broadcast basis) emergence was delayed from two to 10 days, and the plants were severely stunted. The NPAtreated plots were also severely stunted. Two weeks after planting root systems of peanut seedlings from plots treated with 2,4-DEP and 2,4-DES were very restricted with the tap root shortened and enlarged, and the lateral roots very short, enlarged and in many instances curved. These lateral roots were constricted at the point of attachment to the tap root. The tips of these roots were often necrotic, and it was two weeks or longer before normal appearing roots were produced. The root injury was not as severe in the NPA-treated plots with the tap root appearing more normal, but the lateral roots were inhibited at first, and then there was a proliferation of lateral roots from areas along the tap root.

The top growth of the affected plants appeared to be equal to the untreated plants after approximately two months. The root effect was not completely overcome at this time, although normal roots had developed.

Tests were conducted at the Plant Disease Laboratory in 1963 and 1964 to determine whether the time of occurrence of rainfall was important on the phytotoxicity of these berbicides to Spanish peanuts. Irrigation water was applied with perforated aluminum pipe placed at right angles to the row. One and onehalf inches of water were applied either the same day of planting and applying treatments, one day, three days or five days later. The same type of root abnormality, which had occurred in the Stephenville test, was present in the 2,4-DEP and 2,4-DES treated plots in this test where sprinkler irrigation water was applied before the plants had emerged. This root injury was also apparent with the

	11-148	Yield	Average		
Ireatment	Lbs/A* -	1961	1962"	1963°	all tests
Check	0	1245	1481	1926	1557
NPA	3	1094	1390	1708	1398
2.4-DEP	2	1075	1131	1761	1340
2,4-DES	2	1238	1163	1736	1399

<b>TABLE</b> I	. 5	SUMMARY	OF	STATE	WIDE	PEANOT	HERBICIDE	TESTS	1961 - 63	
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\* Broadcast Basis

<sup>1</sup> Average of 4 tests

<sup>2</sup> Average of 3 tests

<sup>3</sup> Average of 4 tests

TABLE II.	TESTS RECEIVING	RAINFALL BETWEEN APPLICATION	OF
	HERBICIDES AND	EMERCENCE OF PEANUTS	

		Yield of clean pods lbs/A						
Treatment	Lbs/A*	. Stophenville			Coman- che	Prairie View	Pearsall	
		1961	1962	1963	1961	1962	1963	
Check	0	1059	1636	528	1055	702	2916	
NPA	3	762	1595	309	808	511	2336	
2,4-DEP	2	847	1048	334	592	645	2530	
2,4-DES	2	839	998	296	796	430	2730	

\* Broadcast Basis

emergent (cracking) stage application of these same materials when 1.5 inches of water were applied the next day after application of the treatments. NPA caused some stunting but there was more extensive branching of the roots, which were slightly larger than the nutreated.

In the 1964 test 1.5 inches of water were applied six days after plants had emerged. Roots were affected with the tips becoming enlarged in the 2,4-DEP and 2,4-DES plots. Plants in the NPA treated plots were also affected but to a lesser degree. In the 2,4-DEP and 2,4-DES plots there was a general yellowing of the leaves and some dying off of plants following post-emergence application of water. The root system was not as restricted in 2,4-DEP and 2,4-DES plots where post-emergence application of water was made, because the roots were better developed at the time the chemicals were leached into the root zone.

A summary of the tests on peannts conducted in 1961-63 is recorded in Table I. There was an overall reduction in average yield per acre of clean pods from the three herbicidal treatments, excepting with 2,4-DES in 1961, and only a slight decrease in 1962 with NPA. A more accurate evaluation of the effect of phytotoxicity of these herbicides can be made if tests are selected on which rainfall occurred before emergence of the peanuts as is recorded in Table II. Rainfall of from one to four and one-half inches occurred on six of the eleven tests conducted during 1961 to 1963. 2-4-DEP and 2,4-DES at 2 lb. per acre caused a reduction in yield in all six tests, and NPA-treated plots were lower than the nntreated check in five of the six tests.

Table III is a summary giving the average acre yields of clean pods from treatments receiving rainfall between application and emergence and also averages from tests not receiving rainfall during this time. Tests receiving rainfall had an average reduction in yield of 262 lbs with NPA at 3 lbs per acre, 301 lbs with 2,4-DES at 2 lbs per acre, and 317 lbs with 2,4-DEP at 2 lbs per acre. In the 1963 Stephenville test plots receiving 3 lbs of either 2,4-DEP or 2,4-DES showed more than 40-percent reduction in yield. In tests not receiving rainfall during this period no phytotoxicity was observed, and there were no significant differences in yield between these treatments.

T to b	71./48	Average yields of clean pods lbs/A			
Treatment	LDS/A*	With rainfall <sup>1</sup>	No rainfall <sup>2</sup>		
Check	0	1316	1839		
NPA	3	1054	1837		
2.4-DEP	2	999	1788		
2,4-DES	2	1015	1871		

TABLE III. SUMMARY OF PEANUT HERBICIDE TESTS 1961-63

\* Broadcast Basis

<sup>1</sup> Average of 6 tests (includes 1 irrigated test) receiving rainfall between time of applying herbicides and emergence of plants.

<sup>2</sup> Average of 5 tests (includes 3 irrigated tests).

Treatment	Lbs/A* Step		enville	Prairie View	Pearsall	Av. %	
		1962	1963	1962	1963	SMK	
Check	0	60.7	65.3	64.0	70.0	65.0	
NPA	3	61.6	60.7	65.5	68.7	64.1	
2,4-DEP	2	57.8	63.5	62.0	68.3	62.8	
2-4-DES	2	56.2	60.7	57.5	68.0	60.6	

TADIE	TV .	PERCENT	OF	SOUND	MATTINE	KERNIET CI
LABLE	1 V .	FERCENT	OF	BOUND	MATURE	NERNELS"

\* Broadcast Basis

<sup>1</sup> From tests receiving rainfall between application and emergence of peanuts.
Grade analyses were run on the various treatments from all tests, and the percent SMK (sound mature kernels) from four of the six tests where phytotoxicity occurred are recorded in Table IV. The percent of SMK was lower for 2,4-DEP and 2,4-DES in all four tests with the latter causing the greatest effect upon the SMK, which averaged 4.4-percent less than the check. The percent of SMK from NPA treated plots was lower in two of the four tests and averaged only 0.9 percent lower than the check. In some tests, where stunting was not apparent, the percent SMK was also lower for certain treatments. This supports the fact that these herbicides can cause a delay in maturity even without visible effect to the plant.

## SUMMARY

Yield of peanuts and percent SMK were significantly lower from plots treated with 2,4-DEP, 2-4-DES, or NPA in tests receiving rainfall of one inch or more between time of application and emergence of the plants. Delayed emergence, severe stunting, and root injury occurred in the 2,4-DEP and 2,4-DES treated plots. The stunting was not visible after two months, but the root condition was still apparent even though normal roots had developed.

This delayed emergence, stunting, and root injury was duplicated in tests by applying 1.5 inches of sprinkler irrigation water either the same day of planting and applying herbicides, one day, three days, or five days later. This condition also occurred with emergent-(cracking) stage application of these materials when water was applied the day after application.

Post-emergence application of sprinkler irrigation water to plots receiving preemergence treatments of 2,4-DEP and 2,4-DES at 2 to 4 lbs per acre caused this same root injury, although the root system was not as restricted as when the water was applied before emergence.

# SUBSURFACE APPLICATION OF HERBICIDES—A NEW APPROACH FOR WEED CONTROL IN PEANUTS<sup>1</sup>

#### ELLIS W. HAUSER

# Research Agronomist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Tifton, Georgia

For preemergence weed control in crops, herbicides generally are applied on the soil surface. However, the effectiveness of must herbicides is greatly influenced by environmental variables when surface applied. An example is the thiolcarbamates which are a group of volatile herbicides. One factor which decreases activity of surface sprayed thiolcarbamates is volatilization from the soil surface into the atmosphere. The degree of volatilization is influenced by the soil temperature, moisture content of the soil, soil adsorption, and other factors.

The use of incorporation tools such as disk harrows and rotary tillers has improved the effectiveness of thiolcarbamate herbicides by mixing the chemicals with the soil, thus reducing volatilization. A more recent innovation has been subsurface application of herbicides. With this method, a band or swath is applied at a specified depth beneath the soil surface. Precision subsurface herbicide placement minimizes or prevents volatilization into the external atmosphere and places the herbicide in position for maximum effectiveness in controlling weeds.

Since the thiolcarbamate herbicides were promising for broad spectrum weed control in peanuts, subsurface placement was investigated. Of particular interest

<sup>&</sup>lt;sup>1</sup>Cooperative investigations of the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, and the Coastal Plain Experiment Station, Tifton, Georgia.

was suppression and control of nutsedge (*Cyperus esculentus* L.) which is rapidly becoming the most troublesome weed in the southeastern peanut belt.

Subsurface herbicides were first evaluated on peanuts at Tifton, Georgia in 1963. The herbicides ethyl N,N-di-n-propylthiolcarbamate (EPTC) and N,N-propyl di-n-propylthiolcarbamate (PPTC) were applied as (a) preplant disk-incorporated, (b) surface preemergence, or (c) subsurface preemergence treatments.

Previous greenhouse research showed that placement at 1.5 in. below the soil level was more effective than placement at 0.75, 3.0, or 6.0 in. The sprays in the field were applied at a depth of 1.5 in. over peanuts planted at a depth of 3 inches.

Control of nutsedge shoots was 76% in the field 6 weeks after application of subsurface EPTC at 1 lb/A. Iu contrast, control of nutsedge with a standard surface preemergeuce rate of EPTC at 8 lb/A and disk-incorporated EPTC at 4 lb/A was 58 and 70%, respectively. Thus, based on the rates used, subsurface placement of EPTC was 8 times and 4 times more effective for nutsedge control than were surface preemergence and disk-incorporation, respectively. Subsurface EPTC at 4 lb/A gave 91% control. EPTC was more effective on nutsedge but more injurious to peanuts than was PPTC.

In 1964, EPTC, propyl ethyl u-butylthiolcarbamate (PEBC), PPTC, and mixtures of these herbicides were evaluated as subsurface sprays. EPTC, disk-incorporated and surface preemergeuce, was included as a comparative check. In contrast to the 1963 results, subsurface PPTC gave somewhat better nutsedge control than EPTC. Subsurface PEBC gave less control of nutsedge than either EPTC or PPTC. Peanut seedling injury was positively correlated with control of nutsedge. As in 1963, surface applied EPTC gave poor and erratic nutsedge control. In coutrast to 1963, disk-incorporated EPTC at 4 lb/A gave 100% nutsedge control at 6 weeks. Equally effective was a subsurface mixture of 2 lb/A of EPTC + 2 lb/A of PPTC. Almost as effective, with improved crop tolerance, was a mixture of EPTC at 1 lb/A, PPTC at 1 lb/A, and PEBC at 2 lb/A. Although crop injury from subsurface treatments was initially moderate or severe, the peanuts eventually recovered and apparently resumed normal growth. Wherever nutsedge was effectively controlled, annual weed control was also excellent. The annuals included crabgrass (Digitaria spp.), crowfoot grass (Dactyloctenium aegyptium (L.) Richter), Florida pusley (Richardia scabra L.), and coffeeweed (Cassia tora L.).

These preliminary experiments indicate that subsurface applied thiolcarbamate herbicides appear promising for the control of certain perennials and difficult annual weeds in peanuts. Preplant disk-incorporation has been erratic from year to year. Extreme variability has characterized surface preemergence thiolcarbamate herbicides. Thus far, surface applied EPTC and PPTC have given better control at lower rates.

The excellent control observed in 1964 with the EPTC-PPTC mixture suggests that mixtures of thiolcarbamate herbicides may give better control than when they are sprayed alone. Further research is needed on optimum subsurface placement and other factors for improving tolerance of peanuts to these subsurface sprays.

# PROGRESS IN SCREENING PEANUT LINES FOR RESISTANCE TO THE SOUTHERN CORN ROOTWORM IN SOUTHEASTERN VIRGINIA

### M. W. Alexander and G. M. Boush<sup>3</sup>

The southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, or 12 spotted cucumber beetle is the most important insect pest of peanuts in Virginia. This pest is native to Virginia and attacks over 200 species of plants. Damage to peanuts by this insect was first reported in 1916 at the Virginia Truck Experiment Station by Fink (3).

Boush et. al (1), estimated that from one-half to two-thirds of the peanut acreage in Virginia is subject to rootworm damage. This damage to peanuts is caused by larvae feeding on peanut pegs and pods, and results in a loss of yield as well as a reduction in grade value.

From the early 1950's until 1958, the rootworm was controlled with soil applications of either aldrin or heptachlor. In 1958, applications of these insecticides failed to give control on about 200 acres in Virginia due to the insects becoming resistant to the cyclodiene group of insecticides (1). The initial area of resistant insects enlarged until by 1961 the entire Virginia peanut belt was affected.

Insect resistant varieties offer the producer an economical method of control and problems associated with chemical residues are eliminated. The use of insect resistant varieties of field crops has been successful against insects such as com earworms on corn, hessian fly on wheat and chinch bug on sorghum. Carnahan (2) recently reported resistance of alfalfa varieties to pea aphid. Fronk (4) reported differential peanut varietal reaction to the rootworm but these studies were made with only one Spanish, one Valencia, and three Virginia varieties.

A preliminary planting of 172 peanut lines available at the Holland Station was made and evaluated in 1959. The study reported in this paper was initiated in 1960, and is still in progress. Changes in evaluation techniques are described, as well as summarized results of observing approximately 2,500 lines.

#### MATERIALS AND METHODS

Based on a promising preliminary planting in 1959, a large scale evaluation program was initiated in 1960. The primary source of lines was the Southern Regional Plant Introduction Station of the U.S. Department of Agriculture. These peanut lines were collected principally from South America and Africa with a few coming from India, China, the Philippines, Australia and Israel. Lines were also obtained from the North Carolina State peanut breeding program. These lines included the Virginia, Spanish, and Valencia types representing a wide range of pod and seed size, seed coat color and other morphological characteristics.

The 1960 rootworm evaluation experiment included 625 lines in single row plots 9 feet long containing 10 plants. These plots were arranged in a raudomized block design with 2 replications. The commercial varieties, Va. 56R and Va. Bunch 46-2, were used as checks. Response to rootworm was measured by visually scoring 3 plants from each replication: 0, or those showing no damage; 1, up to 10% damage; 2, up to 25% damage; 3 up to 50% damage; and 4, over 50% damage. Evaluations were made during the last week in August and the first week in September.

A test consisting of 486 lines which had been classified for type and 265 unclassified lines was planted in 1961 and a second test consisting of 887 lines classified for type was planted in 1962. Data from the classified lines only are

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reported in this paper. Single row plots, nine feet long containing 10 plants were arranged in a randomized block design with 3 replications. A check plot of Va. 56R was planted at the end of each 50 plots.

More damage was observed on immature pods and a more descriptive score was desired, therefore in 1961 the method of evaluation was revised. Fruits were separated into mature and immature lots and the percentage mature fruit was used as an index of maturity. The percent rootworm damaged fruit was determined for each maturity group. The total percentage of injured fruit was determined and used as a measure of infestation. These determinations were made on a composite of the fruits of 3 plants from each plot. Evaluations were made the last week in August or the first week in September both years.

Recommended agronomic practices were carried out each year on the evaluation plots, but no insecticide was used.

#### **RESULTS AND DISCUSSION**

In 1960, all plants evaluated showed some injured fruit. Several lines scored as low as 0.5. in one replication but at least 1.0 in the second replication indicating some variation in infestation. The data obtained are given in table 1 summarized by one-half score intervals; 5.3% had an average score of between 0.6 and 1.0 for the 2 replications. The largest or modal class of the lines was within the range of 1.6 to 2.0 or between 10% and 25% in injured fruit. Most of the lines had above 10% injury in 1960 and 48.3% had over 25% injury. The check variety Va. Bunch 46-2 which had an average score of 2.0 showed somewhat more resistance to rootworm than Va. 56R which had an average score of 3.0. A score of 1.0 was considered to be somewhat resistant. This falls within the requirement for commercial control (90% or above nninjnred fruit).

Score	% Frequeucy
0.6-1.0	5.3
1.1-1.5	15.0
1.6-2.0	31.4
2.1-2.5	25.9
2.6-3.0	19.8
3.1-3.5	2.6

TABLE 1. FREQUENCY DISTRIBUTION OF ROOTWORM INJURY SCORES<sup>®</sup> FOR 625 PEANUT LINES 1960

\* Ave. 2 reps. 1 = 10%; 4 = 50% +

Data from the 1961 and 1962 tests are presented in Figure 1. Spanish, Valencia and Virginia types are separated for comparison. In this study, the group with 0 to 5% injured fruit is considered the resistant group. Twenty-one percent of the Spanish lines were in the resistant group in 1961 and 29% in 1962. The Valencia type had 8% in the resistant group in 1961 and 7% in 1962. Only 3% of the Virginia type in 1961 and 2% in 1962 were in the resistant group. According to the classification in Figure 1, the Spanish lines most frequently contained 6% to 10% injured fruit both in 1961 and 1962. Fruit of the Valencia line most frequently contained 11% to 15% injury in 1961 and 6% to 10% injury in 1962. Most frequent injury in the Virginia lines was 20% or higher both years.

The nature of the apparent resistance is not known. Maturity may be a factor, with less damage occurring to the more mature fruits at the time of infestation. The results obtained in this study appeared to support this postulation since the Spanish lines matured earliest and Virginia types matured latest. However, a correlation analysis run in 1962 within lines of each type showed no correlation between the index of maturity and percent injury. The Spanish lines had





an r value of .0763, Valencia had r = -.0696 and Virginia had r = -.0488. Further study will be necessary to determine the nature of the resistance.

## SUMMARY

Approximately 2,500 peanut lines were evaluated for possible resistance to Sonthern Corn Rootworm Diabrotica undecimpunctata howardi Barber. Least rootworm injury occurred to fruit of the Spanish lines and most injury to the fruit of the Virginia type lines. The nature of this apparent resistance to rootworm attack is not known. Since the Spanish lines which usually mature earliest showed the least injury it was presumed that resistance was associated with early maturity. However, correlation studies of maturity and injury showed no relationship.

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# IMPACT DAMAGE TO PEANUTS AND ITS EFFECTS ON GERMINATION, SEEDLING DEVELOPMENT, AND MILLING QUALITY

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Work was carried on in 1961-62 to study the physical damage done to peanuts when they were subjected to impact forces and to investigate the effects of this damage on the germination, seedling development, aud milling quality of peauuts.

N. C. 2 peanuts, in the hull, were subjected to impact using a machine having a rotating arm with an impact head on one end of the arm. The coutrolled variables included moisture level at time of impact, orientation at impact and velocity of impact. The velocities ranged from 20 feet per second (fps) to 60 fps in increments of 10 fps. The impacts were made to occur on the apical end, basal end or side of the peanuts. Moisture levels corresponded to stackpole dried, windrow harvested, and green peanuts with moistures around 8, 23 or 40%.

The stackpole peanuts were stored immediately after impact while the other moisture levels were dried with natural air to 7 percent and then stored. The storage areas offered control of temperature and humidity.

## HULL DAMAGE

In addition to investigation of kernel damage the hull damage was rated in arbitrary units with ratings assigned to five separate sections of the hulls. Hull damage did not vary greatly with orientation. The apical end of the hull was most vulnerable to damage. At the lower impact velocities the higher moisture level nuts showed the most hull damage. At higher impact velocities the low moistnre level nuts were damaged most due to the brittleness of the hulls. In all cases the intermediate moisture level samples showed the least damage.

#### KERNEL DAMAGE

Kernel damage was given in arbitrary units with separate ratings given to the aricles, stele, cortex and cotyledons of each kernel. Damage determination was facilitated by first treating the kernels to a staining process, employing 2, 3, 5triphenyl tetrazolium chloride to make apparent the damaged arcas, and then disecting and viewing each kernel under a binocular microscope. It was apparent that the kernel in the apical end of the hnll was most vulnerable to damage. Damage resulted to the embryo of this kernel regardless of orientation, moistnre or velocity of impact. The damage increased with increase in moisture. The damage was markedly greater in the peanuts impacted on the apical end. As an example the high moisture level sample impacted at 20 fps had a predicted germination potential of 36% while the intermediate and low moisture level samples had predicted germination potentials of 70 and 91%, respectively. The kernel in the basal end of the hull proved to be well protected from impact damage. The manner in which the kernel is cradled in the hull explains why. Only at high impact velocities did the damage to these kernels become apparent. It would appear that the peanuts should be at a low moisture level when being subjected to impact forces to minimize kernel damage. However, the kernels tend to split at low moisture levels when the velocities rise above 30 fps. The magnitude of the impact velocities will influence the optimum moisture level for mechanical processes. Probably the optimum moisture level for minimum hnll and kernel damage would be around 20%. If the velocities involved are low, a low moisture level would be desirable.

## GERMINATION AND SEEDLING DEVELOPMENT

The germination and seedling development tests tended to verify the hypothesis formulated from the kernel damage evaluation. The germination of the kernel from the apical end of the hull was low. Germination was as low as 4% for the high moisture level nuts subjected to 20 fps impact. These tests showed, in general, lowest germination for the high moisture level samples. The intermediate moisture level nuts yielded the highest germination percentages. The primary roots of seedlings from the apical end seeds were often deformed. The percentage of deformed roots was greater when the peanuts were impacted on the apical end, i.e., the percentage of deformed roots was proportional to the embryo damage.

In the seedling development tests the germination and emergence percentages were higher than anticipated from the damage evaluation and laboratory germination tests. Emergence occurred even with seed having considerable damage to the embryos. This is consistent with the results of work by Teter *et al.* concerning the effects of radicle injury on the development of peanut plants, which showed excellent germination with seed having injured radicles. However, seedling emergence was greatly reduced from the 40 fps., apical end impact seeds. Even though seed with damage to the embryo resulted in emergence, the roots and tops were often irregular. The rate of emergence was also reduced.

# MILLING QUALITY

Milling quality was determined by the amount of split kernels resulting from the shelling of given size samples of peanuts. Milling quality decreased with an increase in impact velocity, the decrease being quite rapid for samples subjected to impact velocities greater than 20 feet per second.

At the intermediate moisture level there was a statistically significant decrease in milling quality for the 20 feet per second impact samples as compared with the check samples. The data for the other moisture levels also suggested a decrease in milling quality of the samples impacted at 20 feet per second. Yet, at this impact velocity for all moisture levels, the hull damage was slight and there was no apparent keruel damage revealed by casual inspection. The decrease in milling quality was approximately the same for the two higher moisture levels and greater for the low moisture level samples.

## THE IMPACT ACTION

Further laboratory work was carried out to obtain approximate values for the coefficient of restitution of typical peanuts and approximations for the average and maximum forces involved in the impact action. Measurements, taken from prints of high speed photographs of peanuts being impacted, allowed determination of these approximations. A camera speed of over 3,000 frames per second gave a time increment between consecutive frames small enough to allow fairly accurate determination of values for velocities and accelerations. To determine the average force during impact the time of contact between the peanut and impact head (t), the mass of the peaput  $(m_p)$  and the velocity of the peaput upon separation from the head  $(v_p)$  were used. Thus

$$\mathbf{F} a \mathbf{v} = \mathbf{m}_{\mathbf{p}} \mathbf{v}_{\mathbf{p}/\mathbf{t}}$$

For finding the maximum force on the peanut during impact is was necessary to determine the maximum acceleration experienced by the peanut. Since this was the maximum time rate of change of velocity it was possible to determine this from the photographic prints. It should be stated that a faster camera speed would yield more reliable and accurate results. The relation,

 $F max = m_p a_{max}$ 

was used to obtain the maximum force.

The coefficient of restitution was obtained by the relation,

$$e = \frac{v_p - v_h}{u_h - u_p}$$

where  $v_h =$  velocity of head after impact

 $u_n = velocity$  of peanut before impact

u<sub>h</sub> = velocity of head before impact

Two independent runs yielded the following force values.

F av = 2.511 and 1.868 grams

F max = 4,513 and 3,736 grams

Four values for the coefficient of restitution were determined.

Orientation	Impact velocity (fps)	e
apical end	30	0.174
apical end	30	0.305
basal end	40	0.222
side	40	0.343

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# NEW TECHNIQUES IN MECHANIZED PEANUT PRODUCTION

## JAMES L. SHEPHERD Agricultural Engineer Georgia Coastal Plain Experiment Station

Many factors have contributed toward the present high degree of mechanization in the production, harvesting and enring of peanuts in the heavy producing areas of the Nation.

Agricultural experiment stations of the University of Georgia pioneered several important contributions which developed into the new recommended "package plan" for peannt production.

In 1946 engineers at the Georgia Coastal Plain Experiment Station began a research and development program which has played a very significant role in mechanizing, from tillage through harvesting and curing, the state's peanut crop of more than one-half million acres. Of particular importance has been the development of principles and facilities for optimum procedure under the "package plan." Basic details of the "plan" were previously published in Proceedings of the 2nd meeting of this Conference; and this will only highlight subsequent additional refinements and developments.

Techniques in land preparation have been developed to a fine point, prescribing systematic procedure, all steps of which are interdependent. Consistently thorough burial of residual litter in soil turning is a first key step. Also, of fundamental importance is the further forming and conditioning of the seedbed in a manner to: (1), maximize preservation of soil moisture; (2) minimize soil compaction and use requirements of labor and equipment; and (3), provide optimum soil environment for plant growth.

The avoidance of disk harrowing during the period between soil turning and planting greatly facilitates observance of the several important precautions. This requires a definite sequence of timely operations which constitute short cuts for higher efficiency with lower costs, as well as optimize conditions for production. Normally, turned soil requires additional pulverizing tool action to prevent clod forming and improve soil texture to the depth of planting. The most effective time for this is during, or close behind the turning, while the soil is moist and soft.

A new device for soil pulverizing, or "combing," during the plow turning operation has proven successful and is ready for general recommendation. The unit which in principle features oriented combing rods, or teeth, functions as an integral attachment to plows of various sizes and nnmbers of moldboard bottoms. The prescribed disk coulters on a plow aid in the burial of litter to a depth below that of the conditioning action of the rods, thereby eliminating interference from the otherwise tronblesome material. Soil "combing" is also feasible to accomplish separate from the turning; during the very important operation of marking row patterns and tractor wheel positions. For this, a somewhat similar rod attachment mounts and operates just ahead of the smoothing blade of the bed forming unit. This operation precisely conditions the seedbed to a tabletop smoothness, providing a perfectly uniform soil profile of high tilth consistency. In effect, soil moisture is sealed in which then, prior to planting, is disturbed only to a minimum depth for controlling weed growth. This is accomplished by very shallow sweep cultivation. Modified type sweeps mounted in staggered and overlapping positions with positive depth control are ideal for this operation.

Currently certain experimental modifications to conventional peanut harvesting plows appear to have merit. The increasing significance of nntgrass (nntsedge) as a pest in peanut production prompts efforts to devise ways of hiving with it until effective control is attained. Plow blades are modified by the addition of flat bar extensions to the rear edges. In principle, after the modification each blade



Soil comb attachment developed at the Georgia Coastal Plain Experiment Station.

carries a "blip" extension in its true inclined plane directly beneath each peanut row. The extension is of sufficient length to provide a total effective blade width under the row of 10". Flat steel bar material is of  $\frac{1}{4}$ "  $\times$  4" size for two-row patterns and  $\frac{1}{4}$ "  $\times$   $\frac{2}{2}$ " for close four-row patterns. Even narrower bar stock may be suitable. These modified plows can operate at greater than uormal depths to avoid troublesome entanglement with nutgrass growth. The "blips" tend to wedge the tap root area of peanut rows upward in a moling effect. This permits lifting vines with peanuts from the soil with minimum losses from nut and stem separation. The same principle also aids in peanut digging when soil contains excessive moisture, as the critical band directly under each row will not tend to settle excessively behind the new blade action.

Studies are underway to reduce the element of risk of damage from weather conditions in windrow harvesting of peanuts. Efforts include techniques by which semi-dry windrow harvesting may be commonly practiced without significant potential hazards. It appears at this time that inverting the peanut windrow simultaneously with the digging and shaking operation may prove advantageous and safe. It is certain, however, that the effect of direct sunlight upon the viability of peanuts is a critical factor, and that systematic observance of safe procedure would be imperative. Digging-windrowing machines which effectively invert peanut plants are presently within the realm of practicality. Two existing versions of machines for two-row production patterns have given generally satisfactory performance on certain varieties of peanuts.

New strategy and application techniques for further increasing efficiency in mechanized peanut production will continue to be developed, and it now appears that further yield increases at lower production costs will be accomplished.

# MARKETING FARMERS' STOCK PEANUTS IN THE VIRGINIA-NORTH CAROLINA AREA

GILBERT W. BIGCS Fruit and Vegetable Branch Farmer Cooperative Service U. S. Department of Agriculture Washington, D. C.

Mr. Chairman and Fellow Confereres and Guests;

It is indeed a privilege for me to appear before this distinguished group. It is a pleasure to see so many different disciplines represented here, all members of the same team, engaged in peanut research. I would like to discuss with you the peanut marketing research that Farmer Cooperative Service, of the U. S. Department of Agriculture has been conducting in couperation with the Department of Agricultural Economics at North Carolina State of the University of North Carolina at Raleigh. The project was aimed at improving the efficiency in marketing farmers' stock peanuts in the Virgima-North Carolina area.

Farmer Cooperative Service conducts research studies and services activities that help farmers market farm products, purchase farm supplies, and supply business services.

### THE FIRST PHASE-GROWER SURVEY

Our work on the peanut project was divided into four phases. In the first phase we surveyed growers:

1. to determine the organization of peanut producing farms and production practices affecting the marketing of farmers' stock peanuts;

2. to determine the number and types of market outlets available to producers and marketing facilities and services offered to peanut producers such as transportation, storage, and market information;

3. to describe and analyze the marketing practices of producers in selection of a time and place to sell; and

4. to examine farmer opinions regarding the present marketing system and alternative marketing techniques.

We published the results of this survey in Marketing Research Report 595. This publication is available from Farmer Cooperative Service, U. S. Department of Agriculture, Washington, D.C.

## THE SECOND PHASE-BUYER SURVEY

During the second phase of the study, we surveyed peanut buyers in the area to determine:

1. the organizational characteristics of first-buyers;

- 2. their current buying practices;
- 3. physical facilities and personnel used;

4. pricing practices; and

5. their opinions on alternative marketing systems.

We published the results of this survey in Marketing Research Report 555. This publication is available from Farmer Cooperative Service, U. S. Department of Agriculture, Washington, D.C.

#### THE THIRD PHASE—ECONOMIC EFFICIENCY IN CONSTRUCTING AND OPERATING BULK STATIONS

In the course of the grower and buyer surveys, we discovered that the shift from bag to bulk handling was just getting under way in the Virginia-North Carolina area. Significant achievements in developing new techniques of harvesting and artificially drying peanuts were also being made. There was a general movement throughout the area to revise the structure and physical marketing facilities so that farmers' stock peanuts could be handled in bulk all the way to the first processors.

The evidence indicated to us that we could make a substantial contribution to the industry by examining bulk handling to determine the most efficient combination of techniques for bulk buying stations that should be constructed in the Virginia-North Carolina area.

This led to the third phase of our work which was undertaken by Dr. E. Walton Jones and Dr. Richard A. King of North Carolina State.

The results of this study were published by North Carolina State as A.E. Information Series No. 107, "Economic Efficiency in Constructing and Operating Bulk Peanut Receiving Stations." This publication is available from North Carolina State at Raleigh.

The third phase of the study covered cost relations from individual bulk peanut receiving stations and was designed to help firms make wise decisions concerning type and size of facility. Objectives were to determine:

1. the alternative techniques that might be used in performing operations at bulk receiving stations;

2. the optimum set of techniques for performing each job under different operating conditions;

the cost of constructing and operating bulk receiving stations when optimum combinations of techniques are used; and

4. the effects on costs of size of operation, length of operating season, size of peanut loads received, length of storage period, and less-than-capacity operation.

Dr. Jones and Dr. King examined the initial investment and annual cost of bulk stations storing peanuts and of those not storing peanuts. They used rates of operation varying from 25 to 1,000 hundredweight per hour for a 200-hour, 400-hour and 600-hour season.

## THE FOURTH PHASE—ECONOMIC FEASIBILITY AND EFFICIENCY OF ALTERNATIVE LOCATIONS OF BULK STATIONS

The fourth phase of the study was conducted by Dr. Billy Ray Miller and Dr. Richard A. King. North Carolina State plans to publish this study in its A.E. Information Series shortly.

The primary objectives of this work were:

1. to determine the optimum number, sizes and locations of bulk buying facilities needed to market peanuts produced in the Virginia-North Carolina area; and

2. to examine adjustments in farmer marketing practices necessary as a result of such changes.

# IMPLICATIONS OF THE SHIFT FROM BAG TO BULK HANDLING FOR THE INDUSTRY IN THE VIRGINIA-NORTH CAROLINA AREA

Our research, conducted during this four phase study, indicated many probable effects of the shift from bag to bulk handling. The overriding conclusion is that bulk receiving stations will be larger and fewer in number than was the situation when peanuts were handled mostly in bags. From this conclusion several implications for the industry may be drawn.

New bulk receiving stations should be designed to use least-cost techniques in order to provide efficient bulk handling of farmers' stock peanuts. Also, scale economies should be considered.

Rates between 250 and 300 hundredweight per hour make it possible to take advantage of most of the scale economies. For instance, nearly \$10,000 a year could be saved in handling 80,000 hundredweight of peanuts during a 400-hour season if one 200 hundredweight station were constructed instead of four 50 hundredweight stations.

There were approximately 200 buying stations purchasing peanuts for 24 shelling plants at 14 locations in the area at the time the study was initiated. Based on our analysis, present sheller demands at 14 locations could be handled through 45 properly located buying stations.

The cost then would be about three-quarters of a million dollars a year less than the system of handling peanuts in bags. Another quarter of a million dollars a year could be saved if shelling plants were optimally located. This would reduce the number of shelling locations from 14 to 7, and the number of bulk buying stations from 45 to 19. In addition to the estimated one-million dollars in savings with optimum location of bulk buying stations and shelling plants, a reduction in shelling costs might be possible. Bulk handling operations require a large capital investment. Estimated total investment from stations storing peanuts ranged from 26 thousand dollars when designed to operate 200 hours at 25 hundredweight per hour to 1.3 million dollars when designed to operate 600 hours at 1,000 hundredweight per hour. For stations not storing peanuts estimated investment ranged from 14 thousand dollars when designed to operate 25 hundredweight per hour to 40 thousand dollars when designed to operate 1,000 hundredweight per hour. Investment in these stations would not vary with length of season, since storage facilities are not required.

The studies showed that approximately 84 percent of the contracts between commissioned buyers and shellers were oral contracts. Financial institutions may require more formal agreements on purchasing practices between receiving station operators and shellers before providing the necessary capital for construction of bulk buying stations. For instance, credit agencies may require that buyers have contracts with established shellers who specify the quantity to be handled over a given number of years before loaning money for construction of a station.

Specialization usually accompanies mechanization and concentration. The buying season for bulk peanuts lasts only a few weeks and expensive equipment may be idle for much of the year. This presents a problem as to what uses can be made of peanut marketing facilities during the off-season. Bulk storage at receiving stations for longer periods than has been customary with bag peanuts may be one solution.

The reduction in the number of buying stations also means that persons currently operating bag receiving stations will need other means of employment and new uses for present facilities. There is the possibility that additional services could be extended to growers by peanut buyers, for instance, custom harvesting and artificial drying. Buyers might also furnish additional hauling equipment.

The reduction in the number of buying stations will require that farmers haul peanuts greater distances. Trailers attached to farm tractors and small pick-up trucks may not be appropriate for these long hauls. Growers might find it necessary to purchase equipment capable of hauling peanuts greater distances to the buying station.

Small growers may find it difficult to finance expensive bulk harvesting equipment and thus get the economies in its use. They may find it necessary to purchase such equipment jointly or hire the work done on a custom basis.

Cortain implications of these changes apply to the general public as well as for farmers and station owners. The public gains if resources freed from peanut buying station operations can be employed to produce additional goods and services. However, it is possible that resources freed by technical progress and economic efficiency may remain idle. Buying station labor that is unemployed loses and so does the general public through transfer payments to the unemployed. The public may also lose when capital investment becomes obsolete or unemployed before the end of its useful life. Also, resources may be underemployed, in the sense that they are not used efficiently. In association with the problems of unemployed resources is the problem of income redistribution as resources are shifted to new uses.

We found the implication of these changes to farmers difficult to evaluate. The present price structure at the shelling plant may be viewed as the support price plus marketing charges. If 'growers continue to operate as small independent operators after the advantages of the new economies are realized, the price structure may not be greatly different from what it is at the present. Gains from size economies might be distributed among buyers and shellers or passed on to the consumer. Adjusting to a smaller set of buying stations could mean that net farm prices would be lower as unit transportation costs increase with greater distances among stations. However, if growers joined together they might share in the economics of hauling and bulk station operation.

# SUMMATION

Thus we see that this shift from bag to bulk handling will have an impact upon all segments of the peanut industry in the area, including growers, commission buyers, and shellers. In addition capital and labor resources of the area will feel this impact. Also, the general public has a stake in this technological change.

Bulk handling along with larger but fewer buying stations seems to indicate that:

Growers—may need to invest in bulk harvesting equipment and equipment for hauling peanuts greater distances to market. This may be difficult for small growers. Growers may also have fewer marketing outlets available to them.

Buyers—may have a larger investment in buying stations. This will necessitate adequate financing. Buyers may need to consider means of utilizing the specialized bulk buying facility. Perhaps storage for longer periods would be desirable. Also, buyers may need to consider opportunities for providing more services to growers such as custom harvesting or hauling.

Shellers-may need to take cognizance of the changes taking place in the industry in order to adjust to them.

The General Public-may need to recognize that the resources released by this technological change must find alternative opportunities in order to be used effectively.

The Peanut Industry in the Area—may need to realize that efficiencies in bulk handling could amount to substantial savings which should be shared by all segments of the industry.

Someone has said "the price of liberty is eternal vigilance." Let us chauge this to read that "the price of progress is eternal vigilance." All segments of the industry including growers, commission buyers, and shellers as well as the general public need to adjust to this change. That groups with a wide range of interests can work together is in evidence here in this Third National Peanut Conference. Let us continue to work together to advance peannt research and thus provide information for making informed decisions so that the price of progress can be secured with a unininum of costs.

# PEDIGREED NATURAL CROSSING—A NEW GENETIC TECHNIQUE<sup>1</sup>

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# INTRODUCTION

A number of factors contribute significantly to the scant supply of genetic information available to peanut breeders. The number of breeders is severely limited. The time available for making cross-pollinations is inconvenient and seasonal. The seed set per pollination is relatively low while the time requirement is high. The productivity of individual plants is comparatively poor. Above all, a suitable and inexpensive technique has not been available for developing the large numbers of hybrid *combinations* necessary to provide proper estimates of the interrelationships among crosses and between progenies within crosses on a sound basis apart from serions bias.

<sup>1</sup> Approved by the Director as Journal Series Paper No. 144.

Utilization of first- and second-generation peanut hybrids for genetic research requires considerable quantities of seed. Peanuts are not particularly easy plants to manually cross-pollinate. From the time van der Stok adapted artificial crosspollination to peanut breeding in 1910 (8) to the present, tedious hand emasculation and pollination of peanut flowers has been the standard method. Two separate operations are necessary: removal of the intact anther sacs from the unopened flower buds (emasculation) during the late evening hours of the day preceding normal flower appearance, and, early the following morning, application of pollen (pollination) from an appropriate variety.

The percentage of artificial pollinations resulting in mature hybrid seed varies widely and is influenced by many factors. Since each successful pollination can be expected to give not more than 2 seeds in most varieties and since the complete operation and record keeping requires about 10 minutes, it is easily seen that breeding and genetic programs are critically limited by the number of crosses a breeder can make.

These factors in concert practically dictate that the predominate effort of the several breeding programs must be consistently directed toward breeding new varieties. Simply stated, the peanut breeder has not been able to afford cruss combinations for genetic investigation *per se*. Consequently the fund of genetic knowledge of the peanut in this country is meager indeed.

#### PREVIOUS STUDIES

Apart from vegetative propagation of  $F_1$  plants from cuttings (1,2), techniques for peanut genetic research have remained unchanged for the past 50 years. In addition to pioneering the pure line selection and artificial hybridization methods of peanut improvement, van der Stok (8) seems to have been the first breeder disturbed by finding a natural outcross in his breeding nursery. Despite the frequent observation of natural-cross hybrids by succeeding breeders, a careful combing of peanut breeding and genetic literature has turned up only meagre evidence that these investigators speculated on the utilization of such hybrids for inheritance studies or breeding purposes.

Murthy and Iyengar (6) observed seedcoat color changes in 2 putative natural hybrids involving a variety with variegated testa. Pelerents (7) obtained natural crossing frequencies in the Congo of sufficient magnitude for use as a crossing procedure, but he rejected this method owing to the lack of a suitable character tu mark the hybrids in a practical manner. Neither of these workers actually attempted to produce natural outcross hybrids for subsequent study.

Occurrence of obvious natural hybrids in peanut nurseries at the Georgia Coastal Plain Experiment Station was of such frequency that exploratory investigations were begun in 1959 with several objectives, including (a) measuring the frequency and extent of natural crossing, (b) identifying responsible agents, and (c) exploring the possibility for the useful application of pedigreed naturalcross hybrids to expedite breeding and/or genetic research.

Suitability of the Krinkle-leaf Spanish peanut as a useful genetic marker was described in a publication earlier this year (4). Analyses of natural hybrid populations resulting from deliberate intercrosses between this distinctive marker and various varieties and strains of peanuts suggested wide application for such a procedure, which was designated tentatively as "controlled natural crossing" (4). Since 'control' consists mainly of insuring identification of the pollen parent, the term PEDIGREED NATURAL CROSSING appears more appropriate.

Additional results, based on data from my own research, still largely unpublished, are now available to further illustrate certain uses of the new procedure.

#### **RESULTS AND DISCUSSION**

Estimates of outcrossing frequency were obtained for peanuts grown in different field areas in 1959 (4) and 1963 seasons (Table 1). Variations in insect

Year	Hybrid combinations	F1 hybrid seedlings	Total plant population	Frequency %
1959	9	609	36,228	1.68
1960	61	843	ca. 75,000	(1.12)
1961	1	27		
1962 S	112	269		
1962 R	24	123		
1963	1	13	2,578	0.50
Total	208	1884		

TABLE	1. Pedicrei	ed Naturai	l Crosse	S FROM J	a Series	OF	EXPERIMENTS	AT
	AGRONOMY	RESEARCH	FARM, 7	TIFTON, (	GA., 195	9-63	Seasons	

populations (5) and other ecological factors undoubtedly influence natural crosspollination frequencies, but a low level likely occurs as a persistent nuisance throughout the peanut belt.

Although the 1959 experiment was designed primarily to obtain a useful estimate of outcrossing from systematic planting arrangements of contrasting types to give a distinctive hybrid, the 609 hybrid seedlings recovered from the 9 different cross combinations clearly indicated the feasibility of employing such material for analysis of qualitatively inherited traits. Second-generation populations from natural hybrids were of sufficient size to establish the inheritance of the Krinkle-leaf marker as a single-gene dominant character (4), to detect and identify a new genetic combination in a white-seeded peanut (3), and to substantiate other evidence confirming the required presence of a flesh-seed factor for visible expression of red seedcoat (3).

In the 1960 experiment, pedigreed hybrids were recovered from 61 of 85 widely assorted genetic and breeding lines exposed to 2 marker stocks (Table 1). Variations in planting arrangements prohibit direct comparisons of crossing frequencies. This experiment not only indicated that a broad range of germplasm can be naturally intercrossed, but a number of the hybrids combine 2 or more distinctive characteristics for character association studies and to give more sophisticated markers in succeeding generations.

The 1962 and 1959 crossing blocks were located in the same general field area. A wide range of crosses was made in 1962 by exposing a group of 146 Spanish type peanuts to the pollinator stock in duplicate hill plantings designed to recover a very few hybrids each from as many combinations as possible. This experiment (1962 S) was a *quick genetic survey*, seeking specifically desirable gene combinations. The 112 new crosses contained 269 pedigreed hybrids or about  $2\frac{1}{2}$  per combination.

In contrast, fairly large numbers of plants from each seed parent were grown in alternate hills with the marked variety in 1959. Comparison of the numbers of hybrid plants *per combination* for 1962 vs. 1959 clearly shows that manipulation of planting design is effective, within certain limits, for providing desired frequencies of  $F_1$  plants.

Outcrossed seedlings from the 1959 experiment exceeded 100 in the 3 (of 9) combinations where more than 7000 seedlings were screened (4). Large numbers of hybrids are of special importance in breeding for variety improvement where a broad base of genetic diversity is sought, but the manhours of labor required to obtain them is prohibitively expensive, particularly so with an overtime night differential pay scale.

Acceptable seed testa color is a prerequisite for prospective new commercial pcanut varieties; therefore, a better understanding of the genetic mechanisms controlling testa color is desirable. Six of the 112 hybrid combinations in the 1962 study exemplify a special utility of the pedigreed natural crossing method: the

Se	ed parent	Pollen	parent	F1 hybrids		
Strain	Seedcoat <sup>1</sup>	Strain	Seedcoat <sup>2</sup>	No.	Seedcoat	
PI 261929 PI 262075 PI 262081 PI 268569 PI 270786 PI 270789	White Yellow (white) White (yellow) Purple (dark) Purple (dark) Purple (ltdark)	Krinkle Krinkle Krinkle Krinkle Krinkle	Flesh Flesh Flesh Flesh Flesh Flesh	3 1 1 1 1 1 10	Lt. reddish-purple Dark purple Very light wine Lt. wine-flesh Red Reddish-purple	

TABLE 2. New Seedcoat Color Phenotypes Detected in a Quick Genetic Survey of Spanish Type Peanut Introductions, 1962-63

<sup>1</sup> Seedcoat colors are subjective descriptions.

<sup>2</sup> The flesh seedcoat of the Krinkle-leaf pollen parent has the genetic formula  $pp rr F_1F_1F_2F_2$  D<sub>1</sub>D<sub>1</sub>D<sub>2</sub>D<sub>2</sub>.

detection of new heritable traits by surveying available materials with appropriate markers. These 6 peanuts testcrossed with the flesh-seeded marker—of known genotype (4)—to give new and previously unreported first-generation seedcoat expression. The hybrid seedcoats not only differ from either parent (Table 2), but from the behavior of any white x flesh (3) or purple x flesh peanut cross described in the literature. Further work is required to establish their genotypic constitution but their occurrence *per se* provides new genetic information.

This is an unusually high yield of genetic information, especially when one considers that only 2 new seedcoat color genotypes have been reported in the United States in the past 24 years (3) and identification for one of these was based partially on information from pedigreed natural hybrid populations.

In a second series of 1962 pedigreed natural crosses (1962 R), approximately 5  $F_1$  hybrid plants per combination were recovered (Table 1). This corresponds to the average number of  $F_1$ 's sought in typical conventional artificial hybridization schedules (W. A. Carver, oral communication, June 5, 1964). The average of 150 seed per  $F_1$  plant equalled the number obtained by my co-worker, J. E. Harvey (unpublished), with 198 conventional  $F_1$  hybrids grown in a nearby planting the same season.

The 208 total hybrid combinations, the 1884 cumulative  $F_1$  hybrid plants, and the frequency with which previously uninvestigated characteristics have appeared (Tables 1 and 2) are all indicative of the versatility of the pedigreed natural crossing procedure for peanut research. The overall average of 9  $F_1$ hybrid plants per combination is nearly double the 5  $F_1$ 's that breeders frequently set as a goal. The 208 crosses obtained in the 5 seasons, 1959-63, compare very favorably with the career total of 286 combinations achieved by Dr. W. A. Carver at the Florida Agricultural Experiment Station (oral communication, June 5, 1964).

The employment of natural crossing to produce hybrids for specific end uses has been exploited with considerable success in some of the more thoroughly investigated crop plants. The practicality of using this method to facilitate genetic research in the peanut depends on the economics involved. That is, the boost in numbers of  $F_1$  plants, in hybrid combinations, and in desirable characteristics obtained by this technique would have to be enough to offset the additional cost of screening and discarding large numbers of non-hybrid seedlings.

A principal advantage of this method of producing pedigreed hybrids is that the number of  $F_1$  hybrids (either separate combinations or seeds per cross) is not dependent upon the limited time available for a single scientist or subprofessional assistant to perform the tedious ritual of conventional crossing operations at inconvenient hours. Screening for hybrids can be done inexpensively on land unsuited for yield trial purposes and hybrids can be isolated, pedigreed, tagged, dug, picked, bagged, dried and shelled by subprofessional workers. The flexibility of the new procedure has been exemplified from results of several years' research illustrating its manifold utility.

A chief disadvantage of pedigreed natural crossing is that the marker stock must have in its genetic makeup those characteristics desirable for variety improvement or genetic study. This major limitation is less restrictive while the level of knowledge of the nature of qualitative character inheritance and of linkage groups is low, but the restriction is not inescapable as information increases.

Another disadvantage of this method is that F1 hybrids differ from those produced by the conventional crossing scheme in that they usually are pedigreed according to parental lines rather than on an individual plant basis within the seed-parental line. (Individual plant pedigrees can be easily come by, but seed handling is less economical). However, since second generation plants are referable to specific F, entities, standard pedigree identification is reestablished.

Maximum utility of the pedigreed natural crossing technique should be expected when this method is employed in conjunction with but not in replacement of the conventional procedure.

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# INTERSPECIFIC HYBRIDIZATION IN RELATION TO PEANUT IMPROVEMENT

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The wild relatives of the cultivated peanut in the genus Arachis number from about 30 to 50 species according to the best taxonomic data we have at present (Krapovickas and Gregory unpublished). The genus includes forms which resemble the peanut in general morphology and others which are very different indeed. According to these authors the species can be grouped into nine series which can be grouped again into three to five sections. The definitive data with respect to a proper subordination of characters is still being assembled. For the purpose of the present discussion I have chosen to illustrate the series of the genus with the more elaborate scheme of five sections.

#### Section

I. Villosoid

#### Series

- 1. A. hypogaea group
- 2. A. villosa group
- 3. A. pusilla group

	Section	Series
II.	Erectoid	4. A. diogoi group
		5. A. benthami group
		6. A. tuberosa group
III.	Repensoid	7. A. repens group
IV.	Rhizomatous	8. A. glabrata group
V.	Prostratoid	9. A prostrata group

This scheme constitutes a working grouping of the species and though not definitive taxonomically, approximates the appropriate dispersion of the species to what are conceived to be evolutionally important aggregates.

Only in the last five years has it been possible to postnlate this grouping of species which is the product of the collecting and taxonomic study by Krapovickas and Gregory. Prior to their 1959 and 1961 collecting expeditions very little material of wild species was available in the living state and much of the herbarium material was not useful. Attempts were made to bring some order out of the chaos but prior to the present endeavor confusion only became worse confounded. Fortunately now the work of revising the classification of the genns Arachis is in an advanced stage and a monograph of the genns will probably appear in the near future.

Chromosome numbers within the genus fell into two series: 2n=20 and 2n=40. All cultivated forms of *A. hypogaea* examined and *A. monticola* have 2n=40 chromosomes, the rhizomatons section also has this chromosome number. All other groups so far counted have 2n=20 chromosomes. The occurrence of the same chromosome number in the cultivated pearut and the rhizomatous group is coincidental and does not imply close affinity.

Attempts were made in the United States in the mid forties to cross A. hypogaea with A. diogoi, A. glabrata, A. repens and A. villosulicarpa (Gregory 1946 and unpublished), fertilization apparently occurred but no viable seed were produced. Following the successful hybridization of A. hypogaea and A. villosa correntina in 1952 by Krapovickas and Rigoni, fnrther attempts at hybridization were made. In East Africa in the early fifties A. hypogaea was crossed with A. diogio and another species probably A. repens (E.A.F.F.R.O. Annual Reports 1958-56) fertilization apparently occurred but again no seed was produced. In Sonth Africa a further attempt to cross A. hypogaea and A. glabrata met with no more success than previous attempts (Tuchlenski 1958).

The cross A. hypogaea  $\times$  A. diogoi studied in detail by Johansen and Smith (1956) showed that endosperm failed to develop and that the embryo aborted. A similar chain of events probably occurs in the other crosses leading to embryo abortion.

Until the recent collections were made the supply of species available for hybridization was almost exhausted. This series of failures after one early success effectively dampened enthusiasm for this enterprise for a time. However, after more living material became available a further series of attempts at interspecific hybridization was imitated in Argentina at Manfredi and in the United States at Raleigh, North Carolina. Both these independent investigations have shown that there is an appreciable number of Arachis species which are cross-compatible with A. hypogaea and a much larger number which are not.

The project in Raleigh has been based on twenty-eight lines of A. hypogaea and A. monticola for nse as female parents and twenty-six different species and collections of wild species for use as males. On both the male and female sides parents have been chosen with care to insure that all sections of the genns were represented in the male parental lines and that the range of morphological diversity and different geographic origins were represented on the female side. In this way it was possible to make comparisons between female as well as male parents. It was found that all lines of A. hypogaea did not behave equally well as female parents, in fact an appreciable number failed to cross with any other species at all; and of these, in a parallel program, some could not be crossed successfully with other lines of *A. hypogaea*. This may be due to sensitivity to emasculation in addition to some inherent genetically controlled incompatibility. This incompatibility was manifested in some lines by the production of empty pods by species which on other lines gave viable seeds and progeny. In some lines success was marginal, both empty and full pods were produced. In some instances the development of the seeds was less than normal, in others the hybrid seeds produced were quite indistinguishable from normal selfed seed.

The hybrids produced have been vigorous for the most part, showing characteristics of both parents, but have been sterile. This is due largely if not entirely to the unbalanced triploid complement of chromosomes 2n=30. Occasionally functional gametes are produced and some progeny have been produced from these triploids. Pietrarelli (unpublished) has produced a number at Manfredi, and at Raleigh we have a single mature second generation plant and expect to have others shortly. One hybrid in particular ( $\times A$ . duranensis) promises to give a fair number of second generation progeny.

To return briefly to the taxonomy of the genus, all the species which have been found so far to be cross-compatible with the peanut belong to the villosoid section. Most are perennials in the A. villosa group but the diploid annual species A. duranensis of the A. pusilla group has also given viable hybrids with two of the twenty-nine females used in the program. The species A. monticola is so freely cross-compatible with A. hypogaea that it can be regarded more as a subspecies of A. hypogaea than a perfectly distinct species.

The cross-compatibility which has been found between the A. hypogaea group and the two other series supports the contention that these three series constitute a natural section of the genus. The consequence of this cross-compatibility is that gene-flow can probably be induced across the interspecific barriers. The nature of these barriers is not yet perfectly clear. However, recent work in India by Raman and Kesavan (1962) on a hybrid A. duranensis  $\times$  A. villosa correntina with almost normal meiotic behavior indicates that at the same level of ploidy interspecific barriers may not in some cases at least be great in the villosoid section. The barrier to interspecific gene-flow between the villosoid species and A. hypogaea may be primarily ploidy level combined with a variable level of genic sterility.

These obstacles to free transfer can be overcome in a number of ways. It should be possible to double chromosome numbers of diploid species and then cross these with A. hypogaea, no successes have as yet been reported with this technique. An alternative method is to propagate the triploid hybrids vegetatively and grow these in the field and harvest whatever seed they produce. On theoretical grounds anything with a genomic constitution from 2x to 6x might be produced together with possible aneuploids. Tetraploid and hexaploid derivatives would be particularly nseful. The tetraploids could be nsed directly in crossing to A. hypogaea lines while the hexaploids could be crossed to other villosoid species and give further tetraploids for crossing back to A. hypogaea. Yet another possibility is that inter-sectional crosses be attempted with the hexaploids, there is the possibility that those which failed with A. hypogaea as female may succeed with the hexaploid.

The Indian workers Kumar et al. (1957), D'Cruz and Chakravarty (1961) claim to have obtained hexaploids from artificial doubling of chromosome number and by spontaneous polyploidization. The genome manipulation that these forms make theoretically possible has not as yet been reported.

In this way it is possible that by manipulating genomes of the wild species desirable characters such as leafspot resistance for example might be transforred to the cultivated peanut. Resistance to other diseases and to adverse climatic factors might also prove to be transferrable. The use of interspecific hybridization may thus supplement the intraspecific variability available to us in the large variety collections and that which has been artificially induced, in the improvement of this valuable crop. More importantly, however, are the potentialities in the material for whole chromosome substitutions on the one hand and for the elevation of peanut breeding to entirely new levels of ploidy on the other to what may be justifiably forecast as a new era in the improvement of peanuts.

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# PROBLEMS AND POTENTIALS OF IRRIGATING VIRGINIA-TYPE PEANUTS<sup>1</sup>

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#### INTRODUCTION

North Carolina grows about 180,000 acres of peannts, 160,000 acres of which are grown in eleven northeastern counties. Preliminary studies of rainfall data from the Lewiston Experiment Station, Bertie County, indicate that in each of the past eleven years (1952-1962) one or more periods of at least two weeks' duration have occurred when crop growth was limited by inadequate soil moisture. During the months of April to September inclusive, the total number of days when soil moisture was depleted varied from 21 in 1955 to 75 in 1952, with the drought period being more frequent and of longer duration in June than in any other month during the growing season.

With the frequency and duration of dronghts that have occurred, it appears that inadequate soil moistnre may be one of the critical factors limiting crop production. It is important to know what effect these periods of drought will have on crop production. For these reasons a cooperative irrigation project was begun by the Experiment Station and the Agricultural Extension Service in 1963.

The present study was designed with several objectives in mind:

1. To determine moisture requirements of peannts for maximum production of quality nuts.

2. To determine the critical period or periods for maintaining soil moistnre for crop production.

<sup>&</sup>lt;sup>1</sup>This paper is published with the approval of the Director of the North Carolina Agricultural Extension Service as Paper No. 1 of the Journal Series North Carolina Agricultural Extension Service, Raleigh, N.C.



### IRRIGATION EXPERIMENT—Charles Smith Farm, Halifax Co., N. C.

3. To determine the amount of supplementary water needed for maximum crop production.

4. To determine the economic feasibility of irrigation of peanuts.

## PROCEDURE

This study was designed for three years (1963-1965). The work is being conducted on the Charles Smith, Jr., farm, located about two miles northeast of Scotland Neck, in Halifax County in northeastern North Carolina. It is an interdepartmental study involving extension and research personnel from the Crop Science, Soil Science, Experimental Statistics, Agricultural Economics, and Agricultural Engineering Departments.

The experiment is being conducted on a Wickham loamy sand soil which has a water storage capacity of approximately one inch per foot of soil depth. The soil pH in 1963 was 5.3, but one ton of lime was applied after the soil tests were taken. Organic matter in the soil was 0.8%. The field was low in calcium, medium high in phosphorus, and medium in potassium. A soil assay was made in April and showed 150 stubby root, 325 dagger, and 175 lance nematodes per pint of soil. No funtigant was used on the soil in 1963, but some will be used in 1964.

No fertilizer was applied to the peauuts at planting as the field was in corn in 1962. Seven hundred pounds per acre of land plaster (Ca  $SO_4$ ) was applied on June 21, approximately six weeks after planting. A three-year rotation of corn, cotton, and peanuts is being followed.

Three moisture treatments were applied: (1) Irrigation when 40% to 50% of available water had been depleted from the upper two feet of the root zone (high moisture level), (2) Irrigation when 70% to 80% of available water had been depleted from the upper two feet of the root zone (intermediate moisture level), and (3) No irrigation. Each irrigation level was replicated four times. Figure 1 shows the layout of the irrigation experiment. Sprinkler irrigation was used with an application rate of approximately .15 inch per hour during the early part of the season, and .20 inch per hour during the latter part of the season. Rainfall was measured with a tube-type volumetric rain gage.

In 1963 each of the plots was 30 feet by 60 feet and consisted of ten 36-inch

rows, 60 feet long. The entire plots were harvested. N.C.-2 peanuts were planted on May 7 at a rate of 95 pounds per acre. The peanuts were cultivated nine times and hand chopped three times to control weeds and grass. The last cultivation was July 31. The peanuts were dusted seven times from June 18 to September 10 with copper sulphate or copper-sulphate-DDT. The peanuts were dug ou October 19 and combined on October 23.

The peanuts were graded both by the Federal State Inspection Service at Scotland Neck and more completely by Dr. Fred Cox of the North Carolina Soil Science Department.

Soil moisture measurements were made with Delmhorst cylindrical gypsum blocks. They were installed at 9 aud 18 inches of depth with two at each depth per plot. Periodically gravimetric samples were taken as a check on the accuracy of the gypsum blocks. Moisture readings were made with an Irrigage Moisture Meter which is calibrated in an arbitrary scale of 0 to 200, with 200 indicating the wettest soil. A reading of approximately 183 represented the level at which irrigation was applied to the high moisture level. This corresponded to a soil moisture tension of approximately .25 of an atmosphere. Irrigation was applied to the intermediate moisture level at a reading of 110, which corresponded to a soil moisture tension of 1.3 atmospheres. Field capacity was assumed to be approximately .15 of an atmosphere tension.

To determine when to irrigate, the readings of the eight shallow blocks (at 9 inch depth) and the eight deep blocks (at 18 inch depth) of the four replications of each treatment were averaged together to give one moisture reading. Then the proper amount of irrigation was applied to return the soil to field capacity.

# **RESULTS AND CONCLUSIONS**

1963 was an unusually dry year in the peanut belt of North Carolina as showu by the rainfall aud irrigation data given in Table 1. For this reason response of peanuts to irrigatiou was probably greater than could be expected in a normal year. Yield data are shown in Table 2. However it appears that irrigation was not begun early enough in 1963 as the peanuts had already undergone a moisture stress period of approximately one month when the first irrigation was applied. Plans for 1964 are to have the three treatments as in 1963, plus three additional treatments, where the soil moisture will be maintained above 50% to 60% of field capacity for the periods (1) 15 June-15 July, (2) 15 July-15 August, and (3) 15 August to 15 September to determine the responses to irrigation during these three periods.

Not only was there quite a response to irrigation in terms of yields, but quality was also improved, as shown in Tables 3 and 4. The irrigated peanuts grossed approximately \$180 per acre more than the non-irrigated, with approximately a \$2 per hundredweight differential in price.

Some suspected problems, such as Southern Stem Rot which had occurred in some irrigation experiments where high moisture levels were maintained, did not materialize. A pale color was observed with the irrigated peanuts that was uot present in the non-irrigated peanuts. For this there is no explanation. The nonirrigated peanuts had external mold on the shells, and the shells were darker than the irrigated peanuts. However there was no visible external or internal damage to the nuts. The non-irrigated peanuts had more cracks than the irrigated peanuts. There was some nematode damage, but this seemed to be as severe in the non-irrigated peanuts as in the irrigated peanuts.

Some other problems encountered were:

(1) The soil moisture measurements were not accurate enough. The gypsum blocks are not reliable in the high moisture range. We are using tensiometers in 1964, and intend to increase the number of gravimetric samples taken; the slow

	RAINFALI	L (inches)	
April 6	1.0	July 1	.15
April 29	.65	July 14	.3
April 30	.65	July 16	.1
		July 19	.2
May 7	.05	July 23	.8
May 16	.25	July 24	.2
May 17	1.0	July 81	.85
May 21	.4		
May 23	.35	August 7	.4
May 26	.2	August 10	.3
May 29	.15	August 18	.9
		August 25	.6
June 2	.9		
June 5	1.0	September 5	.8
June 11	.1	September 14	.2
June 16	.8	September 15	2.45
June 17	.2	September 28	1.4
June 20	.4	-	
June 21	.35		
	IRRIGA	ATION	
Dete		Plot	
Date		High	Low
July 15		18	1.8
July 16		11	1.1
July 29		1.05	

neutron method will also be used to some extent. However, all of these methods are more expensive than the gypsum blocks.

1.125

2.4

.8

.8

.3

1.1

.4

11.775

1.4

(2) Irrigation water was not applied uniformly. This is a problem with any irrigation system, whether sprinkler or furrow.

(3) In an experiment of this type, there is a problem of obtaining maximum production and maximum net returns at the same time. Normally this cannot be doue.

(4) More information is needed about water use rate of the peanut during

Irrigation level	A	В	С	D	Avg.
High Low	$3201.4 \\ 3164.4$	3090.1 2283.8	3653.0 3386.6	2622.3 3286.0	3141.7 3030.2
Non-Imigated	1598.2	2181.6	1461.7	1736.4	1744.5

TANTE	2	PEANUT	VIET DO	(POTNIDE)	A com	0P	8 0%.	Name	١.
LABLE	Z.	PEANUT	TIELDS	T_POUNDS/	ACHE.	OF	0.26	NUTS	1.1

\* Yield increase was significant (5% level).

August 3 August 8

August 20

August 21 August 23

August 27

September 3

September 4

1.275

2.4

.8

.8

.6

.4

1.4

10.075

Plot	#/A	% Fancy	#/A Fancy	% ELK	#/A ELK	% SMK	#/A SMK	% OK	#/A OK
A High	3201.4	23.7	758.7	27.3	874.0	75.3	2410.7	5.6	179.3
A Low	3164.4	45.1	1427.1	28.1	889.2	69.5	2199.3	5.5	174.0
A Check	1598.2	62.2	994.1	29.4	469.9	56.6	904.6	10.2	163.0
B High	3090.1	29.6	914.7	22.8	704.5	68.4	2113.6	6.0	188.2
B Low	2283.8	33.7	769.6	27.4	625.8	68.2	1557.6	6.3	143.9
B Check	2181.6	48.7	1062.4	29.0	632.7	60.0	1309.0	9.3	202.9
C High	3653.0	31.4	1147.0	26.4	964.4	69.5	2538.8	5.8	211.9
C Low	3386.6	48.4	1639.1	30.8	1043.1	70.7	2394.3	4.6	155.8
C Check	1461.7	48.3	706.0	25.8	377.1	56.4	824.4	11.0	160.8
D High	2622.3	36.6	959.8	13.9	364.5	59.5	1557.7	4.6	120.6
D Low	3286.0	37.2	1222.4	20.5	673.6	63.4	2083.3	9.7	318.7
D Check	1736.4	50.4	875.2	30.3	526.1	59.3	1029.7	8.7	151.1

TABLE 3. #/A AND % FANCY, ELK, SMK, AND OK

<sup>e</sup> Grading done by Dr. Fred Cox, Soil Science Department, N.C. State of U.N.C. at Raleigh.

TABLE 4.	AVERACE #	₽/A	AND	70	FANCY,	ELK,	SMK,	AND	OK'.	-
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Plot	#/A	% Fancy	#/A Fancy	% ELK	#/A ELK	% SMK	#∕A SMK	% OK	#/A OK
Hígh Low Check	$3141.7 \\ 3030.2 \\ 1744.5$	$30.08 \\ 41.73 \\ 52.13$	945.0 1264.0 909.4	23.14 26.66 28.74	$726.9 \\ 807.9 \\ 501.4$	66.60 67.94 58.29	2155.2 2058.6 1016.9	$5.57 \\ 6.54 \\ 9.71$	$175.0 \\ 198.1 \\ 169.4$
' Crad	ing by D	r. Fred	Cox, Soi	l Scien	ce Depa	rtment,	N.C. Sta	te of U	Ĵ. <mark>N.C.</mark>
Plot	#/A	% Fancy	#/A Fancy	% ELK	#/A ELK	% SMK	#/A SMK	% OK	#/A OK
High Low Check	3141.7 3030.2 1744.5	56 60 62	$1759.4 \\ 1818.1 \\ 1081.6$	34 40 40	$1068.2 \\ 1212.1 \\ 697.8$	74 70 60	2324.8 2121.1 1046.7	$\frac{1}{2}$ 5	$31.4 \\ 60.6 \\ 87.2$

<sup>2</sup> Grading by Federal-State Inspector at Scotland Neck, N.C.

Plot	Value/CWT Dr. Cox	Value/acre Dr. Cox	Value/CWT Federal-State Inspector	Value/acre Federal-State Inspector	
High	11.79	370.41	11.70	367.58	
Low	11.88	359.99	11.01	333.63	
Check	10.59	184.74	9.27	161.72	

Value of irrigated peanuts over non-irrigated \$180-190/acre.

various stages of growth. This appears to be a basic research problem that can best be solved in a controlled environment.

(5) Peanut irrigation should involve, in an active way, several disciplines to get the needed facts in the shortest length of time. The pale color of the irrigated peanuts mentioned earlier might have been caused by a pathological problem, so a pathologist should have been involved in our program.

Maximum beuefit from irrigation of peanuts has not yet been reached, certainly not by us iu 1963. After two more years of tests, we will have a better idea of when to irrigate Virginia type peanuts and what types of responses to expect.

# THE EFFECT OF SPACING AND FERTILITY ON YIELD AND QUALITY OF DIXIE SPANISH AND EARLY RUNNER PEANUTS GROWN ON RUSTON FINE SANDY LOAM<sup>1</sup>

#### R. W. LIPSCOMB, W. K. ROBERTSON AND W. H. CHAPMAN<sup>3</sup>

Experiments have shown that closer row widths and or thicker than average spacings in the row may increase yields of peanuts. Harris (3) working in Florida obtained yields of 3990 pounds per acre for 12% inch row width and 2750 pounds per acre for 38 inch row width. In North Carolina, Nelson and Welch (5) found that row width of Virginia Bunch peanuts increased yields more than spacing in the rows. Cox (1) also working in North Carolina found that yield and quality improvement of semi-bunch peanuts accompanied increased stand. Some of the newer herbicides are rather effective in controlling weeds in peanuts without significantly reducing yields. This would eliminate cultivation and make it feasible to plant peannt rows closer together. In this experiment Dixie Spanish and Early Runner varieties were spaced in rows 12, 18, 24, and 36 inches apart on Ruston fiue sandy loam for three years.

In order to determine if the row spacing was independent of phosphorus or potassium fertilization a treatment was added which received double the rate of fertilizer applied to the others (800 pounds per acre of 0-14-14 instead of 400). At another location where the soil type was of the same group as Ruston fine sandy loam, results indicated that yields declined with increasing rates of potassium in the row (6). To test this, broadcast applications of potassium were compared with branded applicatious at different rates.

#### METHODS

The Ruston fine sandy loam on which the experiments were located belongs to the Norfolk-Red Bay group of soils (2). They have relatively deep loamy sand and sandy loam surface and subsurface soils overlying friable sandy clay loam at 14 to 30 inches. They are well drained and occur on nearly level to rolling relief in Northwestern Florida and most southeastern states. The soil was sampled to a depth of 6 inches and was found to have a pH of 5.5 and contain 9, 129, 390 and 76 pounds per acre of P, K, Ca and Mg, respectively. Mineral elements were extracted with acid ammonium acetate (pH 4.8). P and Mg were determined colorimetrically, and K and Ca by flame photometry.

#### **Spacing and Fertilizer Study**

Rows were spaced 12, 18, 24 and 36 inches apart. The 24- and 36-inch spacings were repeated with double fertilization—800 pounds of 0-14-14 per acre instead of 400. The Dixie Spanish peanut was used to represent the bunch varieties and Early Runner was used to represent the runner varieties.

Treatments were arranged in randomized block design replicated 4 times and the experimental area was moved each year to eliminate the possibility of detrimental effects from continuous cropping (4). Plots were 12 by 30 feet in size and contained 12, 8, 6 and 4 rows for 12-, 18-, 24-, and 36-inch row spacings, respectively. The center half of each plot was harvested to eliminate border effects. The fertilizer was broadcast and the area disked. The rows were marked off and seed were planted by hand 4 inches apart in the row. Seeding rates per acre were as follows:

<sup>&</sup>lt;sup>1</sup> Published with the approval of the Director of the Florida Agric. Exp. Stations.

<sup>&</sup>lt;sup>2</sup> Associate Agronomist North Florida Station, Associate Soil Chemist, Main Station and Agronomist in Charge, North Florida Station, respectively.

Row Spacing	Number of	Pounds	per acre
	Seed/acre	Early Runner	Dixie Spanish
12 inches	130,680	145	131
18 inches	87,120	97	87
24 inches	65,340	72	65
36 inches	43,560	50	44

Weeds were controlled by hoeing and the peanuts were not irrigated. They were dusted with a sulfur-DDT dust beginning at flowering and at 2-week intervals, until near harvest. The nuts were dug by hand and stacked. Those left in the ground were scratched out, dried and weighed and this quantity was added to the value obtained from the stack. Practically no nuts were lost from either variety during the harvesting procedure.

At picking, a sample of nuts was saved for quality tests. The method of determining quality was similar to that used by Federal graders.

## **Rates of Potash**

Zero, 25, 50, 100 and 200 pounds per acre of potassium (K) were applied broadcast prior to planting and in the row at planting. Treatments were arranged in a randomized block design replicated 4 times in plots containing four rows 30 feet long and 36 inches apart. The peanuts were dusted in a manner similar to that used for the spacing experiment. They were dug, stacked, picked and weighed. They were not graded.

# **RESULTS AND DISCUSSION**

The effect of row spacing on Dixie Spanish nut and hay yields for three years is shown in Table 1. Data in the lower half of the table show where data are significantly different at the 5 or 1 per cent level of probability. The nut yield differences were significant in 1960 and 1962 and the averages of the three years data were significant. Although the 12 iuch spacing vs others approached significance in 1960 the greatest differences occurred in 1962 when the yields increased significantly as the row spacing decreased from 36 to 12 inches. Hay yields increased as row widths decreased every year.

Similar data for Early Runner peanuts are shown in Table 2. Row spacing had no significant affect on nut yields. Apparently the Early Runner variety did not benefit from close spaced rows probably because of the lack of room for

	Year				Year			
Row spacing	1960	1961	1962	Ave.	1960	1961	1962	Ave.
		Lbs. o	f nuts/A			Lbs. al	hay/A	
12" 18" 24" 36"	4110 8950 4040 8530	8780 8550 8790 8580	4410 3930 3540 3240	4080 3810 3790 3450	$7250 \\ 6020 \\ 5690 \\ 4700$	6600 6120 4820 3910	7960 6580 5900 5790	$7270 \\ 6240 \\ 5470 \\ 4800$
		"F"	values <sup>1</sup>		"F" values <sup>1</sup>			
All 12" vs others 18" vs 24" + 36" 24" vs 36"	6.0* 4.7 1.6 1.2	$\begin{array}{c} 0.3 \\ 0.1 \\ 0.3 \\ 0.4 \end{array}$	59.3** 12.2** 45.5** 10.2*	8.8** 15.7** 3.1 7.6*	15.1** 32,3** 6.2 6.7	19.0** 25.8** 26.0** 5.2	15.7** 41.3** 5.7 0.1	60.0* 124.7** 43.5** 11.8**

TABLE 1. EFFECT OF SPACING ON YIELDS OF DIXIE SPANISH PEANUTS ANO HAY FOR THREE YEARS

<sup>1</sup> One and two asterisks indicate significance at the 1 and 5 per cent level of probability respectively.

D	Year			Year				
Now spacing	1960	1961	1962	Ave.	1960	1961	1962	Ave.
		Lbs. of	nuts/A			Lbs. of	f hay/A	
12" 18" 24" 36"	3830 3880 3800 3560	3920 3990 3990 3950	3880 3660 3660 8670	3870 3840 3810 3660	7140 7090 6390 5260	$5150 \\ 4560 \\ 4420 \\ 3170$	6720 5720 5950 4900	6340 5790 5590 4440
	"F" values1				"F" values <sup>1</sup>			
Spacings 12" vs others 18" vs 24" + 36" 24" vs 36"	$1.7 \\ 0.4 \\ 2.1 \\ 2.5$	0.8 0.0 0.8 1.6	1.2 8.4 0.0 0.0	2.8 2.5 2.3 3.5	87.0** 18.8** 12.4** 71.0**	67.9** 24.4** 23.7** 94.7**	121.0** 10.6** 1.3 44.9**	72.0** 21.3** 12.1** 73.3**

TABLE 2.	EFFECT OF	Spacing	ON YIELDS	OF EARLY	RUNNER
	PEANUTS A	ND HAY	FOR THREE	YEARS	

<sup>1</sup> Two asterisks indicate significance at the 5% level of probability.

pegging. However, the Dixie Spanish variety did beuefit to some extent by increased plant population since the upright growth habit apparently allowed sufficient room for pegging. Yields for Early Runner increased as row spacing decreased similar to the Dixie Spanish.

Nut yields of either variety did not always increase as the row spacing decreased and the plant population increased. All plants appeared to live and in no case did yields significantly decline due to increased population. When the yields were increased because of closer spacing, the plants apparently produced fewer nuts per plant but the larger number of plants accounted for the increased yield.

	Dixie Spanish		Early Runner	
	Nuts	Hay	Nuts	Hay
Years	2.8	19.8**	3.1	163.9**
Blocks/years	1.6	5.0**	1,1	6.0**
Treatments	9.4**	47.0°°	2.2	21.6**
Years X Treatments	3,1*	1.6	0.7	28.6**

TABLE 3. "F" VALUES OBTAINED USING THREE YEARS DATA

One and two asterisks indicate significance at the 1% and 5% level of probability respectively.

TABLE 4. EFFECT OF RATES OF FERTILIZER ON YIELDS OF DINIE SPANISH AND EARLY RUNNER PEANUTS AT 24" AND 36" ROW SPACINC

	Ι	Dixie Spanish		Early Runner			
Spacing	400 lbs/A 0-14-14	800 lbs/A 0-14-14	Ave.	400 lbs/A 0-14-14	800 lbs/A 0-14-14	Ave.	
			Lbs/	A Nuts			
24″ 36″	3540 3250	* 3420 3160	$3480 \\ 3210$	8660 3680	3630 3680	3640 3680	
Ave,	3400	3290	3340	3670	3660	3660	
			Lbs	/A Hay			
24″ 36″	5900 5800	6360 6100	6130 5950	5940 4900	5540 4980	$5740 \\ 4940$	
Ave.	5850	6230	6040	5420	5260	5340	

Statistical analyses were run on the three year's data. The "F" values to show the effect of years, blocks/years, spacing and years x spacing are shown for nuts and hay for the Dixie Spanish and Early Runner varieties in Table 3. There were no differences in nut yields between years for either variety. Average yields were approximately 3800 pounds per acre. Blocks/years was also non significant for both varieties which further accentuates the validity of the data. The differences for row spacing were significant for the Dixie Spanish, but not for Early Runner. The years x spacing interaction was significant for Dixie Spanish which indicated that response to row spacing varied with the season. Years x spacing interaction was not significant for the Early Runner which indicated that years had no effect on the response to spacing.

Table 4 shows the effect of additional phosphorus and potassium on nnt and hay yields of Dixie Spanish and Early Runner peanuts. There was no difference between the 400- and 800-pound per acre rates of 0-14-14 for either variety. These data indicate that at the 24- and 36-inch row spacings the annual application of 400 pounds of 0-14-14 per acre was adequate.

Quality data obtained in 1963 from the row spacing-fertility experiment are shown in Table 5. Since there was very little germ or cotyledon damage, these

	Treatment				
Variety	Spacing between rows	% Shrunken	% Seed	Sound & mature	wt (gms) /100 seed
Dixie Spanish Dixie Spanish Dixie Spanish Dixie Spanish	12" 18" 24" 36"	4.5 7.2 4.8 6.0	79.0 78.2 77.5 76.2	74.0 71.0 72.8 70.0	$\begin{array}{c} 46.0 \\ 45.5 \\ 46.5 \\ 45.0 \end{array}$
Early Runner Early Runner Early Runner Early Runner	12" 18" 24" 36"	6.0 6.0 6.0 6.2	76.0 75.2 75.5 76.2	70.0 69.0 68.8 70.0	59.3 57.5 52.0 55.0
Dixie Spanish Dixie Spauish Early Runner Early Runner	0-14-14 400 Jbs/A 800 lbs/A 400 lbs/A 800 lbs/A	5.4 7.0 6.1 6.8	76.8 77.4 75.9 74.4	$71.4 \\70.1 \\69.4 \\67.0$	45.8 47.0 53.5 57.2

TABLE 5. EFFECT OF SOME THEATMENTS ON QUALITY <sup>®</sup> OF 1	NUTS
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\* The visible and concealed germ and cotyledon damages were very small.

	М	lethod of placements	\$*
N IDS/ A	Broadcast	Row	Ave.
		Lbs/A	
0			2050
25	2000	2090	2040
50	2050	2110	2080
100	1980	1950	1960
200	1740	1930	1840
Ave,	1940	2020	1990

TABLE 6. EFFECT OF BROADCAST AND ROW APPLICATIONS OF POTASSIUM ON PEANOT YIELDS

\* Rates of K significant at the 5% level of probability. There was no significant difference between broadcast and row placements. data were omitted. Differences in quality as affected by row spacing were not significant at the 5 per cent level of probability. Quality data differences between the 400- and 800-pounds per acre rates of 0-14-14 at 24- and 36-inch row spacings were non significant.

Data in Table 6 show that yields declined when rates of potassium were increased above 50 pounds per acre. This agrees with data from previous experiments on Red Bay fine sandy loam  $(\theta)$ . There were no differences between broadcast and row applications.

#### CONCLUSION

Dixie Spanish and Early Runner peanuts were grown for 3 years on Ruston fine sandy loam to study the effects of row spacing on yields and quality of nuts and yields of hay.

Dixie Spanish peanuts produced an average of 3780 pounds per acre of nuts each year and the closer row spacing gave better yields 2 out of 3 years. There was no significant difference in quality of nuts. Early Runner peanuts produced an average of 3800 pounds per acre of nuts each year and the effect of row spacing on yields was not significant. Again there was no significant difference in quality of nuts. The thicker stands, resulting from the 12 inch row spacing as compared to the 36-inch row spacing, for instance, reduced the nuts per plant possibly due to light interference or in the case of the runner peanuts due to reduced runners from crowding. Both varieties produced significantly more hay each year as the row spacing was reduced.

Fertilizer at the rate of 400 pounds per acre of 0-14-14 was applied to the peanuts. Doubling this amount did not affect yields.

Yields declined significantly as the potassium application was increased from 50 to 200 pounds of potassium per acre. There was no significant difference between row and broadcast applicatiou of potassium.

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# ISOLATION AND IDENTIFICATION OF OFF-FLAVOR COMPONENTS FROM HIGH TEMPERATURE CURED PEANUTS<sup>1</sup>

# H. E. PATTEE<sup>2</sup>, E. O. BEASLEY<sup>5</sup> AND J. A. SINGLETON<sup>4</sup>

Since the inception of mechanized harvesting and curing processes for peannts some 15 years ago, the problem of "off-flavors" in the raw and processed peanuts and peanut products has been of increasing concern to the various segments of the industry. Extraneous and objectionable flavors in peanuts can arise from many sonrces and are not always attributable to the harvesting and curing process. There is a type of off-flavor, however, which has been shown to occur whenever nnnred peanuts are subjected to increased temperature levels. This type of off-flavor is usually associated with improper mechanical enring and will be discussed exclusively in this paper.

Isolation and identification of the specific compounds which constitute the off-flavor in peanuts has been undertaken as a means of defining the systems which produce the off-flavor and as a step toward developing an objective method of flavor measurement which can be incorporated into a quality control system for better peanut evaluation.

Off-flavored peanuts were produced by drying freshly harvested peanuts at  $52^{\circ}$  C and 50% relative humidity for sixty-two honrs. Normal-flavored peanuts were dried at  $22^{\circ}$  C and 50% relative humidity for two hundred and eight hours.

Concentration of the volatile components from the peanut samples was accomplished nsing vacuum distillation. One thousand grams of freshly ground peanuts were placed in a 1000-milliliter Buchner flask and attached to a 3-bulb vacuum manifold system. Distillation was allowed to proceed for approximately fifteen hours at a pressure of fifty mm of mercury. Three fractions were collected; (1) Salt-ice  $(-10^{\circ} \text{ C})$  fraction, (2) Dry ice-acetone  $(-80^{\circ} \text{ C})$  fraction and (3) Liquid nitrogen  $(-196^{\circ} \text{ C})$  fraction.

Separation of the volatile constituents was accomplished nsing a gas chromatograph unit equipped with two separate flame ionization detectors and columns. The columns were U-shaped,  $\frac{1}{4}$ -inch by 6-foot heavy walled glass tubing packed with 15% diisodecylphthlate on celite, 60-80 mesh, and 15% polyethylene glycol 6000 on firebrick super 22, 60-80 mesh respectively.

Identification of the compounds isolated was based upon comparison of relative retention volumes on the two columns with those of authentic compounds and functional group analysis.

Analysis of the volatile components from high temperature-cured off-flavor peanuts revealed the presence of at least twenty-one components. Eleven of these compounds have been identified. They are formaldehyde, acetaldehyde, ethanol, acetone, isobutyraldehyde, ethyl acetate, butyraldehyde, isovaleraldehyde, ethanol, valeraldehyde, methyl butyl ketone and hexaldehyde. Of the other 10 components one has been identified as either 2-methyl 1-butanol or 3-methyl 1-butanol; another has tenatively been identified as furfural; one has been assigned a ketone functional group, and the remaining seven components remain completely nnidentified.

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FIG. 1. Typical chromatograms of the --196°C fraction from normal and offflavor peanuts.

The authors have attempted to associate specific components to the off-flavor. Preliminary results have shown that when ground normal-flavor peanuts are mixed with the  $-196^{\circ}$  C fraction from off-flavor peanuts, their flavor is changed to that characteristic of the off-flavor peanuts. This would indicate that formaldehyde, acetaldehyde, ethanol, ethyl acetate and an unknown component are predominautly responsible for the off-flavor induced by high-temperature curing.

Typical chromatograms of the  $-196^{\circ}$  C fraction from normal and off-flavor peanuts are shown in Figure 1. A comparison of volatile components from normal and off-flavor peanuts indicates a significant increase in comparable components in the  $-196^{\circ}$  C fraction from off-flavored peanuts. These differences would suggest that off-flavor might he due in part to an increase in amount of the volatile components.

Identification of volatile components from normal-flavor peanuts has only recently been undertaken, thus only the major comparable components have been identified. Further work is presently noder way to complete identification of the volatile components in normal and off-flavor peanuts.

# GAS-LIQUID CHROMATOGRAPHIC DETECTION OF OFF-FLAVORED COMMERCIAL PEANUTS

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### INTRODUCTION

The roasted flavor of individual peanuts or lots of peanuts may be categorized as good-flavored, bland or off-flavored. Classification of commercial lots of peanuts for expected contribution to flavor in peanut butter is desirable, but such classification can only be done at present by persons trained to distinguish the varions categories of roasted peannt flavor. While the organoleptic evaluation of peanuts must remain the basis for final acceptance or rejection of lots of peanuts, peannt butter mannfacturers would benefit from development of rapid objective methods for screening to differentiate good-flavored from off-flavored lots of peanuts.

Application of gas-liquid chromatography to the study of food flavors (1) has permitted relatively rapid resolution and identification of specific compounds responsible for flavor. Mason and Waller (2) have reported on the detection and identification of volatile flavor components from freshly roasted peanuts. Their techniques should be nseful in categorizing peanuts as to relative level of good flavors. At the time our work was started no one had applied GLC techniques to the detection of volatile componneds contributing to off-flavor of peanuts. However, a similar and more detailed GLC study of off-flavored Virginia peanuts has since been made by Pattee, Beasley and Singleton (3) as reported at this meeting.

It is generally recognized that off-flavors in raw peannts are carried through into the roasted product. Therefore, tests on raw peanuts may be as meaningful and more convenient than tests conducted after roasting.

## MATERIALS AND METHODS

Peanuts were dry roasted in an electric rotisserie equipped with a rotating wire basket. The flavor rating of the roasted peanuts was determined by an experienced taster using a composite of the roasted nuts.

Gas-liquid chromatography was performed with a Beckman Model GC2A instrument equipped with a 4 mm I.D.  $\times$  7.5-ft column of 20% diethylene glycol on acid washed Chromosorb W. A ¼ inch  $\times$  12-inch, 30% glycerol precolumn prepared with the same support was need to reduce interference of peaks





caused by injection of water. The column temperature was 70°C with the helium flow set at 30 psig.

Twenty grams of raw peanuts was chopped in a Labconco Mill (Laboratory Construction Company) to about a 10 mesh particle size and placed in a 250 ml round bottom flask. The sample was evacuated and held at a pressure of 0.15-0.25 mm of mercury for 1 honr in a water bath at 50°C. Vapors were condensed in a trap cooled with dry ice-acetone mixture (-80°C). The condensate was thawed (0.5-1.0 ml) and  $5\mu$ l injected into the column. Eluted compounds were detected with a hydrogen flame ionization detector.

Two lots of Virginia peanuts (NC-2 Variety) were obtained from J. W. Dickens, North Carolina State College. These samples are mature peanuts dried at 70°F (No. 61) and 120°F (No. 62) in a laboratory enring device. The other samples tested were obtained from regular commercial channels. They included two good-flavored and two off-flavored lots of Southwest Spanish peanuts, and one off-flavored lot of Southeast runner peannts.

# **RESULTS AND DISCUSSION**

Figure I gives the gas chromatogram of one typical lot of off-flavored Sontheast runner peanuts (No. 44). Five distinct peaks were observed in this offflavored lot, but no flavor component peaks were found in the chromatogram of the good-flavored lot of Spanish peanuts shown here (No. 66). The peak that appears between peaks C and D is cansed by injection of the water contained in the volatiles fraction and can be produced by injection of pure water. Table I gives the results obtained with all samples subjected to the GLC technique. The area representative of the concentration of each component and the total area for each sample are presented. Previous results showed that good-flavored runner peanuts had GLC profiles similar to good-flavored Spanish peannts.

Several observations may be made from these data: (1) good-flavored peanuts may contain some of the same compounds as bad-flavored peanuts but at a lower concentration; (2) bad-flavored peanuts contain large amounts of all or some of the five components; (3) the relative proportion of off-flavor compounds varies among peanuts classified as off-flavored. The data suggest that the types of compounds and their relative amounts might be used to classify peanuts as to type and intensity of off-flavor.

Sufficient numbers of samples of the three major peanut types have not been run to determine possible differences in components of the off-flavor fraction. However, the same five peaks were found in one sample of runners (No. 44) and one sample of Spanish (No. 65), and no additional peaks were found in

Sample	Peanut Ivnea)	Organolepti	с	Peak area, mm <sup>2b</sup> )					
number	realize type .	rating	A	B	С	D	Έ	Total	
69	Sonthwest Spanish	Good	0	5	10	0	4	19	
61	Southwest Spanish	Good	0	0	0	0	0	0	
61	Virginia (NC-2)e)	Fair	0	31	0	0	0	-31	
62	Virginia (NC-2)d)-	Bad	0	0	41	0	20	61	
63	Southwest Spanish	Bad	0	43	10	0	6	59	
64	Southwest Spanish	Bad	0	98	10	0	6	114	
65	Southwest Spanish	Very bad	21	17	175	10	63	286	
44	Southeast Rnnner	Very bad	38	40	234	11	32	355	

TABLE 1. COMPARISON OF ORCANOLEPTIC RATING WITH OBSERVED VOLATILES LEVEL FOR ASSORTED PEANUT SAMPLES

a) All lots from commercial sources except Nos. 61 and 62.

b) Calculated at an attenuation of 20 (hydrogen flame ionization detector).
c) Laboratory cured at 70°F by J. W. Dickens, N.C. State College.

d) Laboratory cured at 120°F by J. W. Dickens, N.C. State College.

any of the samples. It therefore appears that off-flavor development may stem from the same canses or biochemical reactions in all peanut varieties.

The work reported here is admittedly preliminary. No work was done to identify the compounds corresponding to the peaks observed, and no attempt was made to develop a routine evaluation test. The GLC technique is too cumbersome for screening large numbers of peannt samples. However, the excellent research of Pattee, Beasley and Singleton (3) has determined the identity of compounds responsible for off-flavor. Their work has potential value in development of rapid chemical methods that could be need for screening large numbers of peanut samples.

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# THE EFFECTS OF CURING ON RESPIRATION AND OFF-FLAVOR IN PEANUTS<sup>1,2,3</sup>

#### T. B. WHITAKER AND JAMES W. DICKENS<sup>4</sup>

#### INTRODUCTION

Bulk curing of peanuts with heated air is an accepted practice in all peanutgrowing areas of the United States. This method of curing is relatively independent of weather conditions and offers the grower an opportunity to avoid molding and other problems associated with field curing of peanuts. A major problem associated with bulk enring of peannts with heated air is the fact that the flavor of peanuts is adversely affected when the curing temperature exceeds 95°F.

In studies to determine the canse of off-flavors, Dickens (1957a) found that peanuts cured in the absence of oxygen developed more off-flavor than peanuts cured in the presence of oxygen. He also found that mature peanuts developed less off-flavor than immature peanuts while curing at high temperatures. He snggested that anaerobic respiration might be related to the production of off-flavor. The present study was designed to investigate the effects of curing treatment npon the respiration of peannts and to determine whether a correlation exists between off-flavor and the amount of anaerobic respiration that occurred during the curing process.

#### **REVIEW OF LITERATURE**

In 1951 Butt and Kummer reported that peanuts cured at 130°F had an undesirable flavor. Teter (1957), in summarizing research on peanut curing, reported that a time-temperature-moisture relationship affected quality and flavor and stated that a slow drying rate and curing temperatures below 100°F were prerequisites to acceptable quality and flavor.

<sup>&</sup>lt;sup>1</sup> This paper is based on a thesis submitted by T. B. Whitaker to the graduate faculty of North Carolina State of U.N.C. at Raleigh in partial fulfillment of the requirements for the degree of Master of Science in Agricultural Engineering. <sup>2</sup> Acknowledgment is made to the Corn Products Company for its support of this work. <sup>5</sup> Contribution from the Agricultural Engineering Department, North Carolina Agricultural Experiment Station, Raleigh North Carolina in cooperation with the U.S. Department of Research as Paper No. 1838 of the Journal Series. <sup>6</sup> The authors—T. B. Whitaker and J. W. Dickens—are, respectively, Research Assistant in Agricultural Engineering, N. C. State of U.N.C. at Raleigh, Raleigh, N.C., and Agricultural Engineer, Market Quality Research Division, AMS, USDA, Raleigh, N.C.
Dickens (1957a) found that temperature was the main cause of off-flavor. His tests showed that peanuts cured in oxygen developed less off-flavor than peanuts cured in nitrogen or carbon dioxide. He also reported that maturity, determined by hull color, had an effect upon off-flavor. Using these results, he hypothesized that anaerobic respiration occurred in peanuts cured above  $100^{\circ}$ F and that anaerobic respiration produced off-flavor in the peanut kernels. In their textbook Meyer, et al., (1960) defined aerobic respiration as the oxidation of a substrate in which oxygen is consumed, carbon dioxide is formed, and energy is released. Anaerobic respiration was defined as type of respiration that occurs without the utilization of oxygen. Thus, the basic difference between the two types of respiration is in the utilization of oxygen and the by-products produced.

Dickens (1957b) suggested that at high rates of respiration, oxygen does not diffuse into the peanut kernel at a sufficient rate to support the needs of the respiring cells. James (1953) reported that the oxygen concentration in the cells of potatoes decreased with an increase in temperature and Schenk (1959) reported that the rate of gas exchange in curing peannts is inversely proportional to the curing temperature.

Meyer, et al., (1960) stated that increased temperature, immaturity, and high moisture contents are associated with increased rates of respiration. They also indicated that during high rates of respiration, anaerobic respiration would take place because of a deficit in oxygen supply.

James (1953), in his textbook on respiration, defined the respiration ratio as the volume of carbon dioxide liberated in a given period of time divided by the volume of oxygen consumed in the same period of time. Meyer, et al., (1960) reported that the respiration ratio, when nsed with caution, can be nsed as an important indicator of the nature of the respiratory process, and that the ratio can be used to make certain inferences about the nature of the substrate being oxidized as well as the type of respiratiou. Assuming complete oxidation occurred, they stated that a ratio of 0.8 indicates a protein substrate, and a ratio of 0.7 indicates that a fat is being oxidized. Woodroof, et al., stated that peannts contain approximately fifty percent oil, while Altschul (1962) reported that peannts contain approximately twenty to twenty-five percent protein.

Schenk (1961) found that respiration ratios greater than unity occurred in peannts being cured at high temperatures and stated that his measurements supported Dicken's hypothesis. In addition, Meyer, *et al.*, (1960) and others reported that an increase in the respiration ratio above a value characteristic of the substrate being oxidized was indicative of anaerobic respiration.

## PROCEDURES AND EQUIPMENT

## Measurements and Determinations

In order to carry out the objectives of the study, it was necessary to measure the amonnts of carbon dioxide liberated and oxygen consumed by mature and immature peanuts while entring at temperatures of 95°F and 125°F. As suggested by James (1953), the rate of carbon dioxide liberation was used as a measure of the respiration rate. The respiration ratio was also determined from the carbon dioxide and oxygen measurements.

Using the respiration ratio, the rate of anaerobic respiration was estimated at several moisture levels. In addition, the relative total amounts of anaerobic respiration produced by the curing treatments were estimated by computing anaerobic indices for each treatment.

The relative amounts of off-flavor were determined by a taste-test panel. The panel, composed of three persons selected for their experience in peanut taste panel work, ranked the twelve samples from each enring temperature. Using the triangle method, the panel member was given three samples to taste, two of which were identical. The panel member attempted to pair the two samples that were alike and to pick the sample with the most off-flavor. Failure of the panel member to pair the identical samples was interpreted to mean no difference in the two treatments under test. Raw peanuts were chopped in a Waring Blender and tested.

#### Equipment

Measurements of oxygen consumption and carbon dioxide liberation were made in a sealed system consisting of three basic components: a carbon dioxide meter, an oxygen meter, and a container for the peanuts. A Mine Safety Appliance Infrared Analyzer<sup>8</sup> was nsed to measure carbon dioxide from zero to two percent with an accuracy of  $\pm$  0.04 percent. A Beckman E-2 Analyzer was used to measure oxygen concentration on a 16-21 percent scale with an accuracy of  $\pm$  .025 percent. A schematic diagram of the entire system can be seen in Figure 1. The numbered components are identified as follows:

1. Neptune Dyna Pump of the sealed diaphragm type to force air through the system.

2. A specially modified 1-pint glass jar for the peanuts.

3. Heat exchanger consisting of four feet of <sup>1</sup>/<sub>4</sub>-inch copper tubing coiled about an eight-inch diameter.

4. Mine Safety Appliance Iufrared Analyzer for carbon dioxide measurements.

5. Beckman E-2 Analyzer for oxygen measurements.

6. Indicating Drierite to protect the meters from moisture.

7. Adjustable valve to regulate air flow.

# Procedure

Freshly dug peanuts of the NC-2 variety, grown on the Clayton Research Farm, were used. The peanuts were harvested, washed, and shelled by hand. The kernels were separated into mature and immature classes according to the color of the interior of the hulls. Seed from the white hulls were considered immature and those from the dark hulls mature. Kernels from hulls with intermediate colors were discarded.

For each harvest approximately 350 grams of immature and mature kernels were shelled. One hundred grams of each sample were placed in a forced draft oven at 130°C for five hours for determination of percent moisture (wet basis). The moisture determinations enabled calculations of the moisture content of the test samples by weighing them as the test progressed. Two hundred and forty grams of the remaining kernels were used for the respiration measurements.

Measurements of carbon dioxide liberation and oxygen consumption were made on the samples of peanuts at various moisture levels between 60 and 15 percent as they were curing under six replications of each of the following four treatmeuts:

1. Immature kernels cured at 125°F

2. Mature kernels cured at 125°F

3. Immature kernels cured at 95°F

4. Mature kernels cured at 95°F

The immature peanuts contained approximately 60 percent moisture and the mature peanuts contained approximately 35 percent moisture at harvest. At the start of the tests, the peanut kernels were placed into a wide, shallow pan and put into a forced draft oven. When the kernels reached preselected moisture levels, they were placed into the chamber for carbon dioxide liberation and oxygen reduction measurements. After the respiration measurements, the kernels were put back into the pan and dried to the next moisture content. To avoid the possibly injurious effects of high concentrations of carbon dioxide, the respiration measurements were terminated at carbon dioxide concentrations below two percent.

<sup>6</sup> Use of manufacturer's name does not imply endorsement.



FIG. 1. Schematic diagram of system.

# ANALYSIS OF DATA AND DISCUSSION OF RESULTS

# Carbon Dioxide and Oxygen Measurements

Experimental measurements made on immature peanuts cured at 125°F at 56 percent moisture content are plotted in Figure 2. Comparative amounts of oxygen consumed and carbon dioxide liberated during the test were determined by measuring the change in oxygen concentration and carbon dioxide concentra-



FIG. 2. Change in percent carbon dioxide and oxygen concentrations versus time for immature kernels cured at 125°F at 56 percent moisture.

tion during the period of time ( $\Delta$  t) indicated on the graph. Although the curves representing the carbon dioxide liberated tended to be slightly curvilinear, this method facilitated analysis of the data and is considered to be accurate enough for the purpose of the tests. All experimental data were plotted and summarized in this manner.

Before the values selected for the curves were used, a correction was necessary in all cases where the respiration ratio was not one. Each meter recorded the



FIG. 3. Regression curves of the rate of change in carbon dioxide concentration versus moisture content.



FIG. 4. Regression curves of the rate of change in oxygen concentration versus maisture content.

particular gas concentration as a percent of the total quantity of gas in the system. If the respiration ratio was more than one, the total quantity increased; if the respiration ratio was less than one, the total quantity decreased. Therefore, each reading used to determine the respiration ratio had to be calculated using the initial total quantity of gas as a base.<sup>4</sup>

## **Respiration** Rate

The rate of increase in percent carbon dioxide within the system was used as an index of the rate of respiration for each treatment. The curves in Figure 3 are constructed from multiple regression quadratic functions. These equations were computed by using carbon dioxide liberation data from all six replications of each treatment. The correlation coefficients (R) are shown in the figures. The curves indicate that peanuts cured at 125°F have a higher rate of respiration than peanuts cured at 95°F. Furthermore, for a given curing temperature, immature peanuts have a higher rate of respiration than mature peanuts.

These results indicate that the curing treatment affected the respiration of the peanuts. This is in agreement with Meyer, *et al.*, (1960) who stated that the rate of respiration is affected by maturity and the environmental temperature. They also reported that young cells rich in protoplasm will have a higher rate of respiration than more mature cells with less protoplasm and thicker cell walls, and in general, an increase in the temperature will increase the rate of respiration. Figure 6 shows similar curves for the rate of change in percent oxygen within the system.

## **Respiration Ratio**

Using measured amounts of carbon dioxide liberated and oxygen consumed during a period of time  $\Delta t$ , respiration ratios were determined for each test.

<sup>&</sup>lt;sup>9</sup> The derivation of equations to make these calculations is presented in the thesis.





Based on work presented in the review of literature, one may assume that a respiration ratio greater than 0.8 indicates that part of the respiration is anaerobic. Use of this ratio is valid only when the following assumptions are true about the conditions that existed in the respiring peannts:

1. A fat or protein was completely oxidized.

2. There was no shift from a fat or protein substrate to another type of substrate.

3. There was no carbon dioxide fixation,

4. There were no changes in the respiratory pathways.

Figure 5 shows the respiration ratios plotted versus moisture contents from tests in replication two. These curves show that maturity and temperature affect the respiration ratios. The results are in agreement with James (1953), who reported that the respiration ratio increases with an increase in temperature and with Schenk (1961), who reported that the respiration ratio varies with maturity. All four treatments tend to have the same characteristic curve, with the maximum respiration ratio occurring near 25 percent moisture. Beasley and Dickens (1963) report that the maximum rate of off-flavor production in peanuts cured at high temperatures occurs at above this moisture.

#### Anaerobic Respiration

Based on the assumption that a respiration ratio greater than 0.8 is indicative of anaerobic respiration in peanuts, the following equation gives the amount of carbon dioxide liberated per minute by anaerobic respiration.

$$\frac{\mathrm{CO}_2 - 0.8 \ \mathrm{O}_2}{\Delta t} > 0$$

In order to obtain the relative total amounts (percent/minute) of anaerobic carbon dioxide liberated during the entire curing process, the rate of anaerobic carbon dioxide liberation was plotted versus time as shown in Figure  $\theta$ . The total areas under the curves are indicative of the relative total quantities of anaerobic carbon dioxide liberated during the curing process by the different treatments. A planimeter was used to integrate the area under the curves from



FIG. 6. Anaerobic carbon dioxide liberation per minute versus time.

time zero to the time that the peanuts cured to 15 percent moisture content. The area per gram of dry weight is termed the anaerobic index.

The anaerobic indices for all twenty-four replications are listed in Table 1. Listed in the same table is the average index for each treatment. The indices

	Replications							
Ireatment	1	2	3	4	5	6	Index	
Immature 125°F	23.98	19.71b	20.55	15.98	17.98	10.79	18.17	
Immature 95°F	22.14	$1.56^{\circ}$ 16.95	10.41b	10.12	8.39	9.47	12.91	
Mature 95°F	5.08	4.57	1.32b	2.26	1.67	1.40	2.71	

TABLE 1. ANAEROBIC INDICES<sup>8</sup>

<sup>a</sup> Area under curves per gram of dry weight.

<sup>b</sup> Indices derived by measuring area under curves in Figure 6.

Table 2. Ranking of Samples According to Flavor for Immature and Mature Kernels Cured at  $125\,^\circ\mathrm{F}$ 

Maturity	Replication	Anaerobic index	Flavor rank°
Immature	2	19.71	12
Immature	3	20.55	11
Immature	1	23.98	10
Immature	5	17.98	9
Immature	4	15.98	8
Immature	6	10.79	7
Mature	3	6.42	6
Mature	1	5.21	5
Mature	5	2.80	4
Mature	4	2.05	3
Mature	2	1.58	2
Mature	6	1.64	1

\* 12 indicates most off-flavor and 1 indicates least off-flavor.

Maturity	Replication	Anaerobic index	Flavor rank*
Immature	1	22.14	12
Immature	2	16.95	11
Immature	3	10.41	10
Immature	4	10.12	9
Immature	6	9.47	8
Immature	5	8.39	7
Mature	1	5.08	6
Mature	4	2.26	5
Mature	2	4.57	4
Mature	6	1.67	3
Mature	8	1.32	2
Mature	5	1.40	1

TABLE S. RANKING OF SAMPLES ACCORDING TO FLAVOR FOR IMMATURE AND MATURE KERNELS CURED AT 95°F

\* 12 indicates most off-flavor and 1 indicates least off-flavor.

are indicative of the relative amounts of anaerobic respiration carried on by each treatment while it dried down to 15 percent moisture. High rates of respiration coupled with low rates of oxygen diffusion into the peanut could cause oxygen stress resulting in anaerobic respiration. Oxygen stress and the amount of anaerobic respiration would be less in kernels more permeable to oxygen or having a lower rate of respiration.

The panel ranked all twelve samples cured at each temperature according to the amount of off-flavor. The results listed in Tables 2 and 3 indicate that the amount of off-flavor is correlated to the amount of anaerobic respiration. The by-products of anaerobic respiration may be the off-flavor components since one would expect the amount of these components to be proportional to the amount of anaerobic respiration.

# SUMMARY AND CONCLUSION

The objectives of this study were to investigate the effects of curing treatment upon the respiration of peanuts and to determine if a correlation exists between the amounts of off-flavor produced in peanuts and the amount of anaerobic respiration that occurred during the curing process.

To carry out these objectives, mature and immature, freshly dug and shelled, whole kernels were cured at temperatures of 95°F and 125°F. The carbon dioxide liberation and oxygen consumption of 240 grans of kernels were used to determine the relative rates of carbon dioxide liberation and oxygen consumption and the respiration ratio throughout the curing process. The total amount of carbon dioxide liberated by anaerobic processes was computed in the form of an index. Comparisons were made between the anaerobic index and the amount of off-flavor detected in the samples by a taste panel. Major results obtained from this study are listed in Table 4.

TABLE	4,	Effects	OF	CURING	TREATMEN	NT ON	RELATIVE	LEVELS*	OF
		RESPIRA	TIQI	MEASU	JREMENTS .	AND	OFF-FLAVO	R	

Measurements and computations	Immature 125°F	Immature 95°F	Mature 125°F	Mature 95°F
Rate of respiration	4	2	3	1
Respiration ratio	4	3	2	ī
Anaerobic index	4	8	2	ī
Off-flavor	4	3	2	ī

\* 4 = highest and 1 = lowest.

This study has demonstrated that there is a correlation between the amounts of off-flavor found in peannts and the amounts of anaerobic respiration that occur during the curing process. The results of this study, coupled with findings of other investigations, strongly suggest a causal relationship between the production of off-flavor and auacrobic respiration. Determinations of the exact nature of this relationship will require further study.

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# EFFECT OF DRYING RATE AND MOISTURE CONTENT ON THE SIZE OF VIRGINIA TYPE PEANUT KERNELS<sup>1</sup>

I. W. DICKENS AND E. O. BEASLEY<sup>8</sup>

# INTRODUCTION

Kernel size is a major factor in determining the grade and price paid for farmers' stock peanuts. For Virginia type peanuts two screen sizes are used in the grading process. Kernels free from defects that ride a screen with 15/64-inch by 1-inch oblong slots are designated sound mature kernels (SMK) while those sound kernels that ride a screen with 22 ½/64-inch by 1-inch slots are designated extra large kernels (ELK).

The effects of cultural practices on percent of SMK and ELK have received considerable study. Ofteu it has been assumed that post-harvest treatment of peanuts could have little effect on the size of kernels, and drying treatment or moisture content have been ignored when screening peanut kernels for grade determinations. This paper discusses the effect of these factors on the size distribution of NC-2 peanut kernels.

<sup>&</sup>lt;sup>1</sup> Contribution from the Agricultural Engineering Department, North Carolina Agricultural Experiment Station, Raleigh, North Carolina in cooperation with U.S. Department of Agri-culture, Agricultural Marketing Service. Published with the approval of the Director of Research as Paper No. 1837 of the Journal Series. <sup>2</sup> The authors—J. W. Dickens and E. O. Beasley—are, respectively, Agricultural Engineer, Market Quality Research Division, AMS, USDA, Raleigh, N.C., and Research Instructor in Agricultural Engineering, N.C. State of the U.N.C. at Raleigh, Raleigh, N.C.

## DISCUSSION OF PROCEDURE AND RESULTS

Size Distribution of Kernels. Because of the distribution of kernel sizes, relatively small changes in the size of the kernels will change the percentage of ELK by an appreciable amount. Figure 1 shows the percentages of two samples of NC-2 peanut kernels that rode various screen sizes. The peanuts were grown in the same field, dug two weeks apart, field cured on stackpoles, and picked and shelled by hand. Three 500-gram samples of kernels from each digging date were screened over a mechanical shaker such as those used by the Inspection Service. Each point plotted in Figure 1 is an average of measurements made on the three samples.

The slope of the curve uear the 15/64-inch screen size is relatively flat with nearly the same percentage of kernels riding a 14/64-inch screen and a slightly lower percentage of the kernels riding the 16/64-inch screen. On the other hand, the slope of the curve is very steep near the  $21\frac{1}{2}/64$ -inch screen with a much higher percentage of the kernels riding the 21/64-inch screen and a much lower percentage of kernels riding a 22/64-inch screen. Inspection of the curves indicates that one would expect little change in percent SMK with a slight change in the size of all the kernels but would expect an appreciable change in percent ELK. This is further demonstrated by the fact that the two weeks difference iu digging date caused no difference in percent SMK but caused a difference of eighteen percentage points for ELK.

Effect of Moisture Content on Kernel Size. To determine the effects of moisture content on kernel size, representative samples were drawn at various moisture levels from peanuts as they were drying from 15 percent moisture to 5 percent moisture at  $70^{\circ}$  F and 50% R.H. The samples were shelled by hand and screened over a mechanical shaker to determine the percent SMK and ELK. The shelled keruels were then dried down to five percent moisture and screened again. Samples of peanuts from three harvest dates were tested. The percent decrease in ELK versus the reduction in moisture content is plotted in Figure 2. Each point represents an average of four replications from a given harvest date. The regression equation and the correlation coefficient are shown on the graph.

The results of the study show that the relationship between moisture loss and percent decrease in ELK is a straight line function between 15 percent moisture and 5 percent moisture. Reduction of moisture content by 1 percentage point caused a 2.62 percent decrease in ELK. The findings appear to be in agreement with Bartlett (1) who found that a reduction in moisture of 1 percentage point caused a loss of 1 percentage point in percent ELK. Bartlett does not give the average level of percent ELK in his tests, but a change of 1 percentage point for a sample containing 38.2 percent ELK would be equivalent to a 2.62 percent change in ELK. Other tests indicate that the effects of moisture content on kernel size is variable and the percent change in ELK per percentage point of moisture may range from 1 to over 5 percent.

Peanut grading procedures presently allow for an adjustment in weight for moisture content, but there is no provision for adjustment in size of kernels for moisture content. For example, if 1000 pounds of peanuts are found to have 50 percent ELK at 10 percent moisture content the total weight of ELK is computed as being 490 pounds ( $50 \times 980$ ) at the standard moisture content of 8 percent. This adjustment does not take into account the fact that the kernels would shrink when dried to 8 percent moisture and probably 5 percent of the ELK would be lost giving only 465.5 pounds of ELK.

Effect of Drying Rate on Kernel Size. Five tests were made to measure the effect of drying rate on kernel size. For each test 3,200 grams of kernels were hand shelled after partially drying in the laboratory to moisture contents ranging from 22 to 42 percent. To insure that all samples in the test had approximately the same distribution of kernel sizes, the kernels were screened to partition them









into 2/64-inch size intervals. Each partition was divided into eight portions over a Boerner sample divider, and portions from all partitions were then combined to form eight samples of about 400 grams each.

Four randomly selected samples were placed in a room controlled at  $95^{\circ}$  F and 25% R.H. (fast-dried samples). The remaining four samples were placed in a room controlled at  $95^{\circ}$  F and 80% R.H. (slow-dried samples). Fans were used to force air through the samples at a rapid rate.

The samples were screened periodically as they dried from 14 percent moisture to about 5 percent moisture. The percent ELK, percent SMK, and percent moisture at time of screening were recorded. For illustrative purposes a plot of the percent ELK versus the moisture content at time of screening for fast-





dried samples and slow-dried samples is shown in Figure 3. Each point is an average of four replications. Table 1 summarizes the measurements made on all samples at 9 percent moisture. The results show that on the average there were 31 percent more ELK in the fast-dried samples than in the slow-dried samples. Very little difference was found in the SMK.<sup>1</sup>

Some previous tests by the authors (2) have not been in agreement with these results. However, the present studies were designed specifically to determine the

<sup>2</sup> Peanuts should not be rapidly dried for commercial purposes, since more of the kernels will skin and split during shelling and handling operations.

			Size of k	ernels at	9 percent	moisture	
Drying test	Initial moisture %	Slow	-dried	Fast-	dried	Percent due to fa	difference ast drying
		% ELK	% SMK	% ELK	% SMK	ELK	SMK
1	42.3	27.1	95.4	38.0	96.2	40.2	0.1
2	22.0	30.1	94.0	31.9	95.5	6.0	1.5
3	27.6	28.5	97.0	41.8	97.6	44.9	.6
4	33.7	30.6	97.3	42.9	98.1	40.2	.8
5	37.4	38.9	99.2	47.2	97.9	23.9	-1.3
Average	32.6	31.0	96.6	40.3	97.0	31.0	.3

TABLE I. EFFECT OF DRVING RATE ON KERNEL SIZE\*

\* Each entry is an average of four replications.

effects of rate of drying on kernel size and within the limits of the test are conclusive.

It should be noted that the peanuts used in these tests were shelled before drying so that the same kernels could be screened at various moisture levels as they dried. Tests on peanuts dried in the hull are more variable because a different sample has to be used for each moisture level and the size distribution of the samples cannot be adjusted prior to drying. Screening tests on four samples dried in the hull to 9 percent moisture in each of three drying tests showed that the fast-dried samples contained au average of 10 percent more ELK than the slow-dried samples. Kernels in the hull dry more slowly than shelled kernels under the same conditions.

Effect of Drying Rate on Count per Pound. Counts were made of the number of kernels per pound in the fast-dried ELK and slow-dried ELK from the five tests described above. Table 2 shows that the fast-dried ELK had an average count of 32 more kernels per pound than the slow-dried ELK. Test two had the smallest difference in count per pound just as the difference in size shown in Table 1 was less for test two. These results show that lighter kernels were retained on the 21½/64-inch screen when the peanuts were fast-dried than when the peanuts were slow-dried.

Effect of Drying Rate on Density of Kernels. Density determinations on the whole ELK were made with a Jolly spring balance. The spring extension caused by a sample of kernels was measured when the sample was suspended in air and then in water at a known temperature. The average apparent deusity of the sample was calculated by the relation:

$$P_{k} = \frac{P_{w}X'}{X' - X}$$

where

 $P_k = \text{density of kernels, gm/cc}$   $P_w = \text{density of water, gm/cc}$  X' = spring extension caused by peanuts in air, cmX = spring extension caused by peanuts in water, cm

TABLE 2. EFFECT OF DRYING RATE ON COUNT PER POUND OF EXTRA LARGE KERNELS

	Count per pound at	5 percent moisture	
Drying test	Slow-dried	Fast-dried	Difference
I	545	581	36
2	577	581	4
3	545	595	50
4	549	581	32
5	540	572	32

1	Sample number	Density of w	/hole kernels /cc	Density of split kernels gm/cc		
5		Slow-dried	Fast-dried	Slow-dried	Fast-dried	
	1	1.020	0.992	1.097	1.093	
	2	1.013	0.983	1.098	1.105	
	3	1.028	0.996	1.097	1.099	
	4	1.027	0.993	1.097	1.100	
	5	0.942	0.881	1.096	1.103	
	6	1.002	0.964	1.098	1.098	
	Average	1.005	0.968	1.097	1.096	

TABLE 3.	Effect o	F DRYING	; RATE	ON THE	DENSITY	ÖF	WHOLE
	KERNELS	AND SPL	JT KERI	NELS OF	PEANUTS		

To prevent the peanuts from absorbing water during the tests the kernels were sprayed with a thin coat of Krylon No. 1303 Spray Coating which was absorbed by the skins and sealed them.

The density of the split kernels was measured by use of a Beckman air comparison pycnometer. The pycnometer would not measure the volume of the whole kernels because the skins were too permeable to air. Measurements on whole kernels were equivalent to measurements on split kernels with the pycnometer since the void space between the cotyledons did not affect the measurements.

The increase in count per pound and size of peanut kernels with increase in drying rate indicate that the apparent density of the kernels is changed by drying rate. The density measurements in Table 3 show that the apparent density of the whole kernels was affected by drying rate with the fast dried kernels having the lower apparent density. Density measurements on the same kernels after the cotyledons were split apart showed that the density of the cotyledons was not affected by drying rate. These measurements indicate that the void space between the cotyledons is increased by rapid drying due to distortion of the cotyledons.

These results are in agreement with Pickett (3) who found that curing temperature (which changes rate of drying) affected the density of whole kernels but did not affect the density of split kernels.

# SUMMARY

Due to the size distribution of Virginia type peanuts the percent ELK in a random sample of shelled kernels from farmers' stock peanuts is very sensitive to a slight change in the size of the kernels while the percent SMK is relatively stable.

The percent ELK in a sample appears to decrease in direct proportion to the moisture content of the sample. In general one would expect from one to five percent decrease in ELK per percentage point decrease in moisture content from 15 percent moisture down to 5 percent moisture.

Rapidly dried samples contained more ELK than did slowly dried samples. Tests showed that samples dried at  $95^{\circ}$  F and 25% R.H. had an average of 31 percent more ELK than equivalent samples dried at  $95^{\circ}$  F and 80% R.H. The count per pound for ELK was higher in samples rapidly dried than in samples slowly dried.

The apparent density of rapidly dried, whole ELK was decreased by rapid drying while the density of the kernel halves was not affected by drying rate. These measurements indicate that the void space between the cotyledons is increased by distortion of the cotyledons caused by rapid drying. The increase in void space increases the size of the kernels and makes them less dense.

## CONCLUSION

Percent SMK is a more stable measurement than percent ELK. The moisture content of peanut kernels when they are screened and the rate at which they are dried will affect the percent ELK in a sample. These factors should be considered when evaluating the quality of Virginia type peanuts.

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# AGRONOMIC, PHYSICAL, CHEMICAL, AND ORGANOLEPTIC EVALUATION OF ARGENTINE AND THIRTY-THREE PEANUT INTRODUCTIONS<sup>1</sup>

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The agronomic, physical, chemical, and organoleptic characteristics for Argentine and thirty-three peanut introductions were evaluated in 1962 and 1963 at the Oklahoma Agricultural Experiment Station to determine the diversity and potentiality of the germ plasm. The introductions grown with Argentine in preliminary tests at Holland, Virginia in 1961 were high yielding, moderately vigorous or vigorons, and had other desirable characteristics that prompted further investigations into the possibility of obtaining superior strains. The Oklahoma peannt investigation number (Okla, P-No.) assigned each introduction, and the USDA plant introduction number (P.I. No.) and general information obtained in preliminary tests are shown in Table I. The introductions tested included seven from Uraguay, ten from Nyasaland, thirteen from Cuba, and one each from Australia, Jamaica and Argentina.

The pod yields and No. 1 kernel yields (%SMK  $\times$  pod yield) for Argentine and the thirty-three introductions are shown graphically in Figure 1. Argentine (P-2) had the highest average pod yield in the two tests, ranking third in 1962 (exceeded by P-294 and P310) and second in 1963 (exceeded by P-301). The pod yield advantage of the introductions over Argentine reported in preliminary tests in 1961 was not evidenced in the Oklahoma tests.

Less differences occurred among entries for the two years for the mean yield of No. 1 kernels compared with pod yields. The mean yields of No. 1 kernels were 1236 pounds per acre in 1962 and 1159 pounds per acre in 1963 differed by only 77 pounds compared with the mean acre pod yields which were 1905 pounds in 1962 and 1591 pounds in 1963 a difference of 314 pounds.

The plant characteristics measured included plant height, plant width, and leaflet area. The greatest diversity was evident with respect to plant width and leaflet area. The mean plant width was 28.5 inches in 1962 and 33.2 inches in 1963. The mean leaflet areas for ten leaflets per plot, as determined by an area-photometer, were  $16.7 \text{ cm}^2$  in 1962 and  $22.1 \text{ cm}^2$  in 1963.

<sup>1</sup> Manuscript No. 1046.

Okla. F-No.	P.I. No.	Origin	General <sup>a</sup> Vigor	Relative <sup>8</sup> Maturity	Dor	mancy <sup>+</sup> Fresh	Pod <sup>s</sup> Type	Fercent Meats	Testa Color	Seed Size (gms/100)	Branching <sup>a</sup>
293	259591	Uraguay	Vigorous	2%	5	2	IS	75.9	Flesh	48.9	Mod. Profuse
294	259805	Nyasaland	Vigurous	<u>66</u>		3	ŝ	78.4	Flesh	37.8	Mod. Profuse
295	259662	Cuba	Vigorous	24	5	ĭ	š	76.6	Flesh	56.7	Mort.
296	259648	Cuba	Vigorous	24	5	2	ĭs	74.0	Flesh	50.6	Mod.
297	259600	Australia	Vigorous	22	4	2	îŝ	74 2	Flosh	49.7	Mod.
298	259681	Cuba	Mod Vigorous	24	ŝ	4	ŝ	77 3	Fleeh	49.7	Mod
299	259617	Cuba	Vigorous	24	3	î	v	75 1	Red	83.0	Very Profuse
300	259585	Iamaica	Vigorous	12	4	3	ŝ	75.0	lilesh	36.3	Tery Trondse
301	259728	Uraguay	Vigorous	2/0	5	ž	ŝ	78.0	Flesh	38.3	Mod.
302	259774.	Nyasalandi	Mod Vigorous	16	ä	ã	ş	75.8	Flesh	35.9	Mod
303	259665	Cuba	Mod Vigorous	21	A	ĭ	š	74.8	Fleeh	50.6	Mod
304	259814	Nypeoland	Mod Vigorous	12	5	3	ž	77 5	Fleeh	30.5	Mod Profuse
305	259777	Nyasaland	Vigorous	16	5	ä	i i i	77 1	Flosh	35.0	Mod
306	259736	Cuba	Mod Vigorous	16	5	2	ŝ	81.5	Fleeb	97.9	Mod
307	259800	Nynealand	Mod Vigorous	16	3	ĩ	č	77.0	10 level	36.3	Mod Profuse
308	250775	Nyasaland	Mod Vigorous	12	3	4	2	74 5	Flosh	54.5	Mod. Sparse
309	259826	Nyncoland	Mod Vigorous	16	3	2	ŝ	76.1	Flech	37.8	intodi topanie
310	259800	Nyasaland	Mod Vigorous	16	22	1	ŝ	77 9	Flash	36.3	Mod Profuse
311	050504	Traduau	Vigorous	12	4	2	5	10.2	Flesh	43.0	Wall
312	259663	Cuba	Vinorous	26	4	Ÿ	č	78 7	Floch	57.0	Mod Profuse
313	259599	Uradiav	Vigorous	22	-1	à	i's	76.9	Floub	49.0	Mod. Frofuse
314	259675	Cuba	Vigorous	24	Ē	ŏ	5	77.7	Floch	40.7	Mod. Horose
315	250772	Nuncoland	Vigorous	24	R.	1	0	781	Floab	10.0	Mod. Spore
316	259650	Coba	Mod Vironus	26	4	Ť	ů.	70.1	Flesh	40.7	Mod. Sparse
317	250660	Cuba	Winoroux	78	0	1	ě	50.6	Flesh	44.2	Mod
218	250677	Cuba	Mod Vistorour	23	4	0	é	77.1	Floab	59.5	Mod
310	950749	Tramov	Mod. Vigorous	23	19	3	e e	76.0	Flesh	401	Mod.
300	050670	Cuba	Wide vigorous	73	3	1	6	10.4	Florb	40.1	Mod
201	950703	Cuba	Mad Victor	14	1	† †	ě	60.2	L'IL	44.0	Mod.
300	250805	Magaland	Winerous	2/2		1	5	79.4	Flock	27.0	Mod Profuss
303	250503	Tiragan	Vigorous	78	1	3	2	70.1	Floot	47.0	Wall
204	050507	Uniguay	vigorous	17	1	0	ë	10.0	Piesn	40.0	Mad
305	250880	Cubo	Manual	72	3	2	5	76.7	Flesh	50.0	Mod.
040	200000	Canal	vigorous	73	-1	1	3	10.1	eresn	00.0	MOG.

TABLE U. PEANUT INTRODUCTIONS: THE ORIGIN, GENERAL VIGOR NOTES, RELATIVE MATURITY, SEED DORMANCY, POD TYPE, PERCENT MEATS, TESTA COLOR, SEED SIZE AND TYPE BRANCHING FOR PEANUT INTRODUCTIONS INCLUDED IN THE STUDY-

<sup>1</sup> Catalogue of Seed, Southern Regional Flant Introduction Station. Experiment, Georgia, Regional Project S-9 pp. 27-35. 1964. <sup>2</sup> General Vigor and Branching: Mod. = Moderately. <sup>3</sup> Relative maturity. Time from planting to digging at Beltsville, Maryland, indicated by ½ (earliest) to 6/7 (latest). <sup>4</sup> Seed Dormancy: Number of days required for peanut seed to genuinate when planted in the germinator about two (fresh) and 14 days (two weeks) after digging.

<sup>6</sup> Pod Type; IS = Improved Spanish; S = Spanish; V = Valencia.



FIG. 1. Yield Data: Mean pod and no. 1 kernel yield of the 33 introductions and Argentine grown in 1962 and 1963.

The physical factors studied included grade, seed size and fruit measurements. The 72.8 percent of sound mature kernels for the 1963 test exceeded the 69.0 percent for 1962 by 3.8 percent. Percentages of other kernels were 3.8 and 1.9 percent for the 1962 and 1963 tests, respectively. Samples of several introductions contained no damaged kernels and data obtained were variable. Percentages of total kernels were concurrent with the percentages of SMK in both years, but were highest in 1963.

The seed size in grams per 100 seed for accessions ranged from 35.8 (Argentine) to 52.0 (P-312) in 1962, and 37.2 (P-299) to 55.4 (P-312) in 1963. The mean weights were heavier in 1963 for each of the introductions than in 1962. The seed of the seven introductions obtained from Uraguay were similar in size for the two years ranged from 39.0 to 46.9 grams per 100 seed, with a mean of 43.5. Introductions from Nyasaland had small seed but contained considerable variability. The seed ranged from 38.3 to 51.4 grams per 100 seed for the two years with a mean of 42.1. Introductions from Cuba had the largest seed, except for P-299. They ranged in size from 37.0 to 53.7 grams per 100 seed for the two years with a mean of 47.1. Seed of introductions from Australia, Jamaica, and Argentina averaged 51.2, 38.5, and 89.6 grams per 100 seed, respectively, for the two years.

Pod measurements were taken at three width positions, one length position, and two thickness positions. The mean widths at the basal or widest portion of pod (position 1) were 1.24 cm. in 1962, and 1.21 cm. in 1963. The mean widths at the constriction (position 2) were 1.15 cm. in 1962 and 1.13 cm. in 1963. The mean width at the distal portion of the pod (position 3) was 1.12 cm. for each year. Seven accessions consistently had wide pods. The mean pod lengths (position 4) were 2.51 cm. in 1962 and 2.42 cm. in 1963. There were 27 accessions that had longer pods than Argentine which had a mean of 2.31 cm. Pod thicknesses at the ventral basal suture (position 5) were 0.059 inch in 1962 and 0.048 inch in 1963. At the dorsal distal suture (position 6) the mean pod thick-



FIG. 2. Chemical analysis: The jodine number and the oil and protein contents of the 33 introductions and Argentine Grown in 1962 and 1963.

nesses were 0.034 inch in 1962 and 0.028 inch in 1963. In 1962 the mean pod measurements were wider, longer, and thicker at each position than in 1963, except for the pod width at the constriction which was equal.

The iodine number and the oil and protein contents of raw peanut samples for the thirty-three peanut introductions and Argentine grown in 1962 and 1963 are presented graphically in Figure 2. The iodine numbers ranged from 97.9 to 102.0 in 1962 and from 97.7 to 100.0 in 1963. The iodine numbers for the various entries were concurrent for the two years with two notable exceptions. P-312 was slightly higher in 1963 than in 1962 and P-319 was one of the highest in 1962, but the lowest in 1963. The oil contents ranged from 46.53 to 50.53 percent in 1962 and from 46.60 to 50.19 percent in 1963. The mean oil contents for the strains evaluated were 48.22 percent in 1962 and 48.77 percent in 1963. The oil contents for both years were generally the same with the exception of P-294 which differed by 3.42 percent in the two years. Argentine ranked second for the mean of two years and was exceeded 0.25 percent by P-324. The protein contents ranged from 29.54 to 33.06 percent in 1962, and from 31.94 to 35.75 percent in 1963. The mean protein contents for the strains evaluated were 31.29 in 1962 and 34.02 percent in 1963. Results obtained for the various entries were concurrent for 1962 and 1963, but the protein contents in 1963 were slightly higher than in 1962. Conversely, the iodine number of 1963 was lower than in 1962. The diversity among the introductions studied with respect to iodine numbers and oil and protein contents is illustrated below:

Okla.	P-No.	Iodine Number	Qíl	Protein
293,	295	High	High	High
294		· Low	Low	Low
303,	316	High	Low	High
301,	305, 315,	324 Low	High	Low

In peanut butter analyses a five member taste panel rated several peanut butter samples for the various strains superior to, equal to, or inferior to reference samples. The accessions ranking high organoleptically were P-294, P-304, P-309, P-314, P-315, P-318, P-322 and P-323. Five, two, and one of these accessions originated from Nyasaland, Cuba, and Uraguay, respectively. Six accessions received rather low scores. The Argentine reference sample was exceeded in mean preference rank only by P-309 and P-318. The oil content for the peanut butter samples were similar to those obtained for the raw samples. The fatty acid distribution in the triglycerides of the peanut butter were determined for 1962 grown samples by Mason *et al.* (1). Approximately 93 percent of the fatty acids in the oil of various introductions cousisted of palmitic, oleic, and linoleic. The remaining seven percent of the fatty acids consisted of stearic, linoleuic, arachidic, behenic, and lignoceric.

The results obtained in 1962 and in 1963 showed that the germ plasm from Uraguay, Nyasaland, Cuba, Australía, Jamaica and Argentine contained diversity for many of the variables measured. Certain accessions had oue to three unusual features, but uone were outstanding for several characteristics.

In the agronomic evaluation Argentine ranked first in pounds per acre of pods and second in pounds per acre of No. 1 kernel yield. There were two accessions from Uraguay, four from Nyasaland, and one from Argentine that ranked rather high in pod yields.

The plant characteristics, plant width and leaflet area varied between years and within years.

There was considerable diversity among strains for grade, seed size and fruit measurements. Thirteen accessions along with Argentine were consistently high in mean percentages of total kernels. The seed size was variable among introductions within years. Argentine had the smallest seeds of the strains tested and P-312 the largest. Pod width measurements were variable at three positions. The pod length for the two years was rather consistent for each introduction. The pod thicknesses determined for the accessions at two positions varied widely.

The chemical evaluation for the introductions and Argentine indicated wide differences among strains in protein and oil contents and iodine numbers.

There were eight accessions ranking high organoleptically. Five of these came from Nyasaland, two from Cuba and one from Uraguay.

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# QUALITY EVALUATION OF MECHANICALLY CURED PEANUTS

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# INTRODUCTION

Some properties of peanuts can be substantially changed by curing at excessive temperatures. Milling properties are adversely affected when peanuts are over-dried or dried too rapidly. Drying studies conducted at North Carolina State College (2) showed that curing temperatures above  $95^{\circ}$  F. result in poor flavor. When temperatures above  $100^{\circ}$  F. are used, serious flavor deterioration is encountered, especially in peanuts which are not fully matured.

This paper presents the results of quality evaluation tests on peanuts dried in both a commercial-scale and a laboratory-scale dryer. These drying studies were described in the paper just presented by Mr. Hutchinson. The experiment was designed to study the effect of 4 air velocities, 2 drying methods, and 2 drying temperatures in the laboratory-scale dryer and the effect of 3 drying temperatures

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	TABLE I. SCHEDULE OF D'RYING TESTS
Air flow (FPM) Tempdrg. (F) Method	Laboratory-scale drying tests 25, 50, 75, 100 105, 115 A—Heated air in same direction throughout test B—Heating and cooling in alternate steps with air flow in same direction
Air flow (FPM) Tempdrg. (F) Method	Commercial-scale drying tests 75 125, 135, 145 A—Heated air direction reversed every hour with no cooling B—Air flow direction alternated every hour with cooling for one hour—cooling air in same direction as previously heated air treatment C—Air flow direction alternated every hour with cooling air direction reversed from last heated air treatment

and 3 methods of drying in the commercial-scale dryer on both the flavor and skin slippage of 3 types of peanuts (Table 1). In addition to the flavor and skin slippage evaluations, maturity tests and moistnre distribution tests were made.

#### METHODS AND MATERIALS

Taste panel evaluation. The shelled peanut samples remained in plastic bags on an average of 26 days before roasting and processing into peannt butter for taste panel evaluation. Any off flavor due to the "greenness" of the kernel was thereby eliminated. Before roasting, peanuts were sampled and tested for moisture content and graded. Twelve samples of the 26, ranging in moisture content from 9.0-9.9 percent, showed some evidence of mold damage; 12 of the 16 from 10.0-10.8 percent moisture were moldy; and all four samples at 11.0-12.0 percent moisture were moldy and showed evidence of mold damage after roasting. This corroborates the findings of other investigators (3) that peanuts may mold if stored above 8 percent moisture content.

All moldy and otherwise damaged kernels were picked out of each sample before processing into peanut butter so that their effect on flavor would be minimized.

The peanuts were roasted in a CE Model R20 Rotisserie Oven fitted with a  $14 \times 6$  inch revolving cylinder of stainless steel mesh having solid stainless ends and a  $2 \times 6$  inch door. The oven thermostat was set at 400° F. Before each roasting, the oven was preheated to this temperature immediately preceding the insertion of each sample.

The taste panel evaluations were conducted at the Food Processing Laboratory at Experiment, Ga., under the supervision of Dr. Sam Cecil. The taste panel was composed of 10 experienced judges and the samples were evaluated at the rate of one set in the morning and one set in the afternoon of the day following the roasting and grinding of each group of samples. The samples comprising each set were coded and presented in a randomized complete block design, each judge constituting a block, with a reference sample identified as "normal treatment" added to each set: Since this reference sample was identical with the initial or control sample, it served as a measure of sampling error and panel reproducibility.

The score sheet used was one developed by the Market Quality Research Division (Table 2). Provisions were made on the sheet for checking samples as having none, slight, moderate, or extreme off flavor. Numerical values of 4, 3, 2, and 1, respectively, were assigned to the flavor designations on the score sheets. Samples were also evaluated as being smooth or coarse, oily or dry, and appropriate remarks by the panelist were recorded in the space provided.

#### TABLE 2. SAMPLE BALLOT FOR TASTE PANEL

Name\_\_\_\_\_ Date\_\_\_\_\_ Evaluate the coded sample for OFF-FLAVOR. The reference sample (R) is a control which has received normal treatment.

is the coded sample:	
Yes	No
Smooth?	
Coarse?	
Oily?	
Dry?	
QE Ballot No. 8	-
	Is the coded sample: Yes Smooth?

Maturity evaluation. The test was based on a technique suggested by  $Bailey^2$  and involved shelling 100 randomly-selected peanuts by hand, observing the color of the inside of the shell and the general appearance of the kernel, and classifying each peanut to 1 of 3 categories, depending on both the degree of darkening of the inside of the shell and the amount of wrinkling of the kernel. These categories represented mature, semi-mature, and immature peanuts. A maturity score was obtained by adding half the number of peanuts in the semi-mature category to the number in the mature category. Admittedly, this is a highly subjective technique, but with some experience a reasonably accurate estimation can be made of the maturity of Spanish and Runner types. On the other hand, much less accuracy is obtained with the Virginia type, because of less darkening of the inside of the shell.

Skin slippage test. This test involved the weighing of 500 grams of a representative sample of shelled peanuts taken from the gravity table in the shelling plant, pouring the peanuts into a large shallow pan, and picking out all splits, bald-face, and those with less than half of the skin remaining. These skinless, part-skinless, and split kernels were weighed and this weight was used to determine the percent skin slippage in the 500-gram sample.

Moisture distribution measurements. According to Holly (5), moisture content in the peanut skin is a good indication of maturity, high skin moisture content relative to the total moisture content of the kernel being indicative of immaturity. The converse of this is true for mature kernels. Direct current resistance measurements and electrical capacitance measurements were made of the peanut samples, because the surface moisture of the peanut is inversely related to the logarithm of the d.c. resistance and the total moisture is directly related to the total capacitance of the peanut (4). When the resistance and capacitance values are plotted on a semi-logarithmic graph the immature samples would be expected to be offset from the mature sample points because of the higher skiu moisture.

# RESULTS AND DISCUSSION

Statistical treatment of taste panel data. An analysis of variance and Duncan's multiple range test were made of the results from the taste panel evaluation of the laboratory-scale drying.

While there were a few erratic scores, reference samples and the initial samples, which were identical, were scored about the same—references averaging 2.84 and initials 2.81 in the laboratory-scale drying tests.

There was no indication that air velocity, temperature, or method of drying had any adverse influence on the quality of the peanuts obtained from the laboratory-scale drying tests. In fact, treatment results of Spanish averaged 0.10 higher scores. Runners dried at 105° F. averaged 0.42 higher and Runners dried at 115° F. averaged 0.91 higher than the corresponding initial or control samples. The average results for the 2 methods of drying were not significantly different

<sup>&</sup>quot;Wallace Bailey, Agricultural Research Service, Beltsville, Md., suggested this method in a discussion with the senior author.

	Spa	nísh			Runner				Vir	ginia	
Load	R	I	Average R and I	Load	R	I	Average R and I	Load	R	I	Average R and I
1-38	3.83	3.63	3.78	1-8R	3.63	3.50	3.56	1-3V	3.13	3.38	3.26
4-6S	3.63	3.75	8.69	4-6R	3.88	3.38	3.63	4-6V	2.88	8.00	2.94
7-95	3.88	3.88	3.88	7-9R	3.00	3.50	2.25	7-9V	3.25	2.88	3.06
10-128	3.50	3.50	3.50	10-12R	3.38	3.25	3.32	10-12V	3.50	3.88	3.44
13-155	3.38	3.50	3.44	13-15B	3.13	2.88	3.00	13-15V	2.50	2.38	2.44
16-188	3.25	3.25	3.25	16-18B	2.75	3.00	2.88	16-18V	2.63	2.50	2.50
19-215	3.38	3.38	3.38	19-21R	3.63	3.50	3.56	19-21V	3.00	2.88	2.94
22-248	3.38	8 25	3.32	22-24R	3.00	2.63	2.82	22-24V	3.38	3.25	3.32
25-278	2.38	2.75	2.56	25-27R	8.50	3.50	8.50	25-27V	3.13	3.13	3.13
Averages	3.40	8.43	3.42		8.32	3.24	3.28		8.04	2.98	8.01

TABLE 3. R AND I TASTE SCORES

for the two types, being 3.20 for Spanish and 3.01 for Runners. The difference lay in the scores of the control samples, which averaged higher for Spanish.

The analysis of variance made on the data from the commercial-scale tests was based on a "between load" rather than on a "within load" comparison. The "between load" analysis of the commercial-scale drying tests was more meaningful in regard to the effects of temperature and methods of drying on taste panel scores. These data were divided into two sets. The first set was composed of loads of peanuts which received all 3 temperature treatments plus the reference and initial samples with no treatment. The second set of data was composed of loads of peanuts which received all 3 method treatments plus the reference and initial samples with no treatment. This procedure was need to study both temperatures and methods, free of load effects. The load effect consisted mainly of differences in maturity and initial moisture of the peanuts.

Analysis of the reference and initial samples (called R and I) from each load that received normal drying at ambient temperatures established the fact that types as well as loads within types differed significantly in taste scores. In other words, the panelists showed types and loads within types differed in degree of off-flavor even before any special temperature or drying method treatments were applied. Table 3 shows the R and I results.

The differences between R and I within each load gave a measure of sampling error and panel reproducibility (i. e., reproducibility of averages for the panelists). These differences gave a variance of .025, which means that on the average the R and I will differ due to sampling and panel error by 0.2 score points with practically no two sub-samples expected to disagree by more than 0.6 score points. Therefore, *loosely* interpreted, we would expect to find two results from the same load, such as the scores for 125° and 135°, to be within 0.6 of each other, unless temperatures really have an effect on scores, in which case we would get differences greater than 0.6.

	Tempera-	T . 1.1		Methods			
variety	tures	Loads	Α	B	С	- Average	
Spanish	125° 135° 145°	1-85 7-95 13-155	3.25 3.38 2.88	3.38 3.38 2.88	3.50 3.63 3.00	3.38 3.46 2.92	
	Average		3.17	3.21	3.38	3.24	
Runner	125° 125° 135° 185° 145° 145°	13-15R 22-24R 1-3R 25-27R 7-9R 19-21R	3,50 3,38 3,25 3,75 2,13 8,25	3.63 3.38 2.13 3.75 3.13 3.50	3.13 3.38 2.63 3.25 3.25 3.75	3.42 3.38 2.67 3.58 2.84 3.50	
	Average		3.21	3.25	3.23	3.23	
Virginia	125° 135° 145°	13-15V 1-3V 7-9V	3.00 2.38 2.38	2.25 2.25 2.25	2.75 2.50 2.38	2.67 2.38 2.34	
	Average		2.59	2.25	2.54	2.46	
	Average, all varieties		3.04	2.99	3.10		

TABLE 4.	TASTE PANEL	SCORES OF ]	Peanuts	WHEN	DIFFERENT	METHODS
	OF DRYIN	G (A, B, A)	vd C) W	ERE AI	PPLIED	

<sup>1</sup> For a given variety, each load was dried at oue of the three temperatures  $(125^{\circ}, 135^{\circ}, \text{ or } 145^{\circ})$ . Therefore, some of the differences from load to load are due to temperatures.

Methods of drying and temperatures gave significantly different taste results when the effects of loads and varieties were eliminated. The scores when all three methods were applied to each load are given in table 4 and the scores when all three temperatures were applied to each load are given in table 5.

From table 4, the averages of A = 3.04, B = 2.99, and C = 3.10, although close, show that C method, which is the least harsh of the drying methods, is preferable when analyzed statistically. Whereas, considering each type separately no significant differences were found. It is likely that this is due to small sample sizes.

Table 5 averages of  $125^{\circ} = 2.87$ ,  $135^{\circ} = 2.71$ , and  $145^{\circ} = 2.74$ , show that the temperature of  $125^{\circ}$  is preferable and this is confirmed in the analysis. Again, looking at each variety separately, this is not conclusive except for the Virginia type.

There is also some evidence that the temperatures and methods interact. This showed up in the analysis of the Spanish data from tables 4 and 5. For example, Methods A and B show highest scores from the temperature  $125^{\circ}$ , whereas Method C shows the lowest score with this temperature. This was not significant for Runner and Virginia analyses but it appears that interactions may be present in these types.

To establish or deny this type disagreement it will be necessary to conduct additional experiments, the design of which should be different than the one currently used. From the present data, one can say that higher temperatures and harsher drying methods result in lower taste scores, but exactly how and to what degree for each type is still unknown.

Tables 6 and 7 show the average results of treated and untreated (R and I) samples. The analysis indicated significant differences between treated and untreated for all varieties combined.

	16.4.1	T ] T	7	Temperatures			
Variety	Method	Loads.	$125^{\circ}$	135°	$145^{\circ}$	- Average	
Spanish	A A B B C C	4-68 22-24S 10-12S 25-27S 16-18S 19-21S	$\begin{array}{c} 3.13\\ 3.00\\ 3.38\\ 2.75\\ 3.25\\ 3.25\\ 3.25\end{array}$	2.50 2.88 2.75 3.00 3.38 3.50	$2.50 \\ 2.75 \\ 3.13 \\ 3.25 \\ 3.63 \\ 3.75$	$2.71 \\ 2.88 \\ 3.09 \\ 3.00 \\ 3.42 \\ 3.50$	
	Average		3.13	3.00	3.17	3.10	
Runner	A B C	16-18R 4-6R 10-12R	2.88 3.38 3.13	$\begin{array}{c} 3.00 \\ 3.13 \\ 3.13 \end{array}$	$3.63 \\ 2.50 \\ 3.38$	$3.17 \\ 3.00 \\ 3.21$	
	Average		3.13	3.09	3.17	3.13	
Virginia	A A B C C	10-12V 19-21V 16-18V 22-24V 4-6V 25-27V	2.88 2.00 2.75 2.50 2.13 2.63	$\begin{array}{c} 2.13 \\ 1.38 \\ 3.38 \\ 2.13 \\ 2.00 \\ 2.38 \end{array}$	$\begin{array}{c} 2.13 \\ 1.13 \\ 3.13 \\ 2.25 \\ 2.13 \\ 1.88 \end{array}$	2.38 1.50 3.09 2.29 2.09 2.30	
	Average		2.48	2.23	2.11	2.27	
	Average, all varieties		2.87	2.71	2.74		

TABLE 5. TASTE PANEL SCORES OF PEANUTS WHEN DIFFERENT TEMPERATURE TREATMENTS (125°, 135°, and 145°) WERE AFPLIED

<sup>1</sup> For a given variety, each load received one of three methods of drying (A, B, or C). Therefore, some of the differences from load to load are due to methods.

Variety	А	В	C	R	I			
Spanish	3.17	3.21	3.38	3.70	3.67			
Runner	3.21	3.25	3.23	3.32	3.25			
Vircinia	2.59	2.25	2.54	2.96	2.88			
All varieties	8.04	2.99	3.10	8.32	8.26			

TABLE 6. AVERAGE TASTE SCORES FOR METHODS A, B, C, R AND I (Each Method was Applied to Each Load)

Table 7. Average Taste Scores for Temperatures 125°, 135°, 145°, R and I (Each Temperature was Applied to Each Load)

Variety	125°	135°	145°	Ambient R	Ambient I
Spanish	3.13	3.00	3.17	3.25	3.31
Runner	3.13	3.09	3.17	3.34	3.21
Virginia	2.48	2,23	2,11	3.09	3.02
All varieties	2.87	2.71	2.74	3,20	3.18

Skin slippage tests. The lack of sample replications within the loads made it impractical to obtain an error of mean square which could be used to test differences of methods or temperatures within a load. Therefore, no statistical analysis of variance was made on these data. However, it can be seen from table 8 that Method A was higher than Method B or C for the majority of loads and averaged higher for each of the varieties and the total, including all varieties.

Table 9, showing skin slippage scores of peanuts when dried at different temperatures, indicates that the scores at a temperature of  $145^{\circ}$  were higher for most loads than temperatures of  $135^{\circ}$  and  $125^{\circ}$  F. This is true for variety averages and total averages. It can also be seen from tables 8 and 9 that the Runner type has significantly less skin slippage. High moisture peanuts were included in the table but were not used in the averages.

XIt.h.	Tempera-	¥		Methods			
vanety	tures	Loads	A	В	С	- Average	
Spanish	$125^{\circ} \\ 135^{\circ} \\ 145^{\circ}$	1-38 7-98 13-158	6.98 15.64 14.44	$2.00 \\ 4.50 \\ 7.58$	$3.90 \\ 14.72 \\ 5.70$	$\begin{array}{r} 4.29 \\ 11.62 \\ 9.24 \end{array}$	
	Average		12.35	4.69	8.11	8.38	
Runner	125° 125° 185° 185° 145° 145°	18-15R 22-24R 1-3R 25-27R 7-9R 19-21R	$\begin{array}{r} .92 \\ .80 \\ 3.10 \\ .45 \\ 5.62 \\ .20 \end{array}$	.82 .40 .95 .40 1.04 .40	$1.72 \\ .50 \\ .28 \\ .40 \\ .76 \\ .60$	$1.15 \\ .57 \\ 1.44 \\ .42 \\ 2.47 \\ .40$	
	Average		1.85	.67	.71	1.08	
Virginia	125° 135° 145°	13-15V 1-3V 7-9V	$8.22 \\ 10.16 \\ 21.82$	$8.48 \\ 4.32 \\ 8.36$	$10.68 \\ 7.68 \\ 6.68$	$9.13 \\ 7.89 \\ 12.29$	
	Average		13.40	7.05	8.35	9.60	
Average, all	varieties		7,36	3.27	4.47		

TABLE 8. SEIN SLIPPAGE SCORES OF PEANUTS WHEN DIFFERENT METHODS OF DRYING (A, B, AND C) WERE APPLIED

<sup>1</sup> For a given variety, each load was dried at one of the three temperatures (125°, 135°, or 145°). Therefore, some of the differences from load to load are due to temperatures.

		r 11		Temperatures			
Variety	Method	Loads	125°	135°	$145^{\circ}$	- Average	
Spanish	A A B B C C	4-68 22-24S 10-12S 25-27S 16-18S 19-21S	3.96 8.18 4.20 4.22 6.14 3.20	$21.04 \\ 2.00 \\ 10.00 \\ 4.26 \\ 9.00 \\ 5.80$	$24.60 \\11.68 \\12.18 \\6.30 \\16.56 \\6.64$	$16.53 \\ 7.29 \\ 8.79 \\ 4.93 \\ 10.57 \\ 5.21$	
	Average -		4.98	8.68	12.99	8.89	
Runner	A B C	16-18R 4-6R 10-12R	$2.30 \\ .72 \\ 1.44$	8.00 .70 .88	$14.22 \\ 1.00 \\ .32$	8.17 .81 .88	
	Average		1.49	3.19	5.18	3.29	
Virginia	A B C A <sup>*</sup> B <sup>*</sup> C <sup>*</sup>	10-12V 16-18V 4-6V 19-21V 22-24V 25-27V	$14.22 \\ 7.66 \\ 2.48 \\ 10.88 \\ 10.20 \\ 4.72$	$\begin{array}{c} 25.38\\ 12.22\\ 7.52\\ 51.50\\ 11.64\\ 14.38\end{array}$	$36.34 \\ 23.74 \\ 7.14 \\ 17.38 \\ 14.50 \\ 13.06$	25.81 14.54 5.71	
	Average**		8.12	15.04	22.41	15.19	
Average, all	varieties**		4.89	8.90	13.39		

TABLE 9. SKIN SLIPPAGE SCORES OF PEANUTS WHEN DIFFERENT TEMPERATURE TREATMENTS (125°, 135°, AND 145°) WERE APPLIED

<sup>1</sup> For a given variety, each load received one of three methods of drying (A, B, or C). Therefore, some of the differences from load to load are due to methods. <sup>a</sup> These loads are composed of high moisture peanuts.

\*\* Averages were computed by omitting scores of high moistnre peanuts.

Maturity tests. The average level of maturity was different for each variety. Spanish was highest, Runner second, and Virginia lowest. The maturity score, however, did not show a significant correlation with the taste panel score.

Moisture distribution tests. Capacitance-resistance measurements of the samples from the commercial-scale dryer did not correlate significantly with either the maturity measurements or the taste panel score. Samples which had been harvested 2 weeks before normal harvest did show a considerable offset from the mature sample curve. This indicates that this technique may be of value. Further testing with this method will be undertaken this fall.

TABLE 10. FINDINGS WHEN STATISTICALLY ANALYZED BY INDIVIDUAL TYPES

Variety	
Spanish :	<ul> <li>(a) R and I significantly different than Methods A, B, and C. Method C significantly higher than A and B.</li> <li>(b) No significant difference between temperatures.</li> </ul>
Runner:	<ul><li>(a) No significant difference between methods.</li><li>(b) No significant difference between temperatures.</li></ul>
Virginia:	<ul> <li>(a) No significant difference between methods.</li> <li>(b) R and I significantly different than temperatures 125°, 135°, and 145°.</li> </ul>

The small number of tests made for each variety could be the cause for varieties not showing significant differences; therefore, it again is apparent that additional tests would likely show both temperatures  $(125^\circ, 135^\circ, \text{ and } 145^\circ)$  and drying methods (A, B, and C) cause lower taste scores (ergo preferences) for such treated peanuts as compared to "untreated" peanuts (i.e. the R and I's).

# CONCLUSIONS

It has been shown by analysis of variance that there are significant differences between the scores of the treated and untreated peanut samples when all 3 types are combined, but when statistically analyzed by individual types the results shown in table 10 were obtained.

The skin slippage tests indicate that both drying incthod and temperature of drying affect skin slippage and that there is a difference in the amount of slippage due to type of peanuts.

The maturity tests indicate a difference in maturity level for each peanut type, and the capacitance-resistance measurements show some promise as a means of evaluating maturity.

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# EFFECT OF RAPID DRYING ON MOISTURE EQUILIBRIUM IN PEANUTS<sup>1</sup>

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Although accelerated drying of peanuts has been practiced for many years, the search for the relation of rapid drying to quality has brought out the effect on germination as about the only clear cut change involved (1). Karon et al, reported that hygroscopic equilibrium does not appear to be dependent on the method of curing, but the rate at which the nuts were dried is uncertain (2). Lately interest in this question was renewed when rapid drying was reported to lower hygroscopic moisture equilibrium in corn (3).

# DRYING RATES

Our peanuts were dried in one-quarter inch mesh wire baskets, 8 x 11 x 2 inches, loaded at 500 gms per basket. These were placed on inverted wire baskets in forced draft ovens at various temperatures. The drying time, based on weight loss to bring the moisture in the whole fresh nut to approximately 6-8%, ranged from 6 hours to 25 hours, depending on the oven temperature. Drying time varied slightly with initial moisture content and with the oven load, but the curves shown here (Fig. 1) are representative of the drying rates applied to the mature nuts used in this study.

In contrast, room temperature drying at about 80-85° F required from 6-9 days, under the same basket loading conditious, to bring the peanuts to the 6-8% moisture level.

The nuts were usually in the drying ovens within three hours from the time they were dug.

<sup>&</sup>lt;sup>2</sup> Paper No. 486, in the Journal Series of the Coorgia Experiment Station, Experiment, Georgia.



FIG. 1. Peanut drying rates.

# EXPERIMENTAL

The above dried peanuts, which had been stored in polyethylene bags, glass jars, and various other containers at  $40-45^{\circ}$ F, 80-85% r. h., for from approximately six months to over three years, were haud shelled and mature, sound kernels were selected. These uuts were placed in monolayers in small wire baskets (50 gms per basket) which were stacked 3-4 deep in 10 inch desiccators. For the 75% humidity atmosphere, the desiccators contained a saturated sodium chloride solution; a saturated potassium carbouate solution was used for the 43% humidity atmosphere.

Since there was no provision for air circulation within the desiccators, equilibrium was established more slowly than if fans had been used in the chambers, but the results should be the same. Early experience indicated that neither number of baskets nor position in the desiccators had any appreciable bearing on the time required to establish moisture equilibrium in the peanuts. The studies were conducted in a room at 66-70°F. The initial and final moistures were determined by drying for five hours in a static oven at 110°C.

The baskets were weighed periodically and when three successive weighings over a period of not less than one week indicated not more than 0.05% change in weight, the peanuts were considered to be at hygrosocopic equilibrium. Final moisture was calculated and compared with the final, oven determined moisture.

# RESULTS

The effect of 160°F drying as compared with room temperature drying on moisture equilibrium is shown in Fig. 2. The difference shown here is typical and is highly significant at both humidity levels.

Drying at 135°F also lowers equilibrium moisture as shown in Fig. 3. Although the change is not of the same magnitude as in those dried at 160°, it also is highly significant. There is the same trend even at 110°F drying. In fact, as the drying temperature increased and drying time decreased, the equilibrium moisture level decreased on all four curves.





Similar results have been obtained for Tenu. Red, Southeastern Runners, Ga. 61-42 (a white skin peanut), Va. Bunch 67, and Dixie Spanish, covering 1960-1963 crop years (Table 1).

Moisture determinations are subject to error, but the calculated and oven determined final moistures at the 75% humidity level were in very close agreement. The standard deviations of final moistures was  $\pm$  .21%. On the lower



FIG. 3. Hygroscopic equilibrium in peanuts dried at four rates.

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	C	-	75% г.h.				43% г.h.			
Туре	стор уєаг	L Room	Drying te 120°F	mperatu 140°F	ne 160°F	D Room	rying ter 120°F	mperatu 140°F	re 160°F	
		%	%	70	%	90	90	%	70	
D. Spanish Ga. 61-42 Va. Bunch 67	$1960 \\ 1960 \\ 1960$	7.85 7.64 7.46	7.57 7.55 7.42	7.85 7.73 7.21		4.98 4.58 4.46	4.78 4.49 4.40	$4.80 \\ 4.74 \\ 4.38$		
S. E. Runner Tenn. Red D. Snanish	1960 1961 1961	7.57 8.21 8.11	7.78	7.43	7.77	4.64 5.05 5.18	4.64	4.61	$\frac{4.60}{4.91}$	
Ga. 61-42 Va. Bunch 67	1961 1961	8.20 7.77	8.32 8.19	7.93 7.69	7 50	5.06 4.71		$\frac{4.81}{4.69}$	4.01	
D. Spanish Tenn. Red	1962 1962 1963	7.84 8.28		7.65 <sup>b</sup>	7.38 7.52	5.23 5.01 5.26		4.876	4.87 4.74	
D. Spanish Ga. 61-42 Va. Bunch 67	1963 1963 1963	8.34 7.58 7.71	7.80ª 7.35ª 7.63ª	7.76b 7.25b 7.22b	7,70 7.85	5.50 4.80 4.93	5.43a 4.51a 4.85a	5.30b 4.77b 4.53b	5.10 4.80	
S. E. Runner	1963	7.80		7.57b	7.28	5.05		4.85b	4.57	

TABLE 1. HYGROSCOPIC MOISTURE EQUILIBRIUM IN MATURE PEANUTS

a—Dried at 110°F. b—Dried at 135°F.

humidity samples, the final determined oven moisture was approximately 0.3% higher, but had about the same standard deviation of the difference (± .22%). Since the standard deviations were smaller than the differences recorded for drying effect, there is reasonable assurance that drying rates did effect moisture equilibrium.





The average decrease in equilibrium moisture, induced by rapid drying, at both humidities employed in this study is shown in Fig. 4. The results, shown in Table 1, of 110°F and 120°F drying have been averaged because of the small number of samples at each temperature. The 135°F and 140°F results were also averaged. Drying at 135°-140°F and at 160°F gave highly significant decreases in equilibrium moisture at both humidities when compared with room temperature drying. When the 110°-120°F dried nuts were tested, the 43% r. h. level was significant whereas the 75% r. h. level was not significant, although it was close. This uncertainty in the latter values does not mean that these temperatures and these drying rates are not in the rapid drying category. Indeed they suggest that only a slower drying rate can be applied without affecting the equilibrium of the peannt.

The decrease in hygroscopic moisture equilibrium in peanuts induced by rapid drying is numerically small. The physical and chemical changes implied in this effect are, no doubt, quantitatively minute also. But these changes are, relative to comparative moisture levels, greater than those found in corn. And fast drying in corn has important practical effects in mold development. Whether there is any like practical significance in fast drving of peanuts remains to be determined.

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# COMPARATIVE RATE OF GROWTH AND ABSORPTION OF CERTAIN NUTRIENTS BY CORN AND VIRGINIA TYPE PEANUTS ON NORFOLK AND WOODSTOWN LOAMY FINE SAND

## D. L. HALLOCK'

The frequent lack of yield response by peanuts to phosphorus and potassium fertilization is in sharp contrast to the usual behavior pattern of corn. This has been observed many times and places throughout the peanut growing area (8). Four possible causes for this anomalism are: a. If corn requires more total phosphorus and potassium for good yields than peanuts, perhaps many soils normally will supply only the requirements of peanuts without fertilization at that time. b. Corn may have a higher rate of nutrient absorption than peanuts and thus demands of corn and not peanuts could exceed the residual nutrient supplying capacity of the soil at certain times. c. Peanuts may utilize nutrient sources or forms less available to corn. d. Rooting behavior. It seems reasonable to hypothesize that any one or a combination of these factors, should it exist, could help cause this fertilization emigma.

Some composition data (2, 4, 8) indicate that the total uptake of phosphorus and potassium by good crops of corn and peanuts is nearly similar although corn

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may require slightly more phosphorus. In a study of plant root growth and activity by use of tagged phosphorus, Hall, et al. (1) noted that four weeks after planting corn roots were 18 inches in depth and 24 inches in radius. The corn obtained 1/2 of its phosphorus from the surface few inches of soil during the first seven weeks. For the whole growing period, two-thirds of the tagged phosphorus uptake occurred at soil depths of 8 or more inches. These investigators reported that in less than one week after emergence, peanut roots had extended to a depth of 16 inches and three weeks after planting to more than 24 inches. Four-week old peanut plants absorbed 74 percent and 14-week old plants 60 percent of their tagged phosphorus from the 8-inch depth principally via the tap root and short lateral roots. The peanuts obtained relatively little tagged phosphorus from the 24-inch depth despite the rapid early downward growth of the tap root. Hall, et al. suggest that this indicated lack of appreciable nutrient absorption from the 0 to 4-inch soil layer may explain the anomalous behavior of peanuts to normal fertilization. On the other hand, Stivers, et al. (6) obtained no yield response to phosphate placed 10 inches directly below the peanut row. However, a liberal application of fertilizer was made on the surface, also.

The objective of this experiment was to compare the rate of growth and absorption of certain nutrients by corn and peanuts. Also, the rate and depth of root penetration by the two crops was observed.

# MATERIALS AND METHODS

Identical experiments were conducted in 1961 on each of two soil types in the peanut producing area of Virginia. One location was on Woodstown loamy fine sand at the Tidewater Research Station, Holland, and the other on Norfolk loamy fine sand, thick surface phase, in a farmer's field near Walters, Virginia. The Woodstown soil is a deep, light colored, moderately well-drained soil with a light sandy clay loam subsoil. The Norfolk soil was somewhat excessively drained with a very light sandy clay loam subsoil nearly 30 inches below the surface.

The soils at both sites had been well fertilized and cropped for many years prior to the establishment of the tests. The exchangeable potassium, calcium, and magnesium levels in the plow layer were 150, 1000, and 60 pounds per acre, respectively, in the Woodstown soil and 65, 325 and 25 pounds per acre, respectively, in the Norfolk soil. Weak acid soluble phosphorus (Truog) levels were 160 and 70 pounds per acre in the Woodstown and Norfolk soils, respectively. Each soil had a pH of 5.6. The organic matter contents of the Woodstown and Norfolk soils were 2.5 and 0.8 percent, respectively.

No phosphorus or potassium was applied at the Woodstown site in 1961. However, 500 pounds per acre of 0-10-20 fertilizer were applied to the cover crop and plowed under by the farmer at the Norfolk site prior to selection of the location. At both locations 100 pounds per acre of nitrogen (ammonium nitrate form) were side-dressed on the corn when approximately one foot in height.

Alternate 6-row strips of corn (V.P.I. 648) and peanuts (Ga. 119-20) were planted in 3-foot rows using conventional farm machinery to give 4 replications of each crop. The corn and peanuts were thinned to approximate 14 and 9-inch spacings, respectively, in the row. Each strip of corn and peanuts was divided into 8 plots 30 feet long representing sampling dates which were assigned at random to the plots. The first samplings of corn and peanuts were May 25 and June 23, respectively, approximately one month atter planting. Seven additional samplings were taken at 2-week intervals which covered the entire growing period of the crops. Foliar samples of both crops were obtained by random selection of six plants per replication at ground level and dried at  $70^{\circ}$ C to determine dry matter content, then ground for chemical analysis. All immature and mature fruits which had formed were included in the samples, At each sampling, pits were dug immediately beside two plants in each replication and the approximate depth of root penetration into the soil determined.

The organic matter content and pH levels of the soil were determined by rapid soil testing procedures (5). The contents of exchangeable soil calcium, magnesium and potassium were determined by flame photometry in the neutral 1.0N ammonium acetate extract of the samples. Also, the contents of calcium, magnesium and potassium in the plant material were determined by use of a Beckman Model Du spectrophotometer with flame and photomultiplier attachments. Available soil phosphorus was estimated by the method of Truog (7) and total plant phosphorus colorimetrically by the use of ammonium vanadate. All plant tissue samples analyzed were dry ashed in a muffle furnace at  $450^{\circ}$ C and the desired constituents dissolved from the ash with 1.5N nitric acid.

### **RESULTS AND DISCUSSION**

#### Root Depth and Dry Matter Accumulation

The relative rate and depth of root penetration and dry matter accumulation, exclusive of roots, by corn and peanuts are shown in Figure 1. Peanut roots attained their maximum depth in both soils in about 70 days after planting. Corn roots required about 90 days to reach similar depths but on the Norfolk soil their maximum depth was seven feet, one foot deeper than any peanut roots were observed. Maximum rooting depth in the Woodstown soil was five feet for both crops, (water table depth during much of the growing season).

The most striking difference between the two crops in rate of penetration occurred in the Woodstown soil, probably principally because of the rather slow development of the corn roots. May and June, 1961, were excessively wet the precipitation being 11 inches above normal<sup>2</sup>. Also, the average temperature of these months and April, too, was 8 to 4 degrees colder than normal<sup>2</sup>. Such



FIG. 1. Relative rate & depth of root penetration & dry matter accumulation exclusive of roots by com & peanuts, Holland, Va., 1961.

<sup>2</sup> Twenty-nine year mean of records obtained at Tidewater Research Station, Holland, Va.

weather conditions undoubtedly reduced the rate of early development of both corn and peanuts, particularly on the more poorly drained Woodstown soil. The rate of early downward root growth by both crops in this investigation was considerably slower than that reported by Hall, et al. (1) and Linscott, et al. (3).

A marked difference both in the rate and amount of dry matter production by the two crops was obtained (Figure 1). Not only did corn produce more dry matter per acre than peanuts but during a particular period, approximately 60 to 90 days after planting in this case, the rate of its accumulation was nearly three times greater than that for peanuts at any time during its growth period. The effect of this growth rate differential on nutrient accumulation is discussed subsequently.

The general pattern of dry matter accumulation was similar on both soil types. However, the corn produced a little more and peanuts a little less dry matter on the Norfolk soil than on the Woodstown soil. Usually, both corn and peanuts on Woodstown soil produce more dry matter than on light Norfolk soil but, as explained previously, adverse weather probably influenced crop growth on these soils, differentially.

Corn grain yields were 106 and 100 bushels per acre for the Woodstown and Norfolk soils, respectively. The yield of peanuts from the Woodstown site was 2,530 per acre and from the Norfolk soil, 2,800 pounds per acre (farmer's estimate). Heavier vine growth by the peanuts on the Woodstown soil accounted for the higher dry matter production on that soil.

# Plant P, K, Ca and Mg Content and Uptake

The relative mean percentage of phosphorus in the whole plant and rate and amount of phosphorus uptake by corn and peanuts, exclusive of roots, are shown in Figure 2. The percentage of phosphorus in corn increased until about 60 days after planting, then decreased to approximately that in 30-day old plants. Although the Truog phosphorus level in the Woodstown soil was nearly double



FIG. 2. Relative mean percentage of P in the tissue & rate & amount of P uptake by corn & peanuts, exclusive of roots, Holland, Virginia, 1961.

that in the Norfolk soil, 60-day old corn on the latter soil contained a higher average percentage of phosphorus than that on the former, but the reverse was true during the period of maximum dry matter accumulation. Similarly, the initial phosphorus content of peanuts was higher for the Norfolk soil but decreased at maturity. Betweeu these extremes that in peanuts on the Norfolk soil decreased gently toward maturity, whereas the phosphorus content of peanuts on the Woodstown soil was somewhat erratic during the first 2 months.

The phosphorus uptake curves in Figure 2 are very similar to those for dry matter accumulation. Both the rate and the amount of phosphorus uptake by peanuts and corn grown on Woodstown soil exceeded that in the crops on the Norfolk soil. On both soils, the rate of phosphorus uptake by corn during a particular period exceeded considerably that of peanuts at anytime during its development.

Figure 3 shows the variability in the percentage of potassium in the whole plants and in the uptake pattern by corn and peanuts during their respective growth periods. A very sharp 5-fold increase in the percentage of potassium in corn occurred when the plants were about 40 days old followed by a marked but less extensive decrease for the next six weeks, then a slight increase near maturity. The potassium content of peanuts grown on the Woodstown soil followed a sionilar pattern, whereas that in peanuts on the Norfolk soil followed a contrasting pattern for the first 60 days. Also, the peanuts on Woodstown loamy fine sand contained nearly one percent more potassium than those on the Norfolk soil during most of the heavy growth period. Total potassium uptake per acre by peanuts grown on the Woodstown soil was more than double that on Norfolk loamy fine sand. This would be expected in view of the much higher level of exchangeable potassium in the Woodstown soil. Corn did uot show this effect. In fact, the corn on the Norfolk soil was slightly higher in K content and



FIG. 3. Relative mean percentage of K in the tissue & rate & amount of K uptake by corn and peanuts, exclusive of roots, Holland, Va., 1961.
absorbed about 50 pounds per acre more potassium due mostly to increased dry matter production (Figure 1).

It is noteworthy that the general pattern of the percentage composition curves for phosphorus and potassium in corn are quite similar. In both cases there is a marked increase early in the season then a less abrupt decrease, followed by a leveling out or possibly a slight increase as the plants matured. Reference to Figure 1 indicates that this period of rapid decrease in the percentage of these nutrients in the plants corresponds very closely to the period of most rapid dry matter production. This relationship is somewhat similar in peanuts, particularly in the case of the Woodstown soil, but to a much lesser extent. As pointed out previously, the maximum rate of dry matter accumulation in peanuts was much lower than that for corn.

In contrast to these results, the percentages of calcium and magnesium in peanuts rather than in corn present a closer relationship to dry matter accumulation. Figures 4 and 5 show that the contents and uptake of calcium and magnesium by peanuts were considerably higher than by corn throughout the growing season. This difference in composition is due principally to the relatively high content of calcium and magnesium in the peanut vines since the fruits are low in both nutrients.

The contents of calcium and magnesium in peanuts grown on both soil types varied considerably until the plants were about 80 days old. Except for the phosphorus content of peanuts grown on Norfolk soil, similar variability occurred in the phosphorus and potassium contents of the peanuts (Figures 2 and 3). Most of this erratic variability occurred before the period of maximum dry matter accumulation, but during the period of principal root depth penetration by peanuts. Thus, differences in utilization of the nutrients and their availability during this period of root development may have been responsible for the variability noted, however no data were available to verify this supposition.



FIG. 4. Relative mean percentage of Ca in the tissue & rate & amount of Ca uptake by corn & peanuts exclusive of roots, Holland, Va., 1961.



FIG. 5. Relative mean percentage of Mg in the tissue & rate & amount of Mg uptake by corn and peanuts, exclusive of roots, Holland, Va., 1961.

The Woodstown soil contained three times as much calcium and six times as much magnesium in the exchangeable form, yet the percentage of these nutrients in peanuts grown on the Norfolk soil was higher during the first 80 days. This may have been due to a competition effect since during this period the peanuts from the Woodstown site contained a much higher potassium content. Also, there was greater uptake of these nutrients by peanuts grown on the Woodstown soil. However, by maturity their contents were similar for both soil types or slightly higher in the case of Woodstown loamy fine sand.

The data represented in Figures 1, 2 and 3 indicate that the rate of phosphorus and potassium absorption by corn during a certain period exceeds considerably that of peanuts at anytime. Furthermore, the rate and depth of root penetration is greater for peanuts than corn. Although this faster root penetration does not necessarily indicate that peanuts absorbed more nutrients at lower depths, a greater portion of the soil profile is accessible over more of the growing period. In this experiment, much of the dry matter accumulation and nutrient uptake by corn occurred before maximum root depths were reached.

It is suggested that differences between coro and peanuts in the rate and extent of nutrient accumulation principally, and to a lesser degree the depth of root penetration, may explain in part, at least, the apparent fertilization anomaly concerning the two crops.

#### **SUMMARY**

The relative rate and depth of root penetration, and rate and amount of accumulation of dry matter, phosphorus, potassium, calcium and magnesium by corn and peanut foliage and fruit grown on two sandy soil types was investigated in a field experiment in 1961.

1. Peanut roots attained their maximum depth in about 20 days less growing time than corn, but corn roots penetrated one foot deeper (7 feet) than peanuts on Norfolk loamy fine sand.

2. Corn produced more dry matter and, during a certain period, at a rate three times that of peanuts during any of its development.

3. Except for potassinm uptake by peanuts on Woodstown loamy fine sand, the rate and amount of phosphorus and potassium nptake by com during its period of rapid dry matter accumulation exceeded considerably that of peanuts at anytime.

4. The phosphorus and potassium contents of corn (aerial portion) increased sharply early in the season, then decreased less abruptly followed by a leveling out or slight increase as the plants matured. The period of rapid decrease in the content of these nutrients corresponded closely to the period of most dry matter production. This relationship is much less striking with peanuts.

5. In contrast, the percentages of calcium and magnesium in peanuts rather than in corn present a closer relationship to dry matter accumulation.

6. It is suggested that differences between corn and peanuts in the rate and extent of nutrient accumulation principally, and to a lesser degree the depth of root penetration, may explain partially the apparent fortilization anomaly concerning the two crops.

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## THE ROLE OF FUNGI IN THE DETERIORATION OF SEEDS1

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Seeds of many kinds of agricultural plants are subject to invasion by a great variety of fungi, both before and after harvest. Field fungi invade the developing or mature seeds while the seeds still are attached to the plants in the field, before harvest. They may cause various sorts of discoloration, may lower the quality of the seed for processing, and reduce its value for planting. Blighted or scabby wheat and barley, resulting from invasion of the kernels by Fusarium, may be toxic to man and to some kinds of domestic animals, and so is undesirable in food or feed. Invasion of the seeds by field fungi is likely to be heavy when

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the weather before harvest is moist. To grow in seeds all of the field fungi require a moisture content in equilibrium with relative humidities between 90 and 100 % - in the starchy cercal seeds a moistnre coutent of 22-25% on a wet weight basis. All or nearly all grains except corn and rice normally are harvested at moisture contents below this, and corn and rice usually are dried to a moisture content below this within a few hours to a few days after harvest. Therefore field fungi do not normally continue to grow in seeds after harvest, but rather are likely to gradually die, the rate of death depending on the temperature and moisture content of the seeds. However, corn stored on the cob in cribs and exposed to the weather may be invaded and rotted by a number of fungi whose moisture requirements are similar to those of field fongi.

Storage fungi comprise mainly about 10 species of Aspergillus and a lesser number of species of Penicillium, all of which invade a great variety of materials other than stored grains and seeds. These storage fungi do not invade seeds of coreal grains, soybeans, common beans, peas or flaxseed (or, so far as we know, any seeds borne above ground) to any serious degree or extent before harvest. Invasion of seeds by storage fungi may result in various types of damage: Reduced germination; darkening of the germ or embryo, commonly known as germ damage or, in wheat, as sick wheat; darkening of the entire kernel, commonly known as heat damage, even though storage fungi rather than high temperature may have been the chief cause of the discoloration; mustiness and heating. Federal regulations specify the maximum amounts of damaged grainswhether damaged by field fungi, by storage fungi, or by other causes-that are permitted in the various grades of the different kinds of grains.

The major factors that determine whether a given lot of grain or of seeds will be sufficiently invaded by storage fungi to reduce its grade are: Moisture content of the stored grain; (which for a number of reasons may not be precisely determined or accurately known, so that the moisture content of the grain or seeds in a given bin can be much higher than shown on the warehouse records); temperature of the grain (devices to measure temperature in different portions of a bin of stored grain are an aid to and not a substitute for intelligent storage practices); the degree to which the grain already has been invaded by storage fungi before it arrives at a given site, and; length of time the grain is to be stored. The moisture content limits that permit invasion of different kinds of grains and seeds by storage fungi are now known fairly precisely. Methods have been developed to determine the numbers and kinds of fungi on a given sample of grain; and to predict, on this basis, the storability or deterioration risk of the grain. With the knowledge available concerning the causes of damage to stored grains and the conditions under which such damage occurs, and with the aid of modern techniques and devices, a warehouseman can know at all times the condition of the grain or seeds in all parts of his storage bins, and can anticipate damage long before it becomes severe enough to result in decrease in grade, price, or quality.

## PEANUT INFECTION BY SOIL-BORNE FUNGI

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The fact that whole or shelled peanuts may mold in storage is implicit in the extensive literature on peanut curing, storage, shelling, and transit problems. It is also evident from the literature (2, 3, 4) that many of the organisms involved

<sup>&</sup>lt;sup>3</sup> Approved by the Director as Journal Series Paper No. 143. <sup>2</sup> Associate Plant Pathologist.

in shell and kernel invasion are fungi which can persist in the soil. In this study the progress of shell and kernel invasion by fungi initially present on the pod surface was followed at different incubation temperatures.

### MATERIALS AND METHODS

The superficial shell mycoffora of a lot of artificially dried, intact pods of the Spanish variety Argentine used in this study was characterized in previous unpublished experiments. In order of decreasing abundance the fungi present were: *Penicillium* spp., *Fusarium* spp., *Aspergillus* spp., *Rhizopus* spp., *Sclerotium bataticola* Taub., *Trichoderma viride* Pers. ex Fr., and species of 6 other genera which were present in small numbers. Cultural assay of surface-disinfested kernels aseptically removed from pods revealed only 4% initial kernel infection, 2% by S. *bataticola* and 2% by *Aspergillus* niger v. Tiegh.

Pods were spaced separately in tightly-closed containers lined with watersaturated blotters; 3 containers (replications) were placed at each of the temperatures, 70, 80, 90, and  $100^{\circ}F (\pm 3^{\circ}F)$ . Equal numbers of pods were placed in dry, sealed Mason jars and incubated at the same temperatures.

After 2, 4, 6, and 8 days of incubation 10 pods from each container were washed in 3% formalin and shelled aseptically. Kernels were disinfested in 0.5% NaOCl, placed on 2% malt-extract agar, incubated at  $82^{\circ}$ F, and examined after 3 and 6 days.

Before incubation and at each sampling the moisture content (wet weight basis) was determined from a pooled sample of pods from each temperature.

### RESULTS

Pods incubated dry yielded kernels which were not infected above the initial 4% level. Pods which were permitted to hydrate in a moist environment became infected and the progress of kernel invasion by 4 soil-horne fungi that were present superficially on the shell is shown in Fig. 1. At 70, 80, and 90°F, S. bataticola was the most abundant kernel-invading fungus. Aspergillus flavus Link ex Fr. and A. niger were more abundant at 90 and 100°F than at lower temperatures. Rhizopus stolonifer (Erhenb. ex Fr.) Vuill. was found at relatively uniform low levels at all temperatures. Small numbers of Fusarium spp. and T. viride were observed from hydrated kernels at all temperatures. No other fungus was recovered from kernels. Bacteria were frequently observed, particularly from pods incubated at 90 and 100°F.

Moisture content of pods incubated dry fluctuated from 7 to 10%. Pods incubated in a moist environment hydrated rapidly and moisture contents were approximately equal regardless of incubation temperature. After 2, 4, 6, and 8 days incubation the mean moisture contents (all temperatures) were 23, 30, 31, and 34% respectively.

### DISCUSSION

These results demonstrate the rapidity with which some soil-borne fungi on the surface of peanut pods can penetrate shell tissue and infect kernels. It is further evident that some fungi such as *Penicillium* spp. and *Fusurium* spp. which were present ou the pod surfaces in large numbers did not rapidly invade shell and kernel tissue. Infection is conditioned by moisture content of the pod or kernel as Diener (2) and Austwick and Ayerst (1) have indicated and as shown by absence of infection in kernels from dry pods. The point at which moisture becomes sufficient for growth of superficial fungi probably lies well below 20%, since infection by 2 days (23% moisture) was evident at 80, 90, and  $100^{\circ}F$ .

since infection by 2 days (23% moisture) was evident at 80, 90, and 100°F. Infection by S. *bataticola* at 90 and 100°F was initially rapid but the 6and 8-day sampling showed a decline indicating death of the fungus in some kernels, perhaps accompanied by cessation of new infections. Recent unpublished



FIG. 1. Progress of kernel infection at 4 constant temperatures by 4 soil-borne fungi which were initially present as pad surface contaminants. Symbols are: square — A. NIGER, triangle — A. FLAVUS, solid dot — S. BATATICOLA, and open circle — R. STOLONIFER.

work on this point suggests that disappearance of S. bataticola results from competitive growth of other fungi, particularly A. flavus.

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## FACTORS INFLUENCING THE PRODUCTION OF AFLATOXIN BY Aspergillus Flavus GROWING ON LABORATORY MEDIA<sup>1,2</sup>

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One of the areas of research in which the Auburn research group is interested is the fundamental physiology of Aspergillus flavus Link ex Fries. The first problem encountered in this research was that of growing the organism in a chemically defined medium in which aflatoxin was produced. The fungus can be grown on many substrates, e.g., peanuts, shredded wheat, and rice. However, the fungus must be grown on a liquid medium of known chemical composition before details of aflatoxin production can be studied.

During the course of this work, it was found that composition of the medium and the environment surrounding the medium greatly influenced the extent to which A. flavus synthesized aflatoxin. Factors that influenced the production of aflatoxin in a liquid medium are discussed in this paper.

On a chemically defined medium such as Czapek-Dox broth, little or no aflatoxin was produced even though the fungus grew well on the medium. When 1% yeast extract was added to the medium, the fungus was able to synthesize the toxin.

The process of aflatoxin synthesis was highly aerobic. Comparisons of standiug cultures with submerged cultures showed that the highest yields of the toxin were obtained under high aeration. Also, comparisons of cultures with varied surface area to volume ratios showed that maximum aeration was couducive to maximum yields. When yeast extract was added to the medium and cultures were incubated in containers having large surface area to volume ratios, three days were sufficient for surface mycelial mats to produce aflatoxin.

Sporulation usually did not occur within the short duration of the experiments, so the process of aflatoxin synthesis was not dependent upon reproductive growth as is the case with some fungal products.

Another factor that influenced aflatoxin production was the presence or absence of nutrient elements. When the 15 elements generally required by plants were added to the medium, increased yields of aflatoxin were obtained. Aflatoxin was not formed if the minerals were added in the absence of yeast extract. Thus, one or more of the added minerals stimulated aflatoxin production but did not replace the required factor in yeast extract. Zinc has been reported to stimulate aflatoxin production. Whether minerals other than zinc also influence the production of aflatoxin is not known.

Only small quantities of aflatoxiu were found to be present in the fungus mycelium when several hundred grams of iuycelium (dry weight) were extracted. Nearly all of the aflatoxin was in the medium surrounding the fungus. Since the aflatoxin did not accumulate in the mycelium, it must have diffused out at about the same rate at which it was formed. The fact that aflatoxin was free to diffuse might explain how materials were occasionally found to contain aflatoxin even though they were apparently free of mold contamination.

In summary, Aspergillus flavus produced significant quantities of aflatoxin in a Czapek-Dox broth that was supplemented with yeast extract and mineral elements and incubated under conditions of high aeration. The aflatoxin diffused into the surrounding medium and did uot accumulate in the mycelium.

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## FIELD OCCURRENCE OF Aspergillus Florus IN PEANUTS

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Peanuts (Arachis hypogaea L. var. Early Runner) were hand picked from plants or the ground immediately after digging and separated into immature, mature, and overnature categories. Peanuts within each category were separated on a basis of pod color, appearance, and location on the plant. Samples of peanuts picked the same day (cured in the field 5-6 days) and samples in storage for 3 days were also collected. Each lot was surface-sterilized (in the field) for 2 minutes in 1:3 Clorox solution, air-dried for 5 minutes, and placed in polyethylene bags in an ice chest. The following day, samples were carefully shelled in the laboratory and microfioral analyses were made by standard serial dilution technique. In addition, 50 peanuts (100 kernels, 100 pod halves) from each treatment were placed on Czapek's agar containing 20% sucrose.

Microfloral data showed that Aspergillus flavus Link ex Fries was present only in the overmature peanuts. Plating studies revealed that 2% of the mature and 8% of the overmature kernels were infected with A. flavus. Of the pods (hulls) plated out, 1% of the immature, 9% of the mature, 55% of the overmature, and 6% of the 3-day-stored lots were invaded by the fungus. Thus, there appeared to be an increase in A. flavus invasion of peanuts with time as measured by maturity of the peanut fruit.

Thin-layer chromatography analyses of extracts from overmature kernels and the shells gave a high level of blue fluorescing pigments with an Rf value of about 0.7. This compound may or may not have been aflatoxin. The Rf value was slightly high for aflatoxin. At the time of the analyses, no internal aflatoxin standard was available, so the results were somewhat inconclusive. Peanut meal prepared from the overmature kernels when fed to ducklings failed to be either acutely toxic or to produce liver abnormalities. Probably insufficient aflatoxin was present in the small sample of overmature peanuts to affect the ducklings. However, when the strain of A. flavus from these peanuts was grown on sterilized kernels in the laboratory, the extract gave positive determination (strong blue fluorescent at Rf 0.416) for aflatoxin, indicating that a toxin-producing strain was present.

## TESTS ON PEANUT MEALS WITH RATS

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Peanut meal made from sound nuts is one of the top vegetable protein supplements in nutritive value. Some of the first research that I did after joining the Experiment Station staff at Auburn in 1922 was with peanut meal as a protein supplement to corn for finishing hogs. A ration containing corn and 20% of high protein peanut meal produced as rapid and efficient gains as a corn and tankage ration, provided that yellow corn was used and the ration was adequately supplemented with minerals. Moreover such a ration produced firm pork instead of the soft pork that was produced when hogs were fed peanuts which were the chief fattening ration used for hogs in the Southeast at that time.

In research on choline deficiency starting in 1940, we used a basal diet containing 25 to 35% of peanut meal and 6% of casein. Both the peanut meal and the casein were extracted with ethyl or methyl alcohol to remove the choline-containing phospholipid. This diet produced excellent growth in rats when supplemented with choline. If not supplemented with choline, it resulted in a high rate of mortality in weanling rats in 8-15 days. If sufficient choline was supplied to prevent this high mortality during the early part of the experiment and then gradually withdrawn from the diet, rats would develop a significant incidence of liver cirrhosis and liver cancer in 50-70 weeks.<sup>1</sup> Control rats fed the same diet supplemented with adequate choline for a similar period of time did not develop either cirrhosis or cancer of the liver.

An experiment completed in 1959, however, presented some very perplexing results. In that experiment 40 rats were fed a diet similar to that used in our choline-deficiency studies except that dried lean beef instead of casein was used with extracted peanut meal as the source of dietary protein; 11 of these rats developed hepatoma (liver cancer) despite the fact that the diet contained adequate choline. In this experiment, 50 rats of the same strain and age received a higher level of lean beef without peanut meal in the diet; not one of these developed a hepatoma. The results clearly indicated the presence of a low level of a tumor-producing agent in the peanut meal used in this experiment.

We then started experiments with peanut meal (Code No. P.M. 1) from the same source without extracting it with alcohol in our laboratory. This was fed at a level of 33.3% in diets containing 7.9% of dried beef or commercial casein. Of 63 rats fed these diets, 55 developed liver cancer.<sup>2</sup> The incidence of tumors, therefore, was 87.3% when the diets contained the commercial peanut meal compared with 27.5% when methanol-extracted peanut meal was used.

While these experiments were in progress, outbreaks of "Turkey X" disease occurred in England and was reported to have caused the loss of over 100,000 turkey poults in 1960. British workers traced the cause of this malady to diets containing certain lots of peanut meal imported from Brazil, Africa and India.3.4 Extracts of the toxic meals prepared by British workers were found to be acutely toxic when tested on ducklings. The finding of dead fungal hyphae in some of the toxic meals led to isolation of a toxin-producing Aspergillus flavus from toxic peanut kernels obtained from Uganda. This toxin was named aflatoxin. Results from the British laboratories published in late 1961 led us to suspect that the carcinogen in the peanut meal used in our experiments was probably of fungal origin. When this meal was fed to ducklings at a level of 50% in the diet, however, it produced no symptoms of acute toxicity. In contrast, a sample of Brazilian peanut meal (Code No. P.M. 12) made available through the courtesy of the British Oil Mills, killed 100% of ducklings in 1-3 days when it was fed at the same level in the diet. It produced symptoms of acute toxicity at much lower levels in the diet as will be shown by Dr. Prickett in the next paper, Chemical assay for aflatoxin on a sample of the domestic meal (Code No. P.M. 1) used in our rat experiments was then made at the Tropical Products Institute of London through the kindness of Drs. Feuell and Nesbit. It was estimated to contain about 0.20 ppm of aflatoxin B1. More recently I have received from the U. S. Food and Drug Administration an assay on this lot of peanut meal by procedures developed in their laboratories; this showed 0.30 ppm of aflatoxin B<sub>1</sub> and 0.15 ppm of B<sub>o</sub>. They also found 0.25 ppm B<sub>1</sub> and 0.15 ppm B<sub>o</sub> in another sample of domestic meal that had proved to be carcinogenic in our laboratory. A third sample of domestic meal assayed 1 ppm of aflatoxin  $B_1$  by the British laboratory and 0.75 ppm  $B_1$  plus 0.50 ppm  $B_2$  by the F.D.A. laboratory. This meal produced symptoms of acute toxicity in ducklings and induced massive tumors in rats in our laboratory. It should be noted that the independent assays by the two laboratories were in excellent agreement considering the small amounts of toxin present; they also were in agreement with the biological tests in our laboratory. It is apparent that, if aflatoxin is the only carcinogen present, it is an extremely potent one.

The peanut meal (Code No. P.M. 1) used in our experiments with rats actually represents 4 different lots of meal totaling 16,000 lbs. obtained from the same

source over the period 1958 to 1961. A large portion of this meal was used iu our basal diets for studies of choline deficiency; this was subjected to extraction with methanol in our laboratory. This extraction, if doue in accordance with our standardized procedure, removes all of the tumorigenic agent from the meal. However, experiments with this meal without the methanol extraction have been continuously in progress from 1959 through 1963. All lots have consistently produced a high incidence of hepatomas. The first tests on ducklings aud the aflatoxin assays were made on a sample from a lot of 6,000 lbs. obtained July 13, 1961. This was a solvent-process meal made from shelled nuts and had a protein content ranging from 56-57%. It had the appearance of an exceptionally high quality meal. When it furnished 75% of the protein in the diet for rats, it produced an excellent rate of growth from weaning to normal mature weight. After the tumors developed to the extent that they involved a large portion of the liver, there was usually a decline in weight. Occasionally, a rat succumbed without any appreciable loss in weight. The time required for the tumors to reach this stage of development has varied from 180 to over 500 days, depending primarily on the level of tumorigenic agent in the diet.

We have couducted long-term experiments with rats on 16 lots of domestic peanut meal from 6 different sources. Only 3 lots did not produce hepatomas in rats. In cooperation with Dr. Diener and Dr. Davis we have shown that toxin producing strains of Aspergillus flavus are found on peanuts in this country. It is evident that this is not just a problem that concerns foreign countries. It is of immediate concern to everyone interested in agriculture, food industry and public health in this country. Fortunately, we have not had any such disaster as was experienced by the turkey producers in England in 1960 but we could, unless we carefully guard against it. Losses of cattle, horses and pigs as a result of feeding moldy feeds have already been reported in this country. 5,6,7. It is not a problem that calls for alarm on the part of anyone. It is a problem, however, that concerns everyone interested in our agricultural and industrial economy and in public health. It is not a simple problem and we do not have nearly all of the answers to its complexities and their solution. It is a problem that embraces a broad spectrum of food and feed crops. However, this does not lessen the responsibility of anyone connected with the peanut industry. The ideal is that such careful attention will be given to every step in the production, harvesting, curing, storing and processing of peanuts that there will be no mold growth at any stage. The ideal can seldom be attained. This makes it essential to improve procedures of grading, culling and inspection of all peanuts going juto food for human consumption or feed for livestock to insure that fungal toxins or carcinogens are not present. It does not solve the problem to pick out damaged nuts from stock for the edible trade, if these damaged pick-outs are converted into peanut meal for livestock feeding. Moreover, if peanut hulls are used to dilute peanut cake, they should be thoroughly examined to insure that they are free of toxic materials.

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## THE TOXICITY OF FOREIGN AND DOMESTIC PEANUT MEALS TO DUCKLINGS

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In continuation of previous studies (1) of the toxicity of peanut meals in the rat, tests were begun in Pekin ducklings because of the reported high susceptibility of this species to the toxins (Aflatoxin\*) of Aspergillus flavus (2). Ten samples of peanut meal have been studied to determine the toxicity and carcinogenicity associated with long-term feeding of diets containing varying quantities of the test samples. These tests extended over periods of one week to forty-seven weeks depending on the toxicity or the amount of toxic meal in the diet. Table 1 demonstrates the gross pathology observed at necropsy as well as the average survival time of ducklings fed the test materials,

Sample number 12 (P.M. 12) represents the most toxic meal tested. Its acute toxicity is so profound that it was necessary to reduce the dietary level to 3% in order to obtain any estimate of chronic effects associated with its use. By comparison, P.M. 10, at 50% in the diet, showed no gross evidence of thmors after 25 weeks on test.

The gross pathology observed at necropsy varies with the time on experiment as well as with the toxicity and quantity of the individual peanut meals. (Table 1). In general, the changes observed consisted of enlargement of the liver with or without evidence of tumors. The tumors observed varied from small (1-3 mm) solid tan-white focal masses to very large solid tumors which essentially filled the abdominal cavity. Other tumor types consisted of irregularly shaped areas filled with blood which are suggestive of hemangiomata and cystic areas of varying size which contained clear green-tinged fluid suggesting a connection with the biliary system. Metastatic tumors or primary tumors in organs other than the liver were not commonly seen, but did occasionally appear in the lung and thymus.

Among the most consistent findings is a massive pericarditis with effusion and adhesions and peritonitis. These observations include animals subjected to acute as well as chronic regimeus, and suggest the presence of a live infectious agent. This suggestion is strengthened by the isolation of live fungi from many of these animals.

Associated with both the acute and long-term tests was an enlargement and increased firmness or, iu many cases, a distinct hardening of the liver, pancreas and kidney. In many instances the liver was so rubbery in consistency that it could not be crushed between the fingers and the pancreas had the consistency of a piece of celluloid plastic. Preliminary histological study indicates that these organs are unfiltrated or, at times, eucapsulated with a "collageuons" matrix whose nature has not, as yet, been determined. The association of this type of change with the toxic components of the peanut meals is further confused by literature reports associating similar fludings with the feeding of a Purina Duck

<sup>&</sup>lt;sup>2</sup> Aflatoxin is the generic name given to the potent toxins associated with contamination of peanut meals with Aspergillus flavus. They have been reported to be coumarin analogs and can be detected by fluorometric methods (3) (4).

D:-+	No.	P.M.	Per- cent	Average time on	Liver Tumors*		- Peri- Per		"Co.	llagen Change	ous" e
Diet	ducks	used	in diet	ment (weeks)	Solid tu- mors	Cystic tu- mors	tonitis	car- ditis	Liver	Kid- neys	Pan- creas
D- 1	5	1	50	37	4	4	5	4	3		3
D- 2	5	2	50	43	4	5	4	4	3	3	4
D- 3	<b>5</b>	3	50	5	2	2	4	3	2	1	1
D-4	5	4	50	82	4	1	2	3	2	2	2
D- 5	5	5	50	47	2		3	3	I	2	4
D- 6	4	6	50	29	3	1	1	-	1		1
D- 7	4	10	50	25			3	3		1	
D- 8	5	11	50	82	1		2		.1	2	4
D-11	5	12	5	5	3		4	4	3	5	2
D-12	7	12	10	3	3	-	4	3	3	4	2
D-13	7	12	15	1	1						
D-14	7	12	20	1	-		-				
D-15	9	12	30	1							
D-16	9	12	40	1		-					
D-17	9	14	30	3	1		2	2			1
D-18	8	14	33	28	1		2	2		T	1
D-19	8	3	20	26	6	3	6	5	4	2	6
D 00	•	14	33	~~	0		0	~	-	0	2
D-20	8	3	33	22	6	2	8	5	5	6	5
D-21	8	12	3	29	6	3	8	5	4	3	5
D-22	10	12	5	12	0	3	8	5	3	3	6
D-23	10	12	1.5	11	9	2	Ø	0	0	ې	0

TABLE 1.	CHRONIC	Tox	TUCITLY	OF	FOREIGN	AND	DOMESTIC	PEANUT
	MEALS	TŬ	DUCK	s—	NECROPSY	FIN	DINGS	

\* The term "tumor" as used herein should not necessarily be construed to be synonymous with neoplasm or cancer. The majority of tumors must still be classified histologically as granulomata.

Growena diet to untreated Pekin ducklings (5) (6). Review of these reports, however, suggests that the possibility of toxin-contaminated grains or of live fungal contamination has not been sufficiently considered in arriving at these conclusions.

Studies of the relative toxicity of Foreign and Domestic peanut meal samples have indicated the desirability of having an assay method which would allow rapid and accurate indgment of the toxicity of any given sample.

A preliminary test has been carried out in week old Pekin ducklings (the most sensitive animal species) comparing four peannt meals representing samples which had previously been studied in more chronic tests in both rats and dncklings.

Comparisons were made after 2, 4 and 6-day feeding periods. While other tissues were saved for study, this report restricts itself to the gross pathology observed at necropsy and histological findings in the liver.

While further work is necessary to specifically define the range of variability and to determine whether specific titrations can be made with accuracy, this test has demonstrated that histological changes occur as early as the second day after the beginning of treatment. In addition, sufficient variation in the time, type and intensity of reaction are apparent in different test groups to strongly suggest that the relative toxicity of individual samples can be jndged.

While we do not feel, at this time, that the toxin content of the sample is, necessarily, the only basis of judgment of the toxicity of the test meals, our results have compared favorably with those reported by English workers attempting to determine the Aflatoxin content of peanut meal samples.

Diet	Species	P.M. used	Number of animals cultured	Number of positive cultures
D- 1	Duck	I	3	2
D- 2	Duck	2	4	3
D- 4	Duck	4	2	0
D- 5	Duck	6	4	1
D- 6	Duck	7	1	0
D- 7	Duck	10	1	I
D- 8	Duck	11	2	2
D-18	Duck	14	2	1
D-19	Duck	3 & 14	3	8
D-20	Duck	3	3	2
D-21	Duck	12 - 14	4	· 2
D-40	Duck		2	1
$D-40(R_1)^2$	Duck		1	0
$D-40(R_2)^2$	Duck		1	0
$D-40(R_{3})^{3}$	Dnek		3	3
Purina	Duck		5	3
D-43*	Duck	-	1	1
D-46	Duck	Semi-		
		synthetic	10	10
- D-47	Duck	Semi-		
		synthetic	5	5
D-48	Duck	14	5	5
D-49	Duck	soybean	5	5
	Baby <sup>5</sup> ducks	2	10	10
1404	Rat	12	5	8
Purina	Rat		2	1

TABLE 2. TOXICITY OF FOREIGN AND DOMESTIC PEANOT MEALS TO DUCKLINGS AND RATS ISOLATION OF FUNGI FROM TISSUES AND BODY FLUIDS

<sup>1</sup> Received a single oral dose of 5 ml, of a 1:20 aqueous suspension of mycelia of Aspergillus flavus.

<sup>2</sup> Received a single oral dose of 5 ml. of a 1:20 corn oil snspension of mycelia of Aspergillus flavus.

<sup>8</sup> Received a single intraperitoneal injection of 5 ml. corn oil-water suspension of A. *flavus* spores. (Spore connt = 750,000 per ml.)

Fed diet containing 3% of live fungus grown on shredded wheat for 4 days, thereafter fed basal diet.

<sup>5</sup> Day old ducklings cultured immediately on receipt from Vendor. No laboratory contact.

No single set of criteria can be used to judge the relative toxicity of the test samples since the gross and microscopic changes are the product of time, concentration of the sample in the diet and/or the inherent toxicity of the sample. Based on this preliminary experiment the following sequence of events reflect the probable process by which toxicity is expressed:

1. At necropsy, the earliest detectable changes are characterized by circulatory phenomena such as sinusoidal dilation in the liver, hydropericardium, pericarditis, peritonitis and renal edema whose etiology suggests either a hypersensitivity reaction due to a toxin, residues of fungal protein and/or the early stages in the establishment of an infection. By the sixth day, the above phenomena are replaced, in part, by decreased size of the liver, pallor and increased firmness to palpation in liver, pancreas and kidneys which suggest the "collagenous" change observed in ducks on more chronic regimens.

2. Microscopically, the hepatic tissue has three major types of reactions which apparently occur in sequence. First, a period of stimulation in which there is an obvious increased reactivity of defense mechanisms (recticulo-endothelial system) such as Kupffer cells, littoral cells and lymphocytes, increased

mitotic activity of periportal tissues and fluid balance shifts indicated by edema.

Secondly, proliferative phenomena occur which can be seen as early as the second day. The major expression of this phase is shown by increased bile duct-cell proliferation (cholangioles), foci of proliferation of reticnlo-endothelial elements within the lobule and later the presence of an abnormal number of fully-formed bile ducts.

Thirdly, indications of cytological disturbances among liver cells include vacnolization (non-lipid), necrosis, occasionally liver cell mitosis suggesting repair, hemosiderin deposition and a lymphocytic reaction suggesting the establishment of a chronic state.

While all above changes are suggestive of the probable natural history of the toxic reaction, much more work is necessary to specifically define them in terms of the pathogenesis of the disease.

Ducklings and rats fed peanut meal suspected of containing toxins resulting from previous contaminatiou with fungi have consistently demonstrated structures within the vasculature and parenchyma of the liver, spleen, kidneys and pancreas which have the morphological appearance of mycelia, conidia or microsporangia typical of Aspergilli or Monilia. On a few occasions definite histological evidence of fungal colonies have been observed in tissnes.

Review of a number of slide preparations of rat tissues from experiments using toxic peannt meals carried out by Dr. Salmon several years ago have demonstrated similar structures. Ducklings on acute toxicity tests have shown evidence of these structures after two days on experiment, and similar findings were observed in the tissues of ducklings from more chronic tests extending over 50 weeks or more. In addition, tissues from control animals housed in close contact with peanut meal-fed animals for extended periods have demonstrated similar changes, but with less frequency, and, of course, without tumors.

Since these observations have only recently been made, a minimal amount of data has been accumulated by which to judge the extent of these findings or to enlure tissues to determine whether they represent viable fungi. Table 2 demonstrates the incidence of positive cultures from various organs and body fluids of suspected animals. Mycological tests were usually carried out in duplicate on Sahouraud No. 1 and No. 2 media, modified Czapek-Dox, Candida medium and Corn Meal Agar. Some of these media contain antibacterial substances to inhibit bacterial growth.

While a specific identification of the fungi has not been made, a large number of contaminants have been characterized as Aspergilli or Monilial species. No bacterial colonies were seen.

The importance of more specific delineation of this problem lies in definitely showing whether the problem of toxicity of peanut meals, cottonseed meals and, most probably, all cereal grains and by-products lies not only in (1) the possibility of toxin contamination, (2) the possibility that a hypersensitivity (Shwartzman) reaction occurs or (3) that live fungi or spores are present which can infect the host when these ingredients are included in the diet. A more remote, but potentially important possibility, is the activation of an existing floral fungal population by the toxin.

Further tests are being planned to more critically evaluate the problem on both a mycological histological and histochemical basis.

Demonstration and further explanation of the results of research described herein was made with photoslides of the gross and histopathological changes observed.

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## POST-HARVEST APPEARANCE OF AFLATOXINS

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The problem of the elaboration of toxic products by fungi growing on food and feedstuffs is part of the overall problem of quality maintenance in the postharvest period. In a general way, we already know how to prevent loss and spoilage in field crops after harvest. The main factors to be controlled are listed in Table 1. Moisture content of the commodity is the key factor and the others interact with it in various ways.

TABLE 1. IMPORTANT FACTORS IN QUALITY MAINTENANCE OF FIELD CROPS

Moisture Content Maturity Harvesting Practices Foreign Matter Insect Infestation Artificial Drying

If we know in general how to prevent loss and deterioration after harvest, why are we having this difficulty with the common storage mold, Aspergillus flavus, now known to have toxin and carcinogen producing strains? Where in the marketing chain from harvest to grading station, to drying facility, warehouse, shelling plant, do certain strains of this organism become active and produce aflatoxin?

I should like to examine and discuss with you the available published data and the very limited information from our own studies on this subject.

Mold growth occurs in peanuts when their moisture content is maintained at 9% or higher. Uneven moisture distribution among kernels may promote mold growth even when the average moisture content is at a safe level. Damage to shells caused by improper harvesting provides an easy entry for fungi. Foreign matter is exceedingly attractive to stored product insects, and insect infestation

	Days from lifting	Toxie samples	A. flavus contaminated
		%	9%
At lifting After windrowing	3-11	3/351* 0/48	1/50* 3/42*
After sun drying	11-24	13/118	30/51

TABLE 2. AFLA	TOXIN DEVELOPME	NT AFTER HARVEST	(5)	)
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\* These positive samples were from broken or insect-damaged pods.

is almost always accompanied by fungal invasion. The growth and metabolism of insects can provide environmental conditions suitable for mold growth.

Whether artificial drying plays a role in suspectibility to mold damage in peanuts is not as yet known. Artificially dried corn, according to reports in the literature, molds more rapidly than naturally dried corn (1). This effect has been attributed to the demonstrated fact that artificially dried corn supports a higher equilibrium moisture content than corn dried at room temperature (2). For a given relative humidity the former will have a higher moisture content than the latter. Hence, a moisture content considered safe for naturally dried corn may be nusafe for artificially dried corn. The latter should be stored at 0.5 to 1% lower moisture than naturally dried corn to prevent mold development (2).

Available information on the appearance of aflatoxin in peannts after harvest originates almost entirely from work done the past two or three years in Nigeria and Senegal, Africa. Investigators here usually worked cooperatively with personnel of the Tropical Products Institute in London and the Pest Infestation Laboratory, Slongh, England.

Let ns look first at data on aflatoxin development immediately after harvest. A report from Nigeria (3) indicates that, except on kernols from broken pods, toxin does not appear until at least six days after lifting. Of 400 samples examined for toxin after lifting and windrowing, only three showed any toxin (Table 2). The toxic kernels were present in broken pods but by no means did all damaged pods contain toxin. A total of 2,400 kernels from damaged and broken pods was examined in the study but only 56 kernels were attacked by A. flavus.

The results given in Table 3 summarize our limited test conducted last fall at the Peannt Research Station, Lewiston, N. C., relative to incidence of mold damage immediately after harvest. Mr. J. W. Dickens originated the test and Dr. U. L. Diener, Auburn University, ran the microfloral analyses.

All field tests (designated by plots 2, E, and tent 5) consisted of 2 windrows of peannts, the inverted and the random. Both windrows were dug and formed using a conventional shaker-windrower. In the inverted windrow the plants were placed (by hand) in an inverted position so that the peanuts would be on top of the windrow. Both the inverted and the random windrows in each plot were dug the same day, snbjected to the same natural weather conditions, and then harvested at the same time.

The weather experienced by plot 2 was mostly clear and warm. The only rainfall was 0.21 inch on the fifth day. The weather while plot E was in the windrow was cool and cloudy, with rainfall of 0.61, 1.77, and 0.60 inches on the third, sixth, and seventh day in the windrow, respectively.

For the third test, a simulated bad weather test (designated by tent 5), the peanuts were moved to tents set up near a water supply. The tents provided shade and a sprinkler hose was used to wet the windrows. Each test contained two windrows, designated "inverted" and "down." The inverted windrow was the same as in the field test. The down windrow was formed with the peanuts on the bottom of the windrow, allowing most of them to be in contact with the ground. The samples in tent 5 were wet thoroughly on the fourth, sixth, seventh, and minth day after digging. All samples were harvested with a windrow combine and cured using unheated air.

The data of Table 3 agree with the Nigerian findings in that A. flavus contamination may not occur for an appreciable period after digging. Another point that emerges from our data is that the amount of damage as determined by official inspection did not correspond to the amount of mold present.

The data also indicate that the amount of mold on the hulls does not necessarily correlate with the amount of mold on the kernels. Dr. Diener has pointed out that the mold count on the kernels in plot 2 was probably a result of contamination during the shelling operation despite special precautions taken in

Treatment	Date	Date	% Mois- ture	% K	ernel dan	nage <sup>1</sup> , <sup>2</sup>	M coloni	lold <sup>a</sup> es/gram	Dominant fu	ingus species	Traces of Asper-
	dug		harvested	Int.	Ext.	Total	Kernels	Hulls	Kernels	Hulls	flavus
Plot 2—Inverted • Random	Oct. 4 Oct. 4	Oct. 15 Oct. 15	7.9 15.3	.46 .15	1.29 1.47	$\begin{array}{c} 1.75\\ 1.62 \end{array}$	87 120	13,834 12,500	P1,P3,C P1,P2,P3 A1,A2	P2,P3 P2,P3,M	x
Plot E—Inverted Random	Oct. 30 Oct. 30	Nov. 14 Nov. 14	10.1 19.0	$\begin{array}{c} 1.16 \\ 2.54 \end{array}$	$\begin{array}{c} 1.07\\ 4.77\end{array}$	$2.23 \\ 7.31$	$2800 \\ 487$	4,134 28,334	Р2 Р3,М,С	C M,C	x
Tent 5-Inverted	Oct. 1	Oct. 16	18.4	.25	1.92	2.17	1013	158,667	P2,C1,C P3 M	C,M	x
Down	Oct. 1	Oct. 16	38.0	.60	2.62	3.22	287	32,334	P,C,M	С	x

TABLE 3. EFFECT OF POST-HARVEST FIELD CONDITIONS ON MOLD DAMAGE

<sup>1</sup> Determined by Federal-State Inspection Service.
 <sup>2</sup> Percentages based on total kernel weight.
 <sup>3</sup> Average of 2 replications.
 <sup>4</sup> Notations refer to the following species: A—Aspergillus, 1 glaucus, 2 niger; C—Cladosporium, 1 tricothecium roseuu; M—Mucorales; P—Penicillum, 1 citrinium, 2 funicolosum, 8 janthinellum.

Tt	Samples			
Item	Total	Fluoresceut		
No rainfall 48 hours before barvest	20	2		
Rainfall 48 hours before harvest	3	1		
Rainfall between the 1st and 5th day after harvest	11	6		
Rainfall more than 5 days after harvest	10	10		
Two rainfalls, one within 5 days after harvest				
and the other after 5 days	4	3		

TABLE 4, EFFECT OF RAINFALL BEFORE AND AFTER HARVE	ŝт (4
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shelling. It is interesting to speculate whether such contamination would occur in commercial shelling operations.

It is also noteworthy that, of the windrows which were exposed to bad weather conditions (plot E and tent 5), the inverted windrow, which allowed the peanuts to dry more rapidly, also had the most mold on the kernels. Diener suggests that the molds would grow most readily on peanut kernels between 10-18% moisture coutents. The inverted peanuts stayed in this range longer than the random or down treatments which never got down to 18% moisture before harvesting and then were dried out to 8% moisture quite rapidly in the curing bin.

Rainfall increased the total amount of mold on the windrowed peanuts. A report from Scnegal (Table 4) shows that rainfall after digging promotes the formation of the toxic substances (4).

Contrary to what might be expected, this report also states that there was no relation between aflatoxin content and degree of maturity, using the number of shriveled kernels as a maturity index. According to the Nigerian investigators (3), A. flacus does not grow readily on immature pods and kernels but quickly invades old pods and their kernels under humid conditions. They are of the opinion that freshly harvested mature pods, while they are still alive and attached to the stems, may have resistance to penetration by A. flacus. The kernels may likewise be unattacked until the seed has passed into a low metabolic state as a result of moisture loss.

Going one step farther in the marketing system, what is the aflatoxin picture at the buying and grading station? There is no definitive information on the situation in this conntry. The annual quality survey (Table 5) shows the average kernel damage on a yearly basis. This is an upper limit for mold damage; the actual amount of the latter could be appreciably lower. Similar data for wheat are included in this table for comparison. It is quite evident how much more severe the problem of deterioration and damage is in the peanut industry.

Discoloration is a common cause of damage in grading domestic peanuts.

Commodity	Year	Damaged kernels	Shrunken broken	Foreign matter & dockage	Loose shelled kernels
		%	70	70	%
Peanuts —runner —SE Spanish —SW Spanish —Virginia —Valencia	1952 <b>-6</b> 2	$1.57 \\ 1.82 \\ 1.15 \\ 1.52 \\ 1.44$		$\begin{array}{c} 4.23 \\ 4.53 \\ 6.76 \\ 2.95 \\ 10.09 \end{array}$	$2.76 \\ 4.99 \\ 2.56 \\ 1.84 \\ 6.22$
Wheat —hard winter	1962	0.3	1.06	0.48	
hard spring		0.4	1.0	1.55	

TARLE 5. QUALITY SURVEYS (5)

	Composi	te sample	Aver	C			
Type aud grade factors	At purchase	When loaded in	First sample from bins	Winter	After 6 months' storage	After 8 months' storage	sample when loaded out
	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Southeast Runners: Sound mature kernels Damaged kernels Kernel moisture	66.5 .8 10.0	66.6 .8 9.2	$66.0 \\ 1.1 \\ 7.2$	$     \begin{array}{r}       65.3 \\       1.4 \\       6.7     \end{array} $	$66.8 \\ 1.3 \\ 5.6$	$63.9 \\ 1.5 \\ 5.2$	$     \begin{array}{r}       65.2 \\       1.7 \\       5.3     \end{array}   $
Southeast Spanish: Sound mature kernels Damaged kernels Kernel moisture	$70.1 \\ 1.8 \\ 8.6$	69.2 2.2 7.6	69.0 2.3 6,4	$     \begin{array}{r}       68.4 \\       2.5 \\       5.9     \end{array} $	67.9 2.9 5.6	$     \begin{array}{r}       64.7 \\       3.5 \\       6.0     \end{array} $	67.6 2.7 5.2
Southwest Spanish: Sound mature kernels Damaged kernels	$\begin{array}{c} 66.2\\ 1.1\\ 9.1 \end{array}$	$65.9 \\ 1,1 \\ 8.6$		65.9 1.5 5.9	$64.9 \\ 1.4 \\ 5.5$	$66.7 \\ 1.4 \\ 4.9$	$\begin{array}{c} 65.8 \\ 1.5 \\ 5.3 \end{array}$
Virginia type: Sound mature kernels Damaged kernels Kernel moisture		65,9 1.3 9.6	65.6 1.4 8.6	$65.4 \\ 1.5 \\ 8.1$		62.2 3.9 6.8	63.7 3.5 6.7

TABLE 6. AVERAGE CHANGE IN GRADE FACTORS OF FARMERS' STOCK PEANUTS DURING BULK STORAGE, BY TYPE OF PEANUTS, 1952-56 CROP (6)

TABLE 7. RELATION OF AFLATOXIN TO FAT ACIDITY (4)

Fat acidity	% Samples containing aflatoxin
< 0.3	8
0.31-1.0	80
> 1.0	70

The Senegal investigators observed that growth of A. flavus on peannts causes some discoloration of the kernels. The discolored seeds showing yellow spots and yellow mold have the highest aflatoxin content, from 20 to 40 p.p.m. Aflatoxin was also associated with white and blackish seeds. A positive correlation was noted between percentages of discolored seeds and the fluorescence test for aflatoxin (4).

Samples containing aflatoxin contents greater than 1 p.p.m. had significantly moister shells than those on which aflatoxin was absent. On the other hand, the Nigerian investigators (5) reported that the incidence of affatoxin in bags of peanuts at time of delivery to stacking areas from buying stations is extremely variable and not correlated with moisture content.

The final marketing step I shall consider is storage. Table 6 is a composite of results summarizing storage of farmers' stock peanuts in different types of bins over five crop years (6). The data show that, in general, sound mature kernels diminish and damaged kernels increase over the storage period. Again we cannot categorically state that this is mold damage. There is indirect evidence that it is such damage, in part. Fat acidity determinations made on some of the crops reported in Table 6 showed an upward trend during the storage period. Fat acidity correlates very highly with mold growth and rather poorly with other major causes of damage such as frost or insect infestation (7). For all bins of the 1952 crop this factor increased from 0.41 to 0.79% for southeast Spanish and from 0.22 to 0.39% for southwest runners. The increase was greatest in bins receiving peanuts with initial moisture contents greater than 10%. The Senegal workers (4) reported a correlation between fat acidity and aflatoxin as shown in Table 7.

We stated at the outset that the general principles for preventing mold dainage were known but it is evident from this brief review that, for peanuts at least, we shall need refinements in practice and safeguards at each step in the marketing process to overcome the aflatoxin problem,

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## **REVIEW OF RESEARCH ON AFLATOXIN AT THE SOUTHERN REGIONAL RESEARCH LABORATORY**<sup>1</sup>

### LEO A. GOLDBLATT<sup>®</sup>

We have been exceptionally fortunate in the past two years because the Congress has granted increased appropriations for our research ou peanuts at the Sonthern Regional Research Laboratory. Before that, for a number of years, we had only a very small research program in New Orleans on peanuts. Our total expenditures for peannt research during that time amounted to only about \$70,000 a year. These welcome increases have permitted us to expand materially our research on peanuts. In addition, because of the unanticipated urgency of the aflatoxin problem, our administrators in Washington have made available to ns during the past year sums from a contingency fund for the purchase of special needed equipment and supplies and employment of temporary personnel. These increases have permitted a rapid expansion of our research program on peanuts. Much of our increased funds have been used for contract research. The increase last year permitted us to initiate research in New Orleans on the isolation and identification of nonglyceride but lipid soluble components of peanuts and processed peannt products and to let a research contract for an investigation of the flavor and aroma components in processed peanut products with the Evans Research and Development Corporation. This year it permitted us to let a contract with Oklahoma State University, Agricultural Experiment Station, for a study of the relation of the carbohydrate, amino acid and protein components of the peanut to the formation of flavor and aroma during roasting.

Also it has made possible immediate initiation of urgent research on the aflatoxin problem in our laboratories as well as implementation of contract research in this area. As of now we have completed arrangements for two contracts for research on aflatoxin; others are under consideration as we develop onr contract program for FY 1965 and active consideration is being given to research relating to aflatoxin with P. L. 480 funds.

One of the two contracts for which negotiations have been completed is to be conducted right here at Anburn under the general direction of Dr. Urban L. Diener with Dr. Norman D. Davis as co-principal investigator. The stated objective of this coutract is: "To develop information on the limiting environmental conditions for the elaboration of toxic fungal metabolites in both intact aud shelled peanuts," The other contract is with Texas Agricultural Experiment Station, Texas A&M University, at College Station, Texas, under the general direction of Professor Carl M. Lymau and with Professor Raymond Reiser as project leader. The contract has for its stated objective: "To develop information relating processing methods; preprocessing history, distribution of immature, mature, and germinating peanuts; and external conditions such as mold incidence.'

At the Southern Regional Research Laboratory we now have a rather extensive program on aflatoxin but we sort of backed into the problem. Of course we became aware of the problem shortly after the British published their reports in 1960, but we did not do any experimental work ou it until last year. We sort of rocked along with the notion that the contamination was due to poor technology aud "it couldn't happen here." Then last year two thiugs happened almost simultaneously that really got us started. The first thing was a report in April 1963 by a research group in California that they had found that a specific feed used for trout had produced a high incidence of hepatoma iu hatchcry trout and that cottonseed meal was implicated. At the time there was some

<sup>&</sup>lt;sup>4</sup> Presented at the Third National Peanut Research Conference, Auburn, Alabama, July

 <sup>&</sup>lt;sup>9</sup> Flocking at the right radiational result research conference, Aubini, Alabana, July 9-10, 1964.
 <sup>9</sup> Southorn Regional Research Laboratory, New Orleans, Louisiana, one of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

question as to the validity of the findings and if indeed cottonseed mcal was the culprit. But if it was, then certainly the possibility that it might be due to aflatoxin had to receive serious consideration. Then in that same month, Dr. W. D. Salmon from here in Auburn reported at The American Institute of Nntrition Section of the Federation of American Societies for Experimental Biology at their meeting in Atlantic City, that a domestic commercial peanut meal, when fed to rats, produced a high incidence of hepatomas. Professor Salmon noted the possibility that solvent residnes might be involved in the carcinogenic effects observed but again, of course, the possibility that aflatoxin was responsible had to receive scrious consideration. So here we were, bombed almost simultaneously from both coasts, California and New Jersey, on two of onr most important commodifies and we got to work on both of them promptly,

Our first experimental work relating to aflatoxin was actually with cottonseed and cottonseed meal to see if we could detect aflatoxin or any of the mold (Aspergillus flavus) which produces aflatoxin. Also, our Engineering and Development Laboratory extracted about 200 pounds of each of five, selected, cottonseed meals to see if we would obtain an extract that would produce hepatoma in trout. Those extracts are now being fed to trout by Dr. John Halver at the Western Fish Nutrition Laboratory of the Department of the Interior in Cook, Washington. I am telling you abont this so you will know that we at the Southern Laboratory are interested in aflatoxin not only as it relates to peanuts but also as it relates to cottonseed. We have a highly trained scientist at the Southern Laboratory devoting full time to the cottonseed-aflatoxin problem. Incidentally, we now know that aflatoxin does indeed produce hepatomas in trout. In fact the trout may be the most sensitive test animal. It has been reported that as little as one part of aflaoxin B, per billion of feed will produce a high incidence of hepatomas in trout.

Most of our work on aflatoxin at the Southern Regional Laboratory relates. primarily to peanuts. One of the first things we did was plan a series of tests designed to obtain information to confirm or disprove the reports concerning domestic peanut meal and to serve as a basis for answering the question as to whether or not a good quality peanut meal in the daily diet of rats produced hepatomas. This plan was developed in discussions with Dr. Floyd DeEds, who is in charge of the Pharmacology Laboratory at the Western Utilization Research and Development Division in California, and with some of our colleagues in Washington, particularly Dr. Sam Hoover, Dr. W. D. Maclay, and Mr. A. M. DuPre'--not to mention our Session Chairman, Dr. G. W. Irving, Jr., who unfortunately couldn't be present. At about the same time I visited Professor Salmon here at Auburn to get at first hand all the information I could concerning his work. During that visit I consulted not only Professor Salmon but also Dr. C. O. Prickett, Dr. U. L. Dicner and Dr. Norman D. Davis, and the Director of the Agricultural Experiment Station, Dr. E. V. Smith. And I want to say that I could not possibly have received a more cordial reception or more cooperation than I got from these gentlemen of Aubnrn.

We finally settled upon long-term rat feeding tests indicated in Table I.

TABLE I. PEANUT MEALS FOR FEEDING TESTS (25 rats of each sex)

- 1. Aflatoxin-contaminated commercial meal
- Meal from highest quality peanuts
   Meal No. 2 + sterilized culture of A. flavus (replicated to permit periodic sacrificing) Meal from "pickouts" Meal No. 1 exhaustively extracted
- 4. Meal from "
- 5.
- 6. Extract from No. 5 in a basal diet

I want specifically to acknowledge the help of Dr. Salmon in having made available to us commercial peanut meals from the same sources he had obtained his meals and the help of Dr. W. K. Bailey first in locating for us a source of high quality peanuts and bird-dogging those peanuts from harvesting through the drying and storage and to Mr. Reed Hutchinson of the AMS for the extra careful handling, shelling and picking of those peanuts all the time maintaining their identity so that we would get the best possible quality of peanuts and know just what peanuts we were getting. If I seem to be overdoing this bit of acknowledging cooperation, I am doing it deliberately because I have been so strongly impressed with the understanding, the spirit of cooperation, and the feeling of a desire to be helpful in our work in this area.

Eventually all the necessary starting materials were obtained, we cultured A. *flavus* on shredded wheat and produced grams of aflatoxin, our Engineering and Development Laboratory prepared the pressed meals from the high quality peanuts and the pickouts and solvent-extracted the commercial peanut meals; we analyzed them all for aflatoxin and for other pertinent characteristics and shipped everything off to Dr. DeEds at the Western Laboratory in Albany, California. He had the rats waiting there and has been feeding them since April. It is still too early to know what is happening and that test will continue for about 2 years.

Another area with which we concerned ourselves at New Orleans was analytical methodology for affatoxin. The fact is that even as of now we do not have what I would call a good method of analysis for affatoxin and it almost seems that each laboratory uses its own variation. One of the major problem areas is right at the start, in the extraction of affatoxin from peanuts or peanut meal. Almost from the beginning methanol has been the only extractant used to remove affatoxin for analysis from peanut meal, although chloroform was also sometimes used. Methanol is a good solvent for affatoxin but it removes a great deal of other material too, and an elaborate separation procedure is necessary afterward to permit accurate determination of the affatoxin content of the extract.

At the Southern Regional Research Laboratory we have for several years been studying the use of an azeotrope of acctone, hexane and water for the extraction of oil from cottonseed. This solvent mixture has a number of advantages over the conventional solvents that are now used commercially to process cottonseed to oil and meal. There is evidence that it is a practical solvent which can be recovered and re-used in the commercial extraction of cottonseed. It occurred

	Primary extract (g.)	Aflatoxin (ppm)
First Extraction Methanol Azeotrope	11.55 3.13	2.5 2.5
Second Extraction Methanol Azeotrope	4.72 0.48	0.5 0.5
Third Extraction Methanol Azeotrope	1.20 0.29	0.02 0.1
Fourth Extraction Methanol Azeotrope	1.06 0.10	None detected None detected
Total Extraction Methanol Azeotrope	18.53 4.00	3.02 3.1

TABLE II. SUCCESSIVE 6-HOUR EXTRACTIONS OF 100 G. PEANOT MEAL



FIG. 1. Thin layer chromatograms of extracts obtained with azeotrope (left) and methanol (right).

to us that the same solvent mixture might be suitable for the extraction of aflatoxin from peanut meal. It turns out that it is indeed a very good solvent for aflatoxin and it has the added advantage that it does not extract as much of the nonlipid constituents from peanut products as does methanol. This is apparent from the data in Table II.

You will note that the rate of extraction of aflatoxin and the total amount of affatoxin found is substantially the same with both methanol and the azeotrope but the amount of extraneous material removed is far less when the azeotrope is used. On complete extraction of aflatoxin nearly 20% of the weight of the peanut meal has been extracted with the methanol while only 4% was removed by the azeotrope, and, of course, this includes the residual lipids in the meal. We have found that the extract obtained with the azeotrope is much easier to clean up in preparation for assay by thin layer chromatography than is the extract obtained with methanol. This is apparent from Figure 1. In the middle section of this slide are shown chromatograms of two standards of fairly pure aflatoxin. On the right are chromatograms of 3 test solutions obtained after extensive purification of a methanol extract and on the left are chromatograms of 3 test solutions obtained after a fairly simple purification of an azeotrope extract of the same peanut meal. You will note that the methanolextract material gives much more streaking and a dirtier chromatogram than does the azeotrope-extract material, despite the fact that it was subjected to a much more elaborate purification procedure. So, we are doing considerable work in the area that I will call development of analytical methodology. That includes study of different extracting solvents, different modes of extraction (we are now getting good results by extraction in a Waring Blendor or on a shaking machine, instead of by means of a refluxing solvent), different modes of purification of the extract and different methods of determining the actual aflatoxin content of the extract. That includes investigation of microbiological and biochemical methods as well as chemical and instrumental techniques.

The azeotrope I spoke about is a ternary mixture composed of very nearly equal parts by weight of acetone and hexane (49%) and 2% water. An azeotrope has certain advantages in processing because, by definition, it distills at a constant temperature (in this case about 48° C) as though it were a single pure compound. However, we are not restricted to using that particular composition and when we extract aflatoxin in a Waring Blendor or on a shaking machine we choose to use a mixture that is richer in water and acetone. Typically we nse a mixture that is composed of about 5% water, 35% hexane, and 55% acetone. We are now investigating four different areas of possible ntility of this ternary solvent mixture. These are:

I. Analysis for aflatoxins

2. Removal of aflatoxins from contaminated peanut and other oilseed meals

3. Conversion of peannts to aflatoxin-free oil and meal

4. Removal of aflatoxin from whole peannts.

The first area I have already discussed. The second we have investigated briefly in the Engineering and Development Laboratory in New Orleans and used it to extract abont 300 pounds of commercial peanut meal for use by Dr. DeEds in the rat feeding tests to which I referred earlier. According to our analyses there was no detectable aflatoxin in the extracted peanut meal we sent to Dr. DeEds. So we feel it can be done but we don't know enough about the process yet to know whether it can be done economically. However, it should be noted that the weight of material extracted with the azeotrope amounted to only about 3% of the weight of the meal used. That includes about 2% of residual lipids in the meal as we don't lose much of the meal in the process. That compares with a loss of about 20%, if methanol is used in the extraction.

The third area is another one we are actively investigating now. As I indicated earlier there is evidence that the azeotrope is a practical solvent for commercial extraction of cottonseed for the production of cottonseed oil and meal. We know that the azeotrope and other ternary mixtures of acetone, hexane, and water can remove aflatoxin from crushed peanuts and peanut meals. The aflatoxin accompanies the oil in the miscella and we have removed aflatoxin completely from oil by normal laboratory alkali refining and bleaching. So it would seem that we have potentially available a practical process for conversion of contaminated peanuts to aflatoxin-free peanut oil and meal. We are now working on the pilot plant scale development of such a process.

The fourth area listed above involves the removal of aflatoxin from whole peannts. The basic thought here is that it might be possible to "wash ont" aflatoxin from the whole peanuts by simple "washing" with a suitable solvent or a solvent to which a chemical is added to destroy the aflatoxin. We are just beginning work in that area.

I would like to mention briefly two additional items. Rather early in our work on aflatoxin we felt the need of a thorough review of the relevant literature. We made such a review and as a byproduct we prepared a Bibliography on Aflatoxin From 1960. This has some 188 references and covers all the literature on the subject through 1963 fairly thoroughly. We plan to revise the bibliography from time to time and copies of the bibliography are available on request.

The other item concerns a snrvey of the prevalence of aflatoxin in peannt stocks in the United States. Actually two surveys were made. In the first survey peannts held in warchouses by the Agricultural Stabilization and Conservation Services were surveyed to determine the extent to which stocks of peannts were contaminated with aflatoxin. In cooperation with the Biometrical Services group in Beltsville a sampling plan was devised to get the maximum amount of information with minimum effort. The plan that was devised called for collecting and analyzing for aflatoxin 137 samples collected from 16 warehouses in 5 states. The peanuts were distinguished as coming from one of 5 states, as one of three types and of four quality classifications. The quality classifications were: sound mature kernels (SMK), other kernels (OK), loose shelled kernels (LSK) and damaged kernels (DK). Each sample was to be assayed for two types of aflatoxin, Type B and Type G. The number of samples was subsequently reduced to 112 but that was the only thing that was reduced. There was no change in the number of states, warehouses, types or quality characteristics.

We have now completed the analysis of all 112 samples for aflatoxin. I do not plan to report the results in detail but it might be desirable to call your attention to two items. First, every sample of Damaged Kernels contained significant amonts of allatoxins. That means that there are toxin producing strains of molds in all the peanut producing areas sampled. You will recall that samples were obtained from 5 states. The peanut mold problem is not confined to only one part of the country. Second, in a few instances we found aflatoxin in a few samples of Sound Mature Kernels, not many, but some. In that connection it should perhaps also be noted that our data indicate that aflatoxin is highly localized. That is, in a given batch many peanuts will not have any detectable aflatoxin but an occasional kernel may have a large amount.

We also participated in another survey of some 150 samples of No. 2 peannts which were in cold storage. In this survey five different groups cooperated in assaying all the samples for aflatoxin by the same procedure. The peanuts were distinguished as one of three types, from two crop years, from about 22 shellers at 36 locations, and of 8 quality classifications such as "composite, as drawn," "composite, Damage removed," "splits, Damage removed," "Large whole, Damage removed," etc. Twenty-one samples were assigned to the Southern Laboratory and we have completed the assay of all 21. I believe the other four cooperators have also now completed the assay of the samples allotted to them.

Finally, although it is perhaps not directly concerned with my topic on Review of Research on Aflatoxin at the Southern Regional Research Laboratory, I believe it is relevant to say that we plan also to include in our studies an investigation of other fungal flora of cottonseed and peanuts to determine if they produce toxins harmful to animals.

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Auburn University, Auburn, Alabama July 9-10, 1964

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