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PROCEEDINGS OF THE FIRST DEFOLIATION CONFERENCE, 29 - 30 JULY 1963

JANUARY 1964

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

PROCEEDINGS OF THE FIRST DEFOLIATION CONFERENCE
29-30 July 1963

compiled by
Vesta Z. Mattie

Crops Division
DIRECTOR OF BIOLOGICAL RESEARCH

Project 1C522301A06101

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[redacted]tion in this document has not been cleared for release to the public.

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FOREWORD

This conference is the first of a proposed series of conferences to bring together all contractors and government personnel who are working in support of the Defoliation Program for the purpose of summarizing findings, discussing problems, and exchanging ideas. The combined efforts of industry and government in discussing problems of mutual interest and exchanging ideas could result in (a) a fresh approach in solving problems and improving programs, (b) new potential herbicides and chemicals of specific value to the military program, and (c) new commercial products for future markets.

This meeting enables industry to keep abreast of our in-house basic research activities. It provides access to scientific papers and data not yet published. It enables government personnel to redefine the objectives of the Research and Development Program on defoliation.

ABSTRACT

This Proceedings of the [illegible] Conference of 1963 summarizes the research program in chemical defoliation sponsored by the U.S. Army Biological Laboratories. Emphasis is given to the agronomic contract program of synthesis and screening of candidate defoliant compounds and contract and in-house basic studies in the mechanism of leaf abscission.

Progress in synthesis and evaluation of defoliant activity of new chemicals is reviewed by contractors affiliated with the defoliation program.

Personnel of the Biological Laboratories and other branches of government report the objectives of the program, supply background information dating from World War II, and discuss phases of in-house research consisting of introductory, primary, and secondary screening in greenhouse and field; aerial application; equipment calibration; taxonomic, biochemical, and physiological investigations; formulation studies; and structure-activity relationship.

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AGENDUM

FIRST DEFOLIATION CONFERENCE
29-30 July 1963

Chairman: Dr. Charles E. Minarik

29 July 1963		
0900-0910	Welcome	Colonel Carl S. Casto Commanding Officer
0910-0940	Importance of Defoliation in Counterinsurgency Operations	Brig. General Fred J. Delmore CG, Edgewood Arsenal
0940-0950	U.S. Army BioLabs Defoliation Program	Mr. A. E. Hayward Chief, Program Coordination Office
0950-1005	Coffee Break	
1005-1020	Defoliation Contractual Effort	Mr. Alex Smallberg Director of Materiel
1020-1115	Crops Division Defoliation Program	Dr. C. E. Minarik Chief, Crops Division
1115-1200	In-house Basic Research Effect of IAA on Bean Leaf Abscission	Lt. Bernard Rubinstein Lt. Fred B. Abeles
1200-1300	Lunch at the Officers' Open Mess	
1300-1400	University of California Contract Program	Dr. Frederick T. Addicott Univ of Calif, Davis
1400-1500	Purdue Contract Program	Dr. A. Carl Leopold Purdue University
1500-1515	Coffee Break	
	SYNTHESIS PROGRAMS	
1515-1535	University of Illinois	Dr. Glenn C. Finger
1535-1600	Ansul Chemicals Company	Dr. Phil J. Ehman
1600-1620	Pennsalt Chemicals Corp.	Dr. Harold J. Miller
1620-1640	Ethyl Corp.	Dr. Rex D. Closson

1640-1700	Monsanto Research Corp.	Dr. Stanley D. Koch
1700-1730	General Aniline & Film Corp.	Dr. Max E. Chiddix
1800	Cocktail Party	
1900	Dinner - Officers' Open Mess Speaker - Colonel Carl S. Casto	

GREENHOUSE SCREENING PROGRAMS

30 July 1963

0830-0900	Crops Division Methods and Results	Dr. E. L. Robinson Mr. J. Ray Frank
0900-1000	Tour of Crops Division Facility	Mr. Kenneth Demaree
1000-1015	Coffee Break	
1015-1035	Pennsalt Chemicals Corp.	Dr. Harold Miller
1035-1055	Ethyl Corp.	Dr. John C. Wollensak
1055-1115	Monsanto Research Corp.	Dr. Stanley D. Koch

FIELD SCREENING

1115-1135	Crops Division	Mr. Kenneth Demaree
1135-1200	US Department of Agriculture - ARPA Program	Dr. Dayton Klingman
1200-1230	Dow Chemical Company	Dr. Mark G. Wiltse
1230-1315	Lunch at the Officers' Open Mess	
1330-1430	Fifth International Conference on Natural Plant Growth Regulators held at GIF-Sur-Yvette, France	Dr. Frederick T. Addicott Dr. A. Carl Leopold
1430-1530	General Discussion	
1530	ETD	

I. IMPORTANCE OF DEFOLIATION IN COUNTERINSURGENCY OPERATIONS

General Fred J. Delmore*

Colonel Casto and Gentlemen. I consider this really an opportunity to be with you today, for several reasons. First, I think that this meeting will allow all the people who are engaged in the program to exchange ideas; and secondly, perhaps in this exchange of ideas it will then be possible to proceed on a straight line so that we can achieve our objectives in a more efficient manner and in a shorter time.

When I heard of this particular meeting with Dr. Minarik and Colonel Casto, I felt it so important that I flew in here this morning to see you and talk with you even though I must fly out again this morning to take care of other things back at headquarters. I will not be able to meet you individually now, but I am going to try to come back here again this evening if I can.

Now, why do I think that this program is exceedingly important; why do I make so much of it? I had the opportunity of seeing firsthand some of the work that has been done in Southeast Asia. I spent five months in that area in connection with this program. I saw firsthand what this particular endeavor can do and what its potentials are for the future, and I was also able to judge for myself what is still needed in order that some of these potentials may be exploited. I will try to give you these on an unclassified basis.

As long as we have known war or conflicts in the field, one of the principles of these engagements and conflicts has been "cover and concealment." The soldier learns early in his career to take cover and to conceal if he is to accomplish what he has set out to do and save his own life. The tactics of World War II gradually seem to be going into the past, a lot of them, and new ideas and new tactics are coming into vogue, in which the guerrilla type is of great importance. All you have to do is to pick up the book written by Dr. Fall, "The Street Without Joy," which involves Route 1, the main highway from Hanoi, North Vietnam, all the way down into South Vietnam, and you will get some idea of what all these guerrilla tactics are about. However, even if you read this and study it, and you study it again, you will find that the tactics that are being used today are a little bit different than those being used in the French Indochina War. When South Vietnam became involved in this present situation, one of the things they had to face was ambush. All the way on up through '59, '60, and '61 the ambush was on the increase, and it became so desperate that the government turned to any means possible in order to be able to cope with this particular situation. We assisted the government of South Vietnam only in advice, and again to supply some of the materials in countering these ambushes.

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* Commanding General, U.S. Army Edgewood Arsenal.

Considerable research has gone on in these laboratories in the use of chemicals that could attack vegetation in order to preclude concealment. Some criticism resulted from the use of these chemicals at first. Well, perhaps with anywhere from 30 days' to 60 days' time required to do the job, and the high amounts needed, the cost perhaps was high. The people in the field became impatient and certainly could not wait for 30 to 60 days to destroy the cover and concealment of these guerrillas, and some criticism resulted. We were able to dispel a considerable amount of this misconception by bringing the program back into proper perspective last year. However, the need still exists for better agents and this is the reason that you are meeting here today. The need still exists to get herbicides or organic-type compounds that will do the job in a quicker period of time than we are able to do at present. The man in the field would like to destroy concealment in a matter of hours. I am not going to say your objectives are hours and I am not going to say it's days or whatever, but it must be shortened if the program is to succeed for future operations. And remember, today we are not engaged in a war in Southeast Asia, we are only advisors assisting that country to carry on, but tomorrow morning perhaps we may be engaged in similar type operations, not over there, but perhaps in some other part of the world. We never know when this is going to happen. We are in need right now of chemicals that will do the job at an earlier time, and in a quicker period. Now there is no question that field commanders will accept something that can destroy cover and concealment for many reasons: for anti-ambush, for spot firing, to clear lanes of fire, to expose logistical installations, or for many other uses. But he needs it, he wants it, but not in the present state. We must have an improvement not only in the ingredient but also in the type of dispersion that is involved.

Now this is another part of the story you are probably not involved in; the hardware part of this thing. I don't know. You are involved in the ingredient that will bring about the results, and this is the important part. We must get it so it not only acts quickly, but it must be logistically feasible. The toxicity gets a great amount of play. This is a big problem. The problem is to make sure it is perfectly innocuous to man and animal and at the same time, will do its job. But I leave that to you people to reach this particular objective. The know-how of this country in being able to solve this problem perhaps is located right here. Whether you are a scientist or whether you represent one of the industrial concerns. I fully recognize that on the part of industry there are certain proprietary rights. There are certain secrets that for competitive reasons and many others, you don't want somebody to know about. I fully recognize it. I had hoped, however, that this would be minimized; that you are in a position to really go the limit among yourselves in order that this problem may be solved. Today this country is not at war. I hope another war never comes. However, we may be engaged in one somewhere, someplace in this world, where an American soldier will have to fight. That American soldier deserves the best of anything we can give him in order that he might survive, and win, so as to protect the freedom of this country as well as all of this entire free world.

The capability of destroying cover and concealment to defend against and fight off guerrilla and other types of tactics is absolutely essential. It is generally agreed, and we will certainly endorse it, that the use of chemicals can play a big part in being able to defeat cover and concealment. However, certain things must be recognized. The systems, to be fully accepted, must have something that will act more rapidly than we have now. It certainly must be more logistically feasible; the toxicity must be taken into consideration; and the cost, because it will be in competition with other systems. There is a big job ahead. These laboratories have been given the responsibility to coordinate and to carry out this particular research. They have engaged you and made you part of this team. I hope that in the near future you will be able to come up with some sort of answers on this problem. I am not in a position to engage in technical discussions. We have plenty of technical people here who will be able to do so. Perhaps six months from now we may be able to call a similar meeting, and be able to discuss progress that we have made. In this progress meeting I am hopeful that we will be further ahead than we are today in the month of July, 1963. We will give you all the support we possibly can. All we need is for industry to work with us. Dr. Minarik, I am going to turn the meeting back to you, and lots of luck. Go as far as you can without committing your companies or giving away proprietary rights or your really deep secrets, but we must have some answers. Thank you.

II. U.S. ARMY BIOLABS DEFOLIATION PROGRAM

A. E. Hayward*

It is my purpose this morning to try to establish a broad but systematic frame of reference so that the subsequent discussions you will have today may fit into this general pattern. Historically, our interest in defoliants and herbicides began with the establishment of Fort Detrick in 1943. In 1946 much of the work in this area was published and the June issue of the "Botanical Gazette" was devoted entirely to a series of papers published from Fort Detrick. Work in this area has continued since that time and, while the program has seen both good times and bad, it is today enjoying a higher level of support than ever before.

General Delmore has stated some of the objectives and some of the problems that pertain to this program. I think it might be well to repeat some of the things he said because I believe they deserve this added emphasis. First, it is obvious that success in this program would allow us to do something about the problem of cover and concealment. When we clear vegetation from roadsides, railways, and canals we substantially reduce the opportunity for ambush and thus allow our own operations to proceed in a more timely manner. Defoliants would also be used, as General Delmore has said, to demarcate boundaries. I don't know if you heard the radio this morning, but two American soldiers were killed by ambush in the demilitarized zone in Korea. Maybe if this demilitarized zone had been plainly marked by defoliating a strip perhaps 200 meters wide this wouldn't have happened. At least, it would be obviously silly to accept the usual explanation that the attackers were lost and thought they were within their own boundaries. Successful defoliation could be used to clear gun emplacements, open up fields of fire, mark areas for bombing, or test whether or not a particular area was camouflage or actual vegetation.

Let me turn now to some of the technical problems that we face. I am quite sure that I will not state all of the technical problems, for I am not gifted with perfect foresight. To borrow one of the quips of the gentleman who will follow me, I am an appointed official, not an anointed one. I can, however, describe some of the major problems and the first of these would be our requirement for agents that have a broad range. We will never know precisely what type of vegetation we may wish to defoliate and so we would like to have materials that will take the leaves off of the vegetation in rain forests, deciduous forests, coniferous forests, and almost any other type of vegetation one could name. Whether these materials are true defoliants, whether they are herbicides or desiccants, does not really matter greatly but they should have a broad range.

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* U.S. Army Biological Laboratories.

Secondly, we need materials that will act rapidly. As General Delmore stated, it is premature at this time to say that these materials should act within an hour or a day or some other finite unit of time, but we certainly want them to act as rapidly as possible. I know that this factor will be discussed in more detail later and I will say no more about it at this time.

It goes without saying that the materials must be applicable by ground and air spray, that they must be logistically feasible, and that they must be nontoxic to humans and livestock in the area affected. Not only should these materials be nontoxic, but it seems to me that it is important that they not have any cosmetic effect. If, for example, a material had a marked red fluorescence and a number of people were obviously stained by the material, then our enemy might derive considerable propaganda value from this fact even though the individuals were not in any way injured by the material.

In a very real sense, this program in defoliation is a little bit unique with respect to the usual military-industrial collaboration. Ordinarily, in military R&D we have a military concept that leads to the statement of a military requirement, technical characteristics, performance specifications, and other strictly delimited aspects. In this program we do not have rigidly specified characteristics. I have stated some of the broad requirements that a successful defoliating chemical should have, but within this general framework we will accept and use materials that will do a job for us. In a few years it may be that we will come up with more definite specifications but at the moment we simply solicit the assistance of you gentlemen in finding materials that can be used successfully within the reasonably broad and general guidelines that General Delmore has stated and that I have repeated.

It has been both a pleasure and a privilege to speak to you gentlemen this morning. I know that I speak for all of us who have responsibility in this program when I say that we are most gratified at the very wonderful response industry has shown and we are confident that your interest and your capability will lead to success in this program.

III. DEFOLIATION CONTRACTUAL EFFORT

Alex Smallberg*

A. INTRODUCTION

There are three areas in this contract program I would like to touch upon briefly because I believe they may be of interest and benefit to you: (a) the importance of our contract program, both to the Government and to you; (b) how we have organized this contract program to bring us where we are; and (c) what I believe lies ahead.

B. IMPORTANCE OF THE PROGRAM

The previous speakers have already told you of the importance and urgency of this program to the military. I would like to add that coupled with this urgency and complexity is our military objective of being second to none. This is a tall order for all of us — it is a challenge not only to the military, but also to industry. It is a challenge that demands our best, it demands your best — nothing else will do.

Our contract program also offers many incentives and advantages to industry. The contractor motivations to participate in military R&D contract programs are some mixture and combination of patriotic, scientific, and economic impulses. I am certain that General Delmore's remarks will keep the patriotic incentive spark alive. On the scientific and economic fronts our program provides several advantages.

(a) It gives each of you a preview and insight into future related R&D contract programs.

(b) It enables you to maintain a satisfactory position with other organizations that are doing similar work and to evaluate your own capability with theirs.

(c) It gives you the opportunity to attract highly trained scientific personnel by offering them a scientific and technical challenge, as well as to train other personnel for subsequent Government programs and/or related commercial research.

(d) It permits you to keep abreast of our previous in-house accomplishments and gives you the opportunity to visit our laboratories and observe the scientific work we are doing and the specialized techniques and scientific equipment we are using.

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* U.S. Army Biological Laboratories.

(e) It also gives your scientists the opportunity to gain access to current research data that may have been assembled in Government reports and papers but may not be published for several years.

In summary, this program offers you opportunities for creative and challenging work that may lead not only to the solution of military problems, but to new knowledge leading to new commercial products, future markets, and ultimately high industrial profits.

C. ORGANIZATION OF CONTRACT PROGRAM

In organizing our contract program there were several aspects that required careful consideration. At Fort Detrick, as I am sure you recognize, we do a great deal of creative and skillful work. Frequently, however, we do not have available the necessary manpower and facilities to do the entire job within the needed time frame. We must turn to outside organizations such as yours to research, develop, and produce our highly specialized items. This calls for a military-industry contract partnership and demands of industry its ability, creative ideas, facilities, and the competence of its scientific and engineering disciplines to achieve the results we need in the shortest possible time.

Our previous contract budgets in this area, particularly during the period 1959 to 1962, were practically nil. The dollar support for our in-house effort was only a little better. We were faced with two major problems: We could not suddenly turn on the contract faucet and turn to one, two, or three contractors who had already demonstrated their special competence to us under any major contract program, nor did we have the total in-house professional competence to monitor and evaluate a wide and dynamic contract program. During this prior period, Dr. Minarik did an outstanding job in keeping the industrial and in-house interest alive.

In 1962 we invited essentially the entire chemical industry to Fort Detrick. We briefed them on the technical and contract aspects of this program and solicited their participation. The industrial interest was very high throughout and the response very good. It became clearly evident that the procurement would be a highly competitive one and that the selection of the contractors considered to be best qualified to perform the contract would be a very difficult decision for the Government to make. The proposals were evaluated in an objective, impartial, and thorough manner by a group of highly qualified Government scientists and consultants who were especially selected for their proficiency in this area. It was clearly recognized that a great deal of highly important capabilities existed in numerous outside organizations. The line between those selected and those not selected was a very narrow one. We are therefore keeping the procurement doors wide open to all highly qualified organizations, and there are many, to enable their future participation in our program as their future interest and capabilities may dictate.

At the onset of the contract program, it was immediately recognized that there would be a dual competition. The first would be the competition to obtain the contract; the second would be the competition between the selected contractors. Each contractor quickly became aware that the contract itself offered no social security and that their contract output would have to measure up to the qualitative output of their competitors. This competition did, and I am sure will continue to, generate a high degree of scientific effort, productivity, and efficiency. The results of the contract effort will be currently and closely evaluated, and the extension of the contract programs will be determined on the basis of careful assessment of the contract accomplishments and the potential benefits they offer to the Government.

In establishing our contracts we have endeavored to provide several things that we felt would be very helpful to the contractor. It is my personal viewpoint that a major reason for R&D contract slippage after a qualified contractor is selected is that Government objectives and contract goals are not always currently stated and clearly understood, and the urgency of the program is not fully realized. During the negotiation of the defoliant contracts we endeavored to describe the immediate and long-range contract objectives, the importance of the contract program, and the management steps that would be taken to plan, monitor, and evaluate the contract effort and results. In addition to making our requirements clear and impressing upon you the importance and urgency of the program, we felt that the contract documents themselves should serve as a suitable tool for the parties. Therefore, our program was planned to provide stability and continuity for the contractor capable of doing an outstanding job. The successful accomplishment of our contract program needs the bold and ingenious efforts of the people assigned to it. Your contract has been designed to allow you a management flexibility that will give your competence and judgment the freest opportunity to do creative work, both in-house and by attracting on a national basis the greatest scientific and industrial competence that might be available. The contract program has Fort Detrick's top management interest and support and has drawn the best capabilities of our research personnel to work closely with the prime contractors to insure that the program focus and direction will not be misplaced or misunderstood. We hope to facilitate the contractor capability by bringing into the program the best research and industrial competencies that may be needed. To the extent that it is practicable and worthwhile, we will do our utmost to integrate outside ideas that we believe are needed for the results we are seeking.

D. WHAT LIES AHEAD

The results of this program are being used in field test evaluations. The assessments that follow may lead to large-quantity military requirements. I am not prepared in this brief time to provide you with a market research analysis. I am sure you all have a great deal of competence and experience in this area.

I have already mentioned commercial and economic incentives. I am not surprised, and I am happy to report, that there have been a large number of patent applications resulting from this program. This may be a partial explanation as to why many of you have proposed to work without profit and on a cost-sharing basis. Continuing to look ahead, we are hopeful, of course, that the needed breakthrough will occur. No one can predict when, where, and how this will happen. We are giving every industrial organization with a capability and interest an opportunity to submit unsolicited proposals and to test their compounds. It is safe to say that our future budgets will be directed toward the organization whose research capability and product can provide the best potential benefits to our program. Your job, as I see it, is to make sure that your organization is the one selected.

In reviewing today's program, I couldn't help but be impressed by the qualifications of the contractors and the representatives who are participating in this program. It further demonstrates that our program needs a special research capability. If I may attempt a simplified definition, "research is the difference between the problem and the answer." The challenge to all of us — the contractor and our Government people — will be to continue to reduce the difference.

IV. CROPS DIVISION DEFOLIATION PROGRAM

C. E. Minarik*

A number of speakers this morning mentioned that our program started during World War II, with the establishment of Fort Detrick in 1943. There was a great deal of interest at that time in destroying vegetation in the South Pacific Theater, and the principal means available was high explosives. I have forgotten how many millions of tons of high explosives were required to destroy the vegetation on some of these Pacific Islands. We were asked to investigate chemicals that were available in large quantities in the United States that could be employed for "defoliating" this vegetation. The work was done by the group then at Fort Detrick under the leadership of Dr. A. G. Norman, who is now at the University of Michigan. The group screened a large number of chemicals, including the commercially available cotton defoliants. The screening program yielded two chemicals of promise, ammonium thiocyanate and zinc chloride. Both of these produced excellent results when used in conventional military spray equipment available at that time, which consisted of the B-25 aircraft and the M-10 smoke tank. The first requirement was to defoliate trees; later this was changed to add target marking. In 1945 we were ready to recommend the use of ammonium thiocyanate in the Pacific Theater. During the course of coordination at high levels in the government, someone decided that ammonium thiocyanate sounded very much like cyanide, which everybody knows is poisonous. If we used this chemical, we would be accused of conducting poison gas warfare; therefore, the plan to use chemicals for destroying vegetation in the Pacific Theater was dropped. The war ended before another "defoliant" could be developed. Much of our work then was classified Secret; however, some of our reports have since been downgraded to Unclassified. One report relates to our work at Fort Knox in cooperation with the Armored Medical Research Laboratory. At Fort Knox we sprayed temperate zone vegetation with ammonium thiocyanate and other chemicals and followed the spray treatment with incendiaries that were dropped from B-25 aircraft. We have some excellent before-and-after photographs of the defoliation that was achieved during these trials. The chemicals used were not true defoliants but were more properly described as desiccants. However, they did accomplish the desired results.

The next program in which we became involved dealt with subtropical vegetation in the Everglades of Florida. In May 1945 Camp Detrick published Special Report 13, "Marking and Defoliation of Forest Vegetation." This report also was originally classified Secret but recently has been downgraded to Unclassified. This program also involved screening additional chemicals, but we again came up with the same chemicals, ammonium thiocyanate and zinc chloride, that had proved so effective at Fort Knox. The vegetation changed color in two or three days. The effect was excellent for target marking. As Mr. Hayward pointed out, target marking, or delineating drop areas and other zones by causing the vegetation to change color,

* U.S. Army Biological Laboratories.

is extremely important in warfare. But more important than this, and this does not involve chemistry, was the fact that we demonstrated that spray materials that had a wide range of droplet sizes did penetrate a forest canopy so that droplets were deposited, not only on the upper canopy, but in the middle, and also on the forest floor. Canopy penetration is a subject that comes up every time one discusses spraying dense tropical vegetation, such as occurs in Vietnam for instance. One says, "Oh, well, it's very dense so you can spray the top; when those leaves fall, you spray the middle layer, and so on." This technique is not necessary. The 1945 trials have amply proved this point.

More recent work conducted prior to our phase-out in FY 1958, just before we hit the leanest of our lean years, consisted of extensive screening of defoliant and desiccant chemicals. We had examined approximately twelve thousand chemicals here at Detrick for vegetational control activity, and those that displayed even the slightest defoliation or desiccation activity were then put into our defoliant screening program. An unclassified report issued in July 1959 lists the most active of approximately 700 chemicals that were screened for defoliant activity. I think some of you contractors have seen copies of this report. Recently, an attempt was made to have it issued by the Department of Commerce but, although Unclassified, it was still considered sensitive.

One of our earliest and most fruitful contracts dealt with the evaluation of candidate defoliants on tropical vegetation in Puerto Rico. Bruns, Cruzado, and Muzik evaluated 51 different formulations on 25 species of tropical woody plants and reported their results in a recent issue of Tropical Agriculture. Incidently, Dr. Muzik, one of the authors, will join our staff to spend part of his sabbatical leave from the Washington State University, continuing his work on absorption and translocation of defoliants and herbicides.

I have briefly reviewed the highlights of our defoliation program to show that we have been engaged in defoliation Research and Development (R&D) for some time and have accumulated quite a backlog of information.

General Delmore has pointed out that we need a chemical that acts more rapidly. At one time we had a requirement for a defoliant that would be effective in 24 hours and a target-marking chemical effective in 15 or 20 minutes. There are certain chemicals that can mark vegetation in this short period of time. Tributylphosphate, for instance, will desiccate certain species very rapidly but will not affect other species. The same is true of certain defoliants. This points up the fact that we must find chemicals with broad host ranges.

Other speakers before me have discussed our objectives in terms of what the compound must do or be like. I would like to reiterate that we need a chemical that is effective in low doses, is inexpensive, readily available or capable of being manufactured in large quantities, nontoxic to man and animals, stable in storage, nonhazardous to the user, and noncorrosive.

The work that I have discussed so far was done in the early days of our defoliation program. A more recent project involved vegetation control at Camp Drum, New York. We were able to have photographers go along on that mission to get before-and-after pictures. A very short film has been prepared that we would like to show at this time. This work was done in 1959 and it was shortly thereafter that we got the call to demonstrate what could be done in Vietnam. Dr. James W. Brown was responsible for this work. He was also responsible for the demonstrations in Vietnam that defoliants and herbicides can be effective for vegetation control in that country. After Dr. Brown's highly successful demonstrations of the potential of herbicides, the United States Air Force was directed to conduct defoliation spray operations. The flow rate of the equipment was too low for the desired ground deposit, and many delays caused the operation to be conducted during the wrong season of the year for the best results. As General Delmore indicated, there were some adverse comments in the press. Dr. Warren Shaw of the Department of Agriculture, who is here today, General Delmore, and a number of others went to Vietnam to assess the results. They reported that the sprayed vegetation had been defoliated to the extent of 75 per cent on the average and in certain targets 100 per cent. I think Colonel Casto will show some slides this evening of areas that were sprayed in February 1962 and photographed in May 1963. You will see that there has been very little regrowth in these areas, maybe five to ten per cent at most.

This brings us up to our current in-house program. Our total budget for defoliation R&D is approximately two million dollars, about three hundred thousand of which goes into our in-house program; the remainder goes out on contract to you folk. We now have 18 professional personnel, military and civilian. I would like to compliment our military personnel for the marvelous job they are doing for us. They have shown great adaptability and enthusiasm for the program and are contributing very significantly to its success. We have four additional professional civilians coming in next month. As I indicated earlier, Dr. Muzik will be here on Wednesday or Thursday, as will Dr. Hurtt from Michigan State. Dr. Szabo and Mr. Wiswesser will be coming in later in the month. In addition, there will be three civilian technicians, giving us a total of approximately 25 people working on defoliation.

What type of work are we doing here? We are engaged in basic research on the biochemical and physiological aspects of leaf abscission. If you will note your program, you will see that I will be followed by Lt. Rubinstein and Lt. Abeles, who will discuss some of their basic research on leaf abscission. Another problem under investigation is the role of ethylene

in abscission. The formulation of candidate defoliants for use in military operations is also an active project. Work in absorption and translocation is planned for Dr. Hurtt when he arrives. Dr. Muzik has elected to study environmental factors affecting absorption and translocation in our growth chambers. This investigation is very important because some of the sprays used in Vietnam were applied when the plants were dormant, and a lack of moisture certainly can influence the plant response to growth-regulator types of chemicals. The mechanism of action is the type of study that Dr. Szabo will be initiating next month. Residues in plant tissues is another subject that is receiving some attention. Correlation between plant response and stage of growth when treated is also undergoing investigation. Other active projects concern: (a) dose-response curves for various chemicals and plant species; (b) taxonomic studies; (c) modification and calibration of aerial spray equipment; (d) evaluation of candidate defoliants on mixed stands of tropical vegetation in 5- to 20-acre plots; (e) relationships of molecular structure and biological activity; (f) primary and secondary greenhouse screening; and (g) field screening using ground spray equipment.

You will note that we are not doing any synthesis in-house. We are relying entirely on you contractors to provide the synthesis effort.

In brief, our in-house program consists of primary and secondary screening in the greenhouse and field; aerial applications; equipment calibration; taxonomic, biochemical, and physiological investigations; formulation studies; and structure-activity relationships. With the exception of one project, our entire defoliation program is unclassified.

V. MECHANISMS OF AUXIN ACTION ON LEAF ABSCISSION

Bernard Rubinstein*

Anyone who is interested either in understanding the nature of leaf abscission or in synthesizing chemicals that artificially induce defoliation is aware of the importance of the internally produced growth regulator, indoleacetic acid (IAA). Under various conditions IAA can either stimulate or inhibit abscission, but little is known as to how this auxin actually works. In our attempts to better understand auxin action, we are trying to ascertain the site of action or, in other words, to determine if auxin acts directly on the separation area or indirectly by influencing the metabolism of the petiole.

A. STUDIES OF SITE OF ACTION

For studies of site of action, intact Black Valentine beans (Phaseolus vulgaris L. var. Black Valentine) were grown in individual pots in the greenhouse. After 14 days the primary leaf blades were cut off, and either plain lanolin or 1000 parts per million (ppm) IAA in lanolin was applied to cover the cut ends of the petioles.

Using this method, it is relatively easy to separate IAA-induced inhibitions and stimulations of abscission (Figure 1). If IAA is applied immediately to six-centimeter (cm) debladed petioles, abscission is completely inhibited. IAA applied ten hours after deblading still produced inhibitions, but they were not as pronounced. However, if 17 hours elapsed between removal of leaf blade and IAA applications, a very marked stimulation of abscission occurred. These results are similar to those first reported by Rubinstein and Leopold** after they had applied alpha naphthaleneacetic acid to abscission zone explants. These results, then, led to the postulation of two stages in bean leaf abscission - a first stage characterized by an auxin inhibition of abscission, and a second stage when auxin applications stimulate abscission.

To examine the effect of an intact primary leaf on the opposite debladed petiole at the same node, the experiments shown in Figure 2 were set up. The first diagram represents the hours to 50 per cent abscission for the one-cm and six-cm controls and the second illustrates that the intact blade inhibits abscission of the opposite six-cm petiole. Hypothesizing that the inhibition was due to auxin production by the leaf blade, IAA was applied to only one debladed petiole at the primary leaf node. The third diagram shows the surprising results. Not only was there no inhibition of the untreated petiole, but there was a marked stimulation of abscission.

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* U.S. Army Biological Laboratories.

** Rubinstein, B., and Leopold, A.C. "Analysis of the auxin control of bean leaf abscission," Plant Physiol. 38:262-267, 1963.

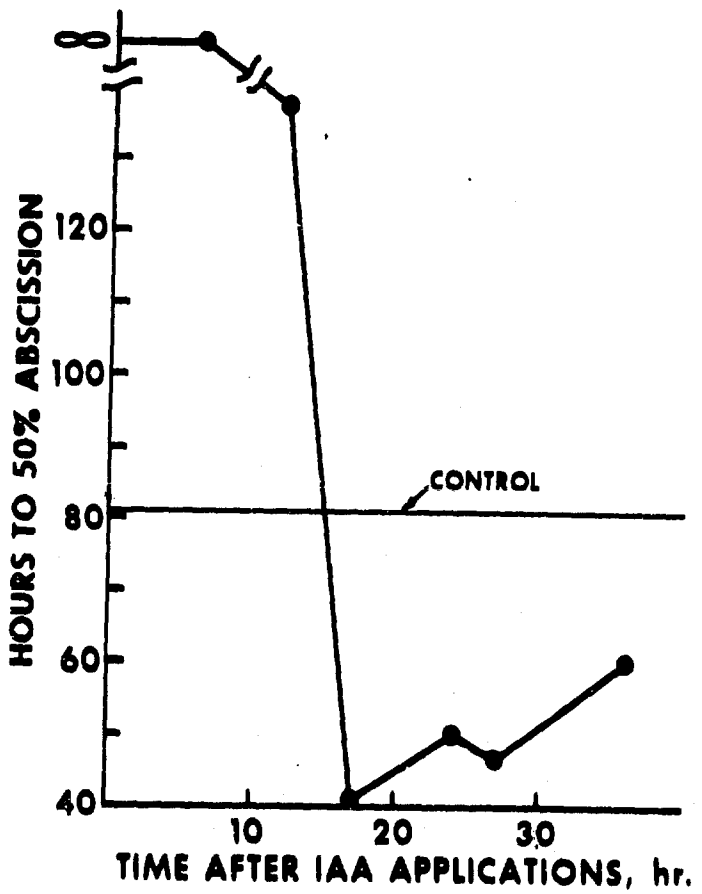
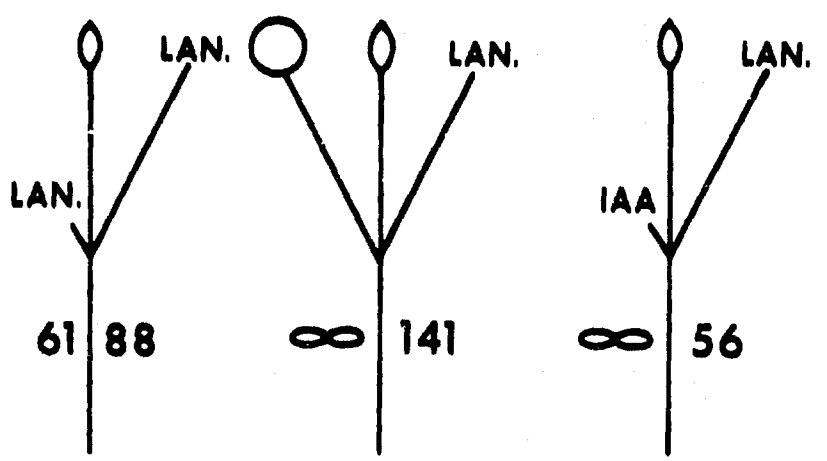


Figure 1. Abscission Responses of Petioles of Intact Bean Plants to Applications of IAA (1000 ppm in Lanolin) at Various Times After Deblading.



Numbers indicate hours to 50% abscission

Figure 2. Effect of Leaf Blade or IAA on Abscission of Opposite Petiole.

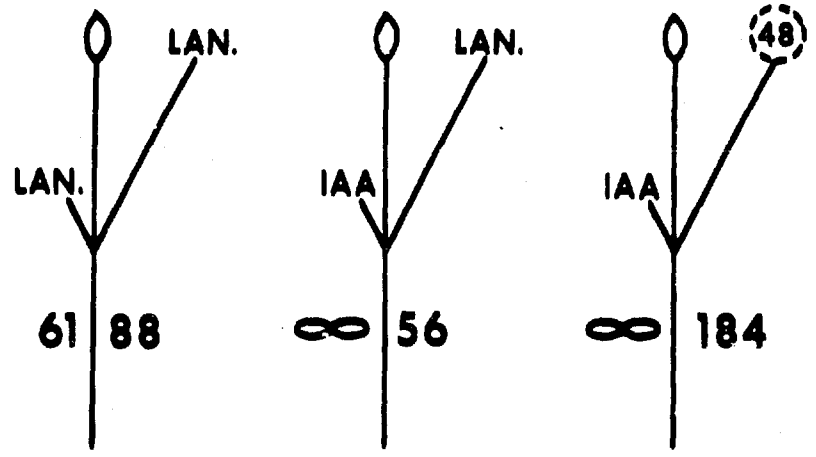
The possibility then existed that auxin on the one-cm petiole was affecting the opposite six-cm petiole after its transition into Stage 2. If this were true, it should be possible, by retaining the untreated six-cm petiole in Stage 1, to inhibit its abscission when IAA is applied to the opposite petiole. Figure 3 illustrates such a situation. The diagram on the left is the untreated control and the one in the center shows the stimulation of abscission by IAA. The plants represented by the third diagram were treated so that IAA was applied immediately to a one-cm petiole, but the opposite leaf was not debladed until 48 hours later. It can be seen that by keeping the leaf intact and retaining the six-cm petiole in Stage 1, an inhibition of abscission was produced.

Thus, we have a system to determine the site of auxin action. By applying IAA on one petiole, we can assume that auxin reaches the abscission zones of the opposite petioles at exactly the same times, and if the abscission time changes as the length of the untreated petiole is varied, it would be suggestive of an indirect auxin action, that is, an effect on the petiole itself and not on the abscission zone.

To investigate the effect of length of petiole on the first stage, or the stage when auxin inhibits abscission, experiments were set up as diagrammed in Figure 4. One leaf at the primary node was trimmed to one cm and treated with IAA; the opposite leaf was left intact for 48 hours and then cut so as to leave either a six-cm or a one-cm petiole (upper right and lower left diagrams, respectively). When compared with the control (lower right diagram), the results indicate that the inhibition was apparent only with six-cm petioles, thus implying an indirect site of auxin action during Stage 1. Abscission of the one-cm petiole upon which auxin was applied, however, was inhibited.

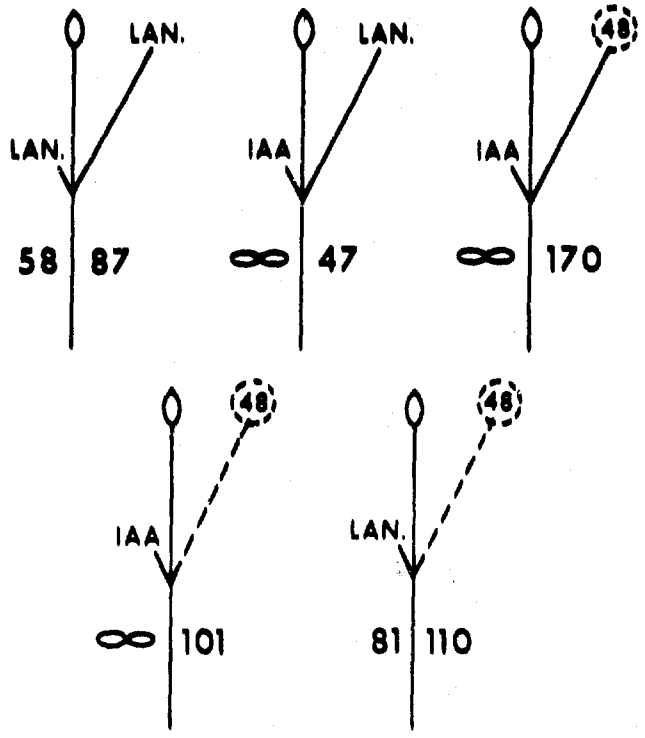
The localization of auxin action during the second stage, when IAA stimulates abscission, is outlined in Figure 5. Again IAA was applied immediately to one-cm petioles, but this time the opposite leaves were debladed at the same time and trimmed to one or six cm. By comparing the abscission rates of the untreated petioles in the center and right diagrams, it can be seen that regardless of length, the petioles were accelerated equally, thus implying an action of auxin directly at the abscission zone.

From the data so far, we have concluded that the action of auxin is primarily direct, that is, on the separation area and its associated cell layers. It is true that Figure 4 implied an indirect site because six-cm petioles were inhibited longer than one-cm petioles, but it should also be noticed (Figure 5) that IAA applied even to one-cm petioles produced marked inhibitions. It may be, then, that lengthening the petiole serves only to intensify the inhibitory effect. Results of experiments illustrated in Figure 5 suggest a direct auxin action during Stage 2.



Numbers indicate hours to 50% abscission

Figure 3. Effect of IAA on Abscission of Opposite Petioles Debladed at 0 and 48 Hours After Treatment.



Numbers indicate hours to 50% abscission

Figure 4. Effect of IAA on Abscission of Opposite Petioles Trimmed to Various Lengths at 48 Hours After Treatment.

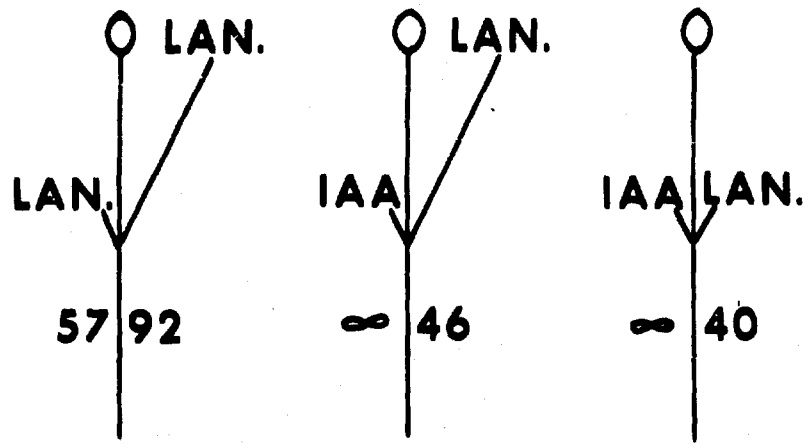


Figure 5. Effect of Length of Petiole on the Acceleration of Abscission by IAA.

Since it appears that auxin acts at the abscission zone during both stages, we have gained confidence in using excised abscission zones to represent processes occurring in the intact plant. A further analysis of the physiological and biochemical aspects of auxin effects on these abscission zones was then attempted by measuring ethylene production under different experimental conditions.

B. ETHYLENE PRODUCTION STUDIES

Since 1935, the leaves of various plants have been reported to give off ethylene, but no one has examined the abscission zone itself nor related the ethylene evolution to naturally occurring leaf abscission. Dr. F.B. Abeles of this laboratory and I are currently investigating the production of this gas by using excised abscission zones in order to learn more of the mode of action of auxin and other abscission stimulators. Ethylene in our experiments was measured by an F&M Model 720 gas chromatograph equipped with a flame-ionization detector. A two-foot activated alumina column was used at a temperature of 45°C; helium flow rate was 110 milliliters per minute.

The bioassay consisted of one-cm-long abscission zone explants from primary leaves of Red Kidney bean (Phaseolus vulgaris L. var. Red Kidney). They were placed in agar that had been poured into bottles that could be fitted with gas-tight vaccine caps. Gas samples were withdrawn with a syringe and injected into the gas chromatograph.

We first wanted to determine if naturally abscising explants evolve ethylene. To do this, we removed air samples from sealed gas collection bottles at various times until abscission occurred. The explants did give off ethylene — a rapid evolution at first, followed by a slow but regular increase for more than 96 hours. Fifty per cent abscission occurred at 48 hours, when the concentration of ethylene was approximately 0.34 milli-microliter (mμl) per explant.

To observe the effect on ethylene evolution by auxin applications during the two stages, the following experiment was set up. After cutting, explants were either left in plain agar or placed immediately in alpha naphthaleneacetic acid (NAA). Other explants were placed in NAA at either 24 or 48 hours after cutting. It was found that NAA always induced marked stimulations of ethylene evolution (more than three mμl per explant per 12 hours) no matter when the auxin was applied to explants. Abscission was inhibited by all NAA treatments, however, except the 48-hour one when an acceleration occurred. These results led to the hypothesis that ethylene is effective only during the second stage of abscission.

To test this hypothesis, one ppm of ethylene was added to explants in plain agar at either 0 to 12 or 48 to 60 hours after cutting (Table I).

TABLE I. ABSCISSION RATES OF BEAN EXPLANTS AFTER ETHYLENE EXPOSURES (1 ppm) AT 0 TO 12 OR 48 TO 60 HOURS AFTER CUTTING

Ethylene Added, time	Hours to 50 Per Cent Abscission
Control	84
0 to 12 hours after cutting	80
48 to 60 hours after cutting	55

It can be seen that ethylene applied immediately had no effect on abscission rates but that ethylene applied during Stage 2 markedly accelerated abscission.

From these data we feel that endogenously produced ethylene may regulate leaf abscission. The gas was found in sufficient quantities to stimulate abscission, but it acted only during the second stage of abscission and had no effect during Stage 1. Thus, even though immediate auxin applications stimulate ethylene production, the gas is not active because auxin simultaneously retains the explant in Stage 1. The experiments so far suggest that abscission is controlled by factors even more basic than ethylene. It seems that the nature of the change from the first to the second stage is of vital importance for the induction of abscission, since it is during the second stage only that ethylene is active.

C. SUMMARY

Experiments have been performed to investigate the mode of action of auxin as it both inhibits and stimulates leaf abscission. Intact bean plants were used to determine first the site of auxin action. After applying indoleacetic acid to one debladed petiole and observing abscission rates of the opposite untreated petioles at the same node, one can conclude that auxin acts directly at the abscission zone during both the first stage, when it inhibits abscission, and during the second, when it stimulates abscission.

To characterize a mode of auxin action, the evolution of ethylene was measured from excised abscission zones. When alpha naphthaleneacetic acid is applied to the explants, ethylene production is stimulated during both stages. Ethylene additions to control explants, however, suggest that the gas acts only during the second stage, so it is proposed that the stimulatory action of auxin during Stage 2 is due to the production of ethylene.

VI. ABSCISIN II, AN ABSCISSION-ACCELERATING SUBSTANCE FROM YOUNG COTTON FRUIT*

K. Ohkuma, O.E. Smith, J.L. Lyon, and F.T. Addicott**

ABSTRACT

Crystalline abscisin II, with a tentative molecular formula of $C_{15}H_{20}O_4$, has been isolated from young cotton fruit. It accelerates abscission at rates as low as 0.01 microgram per abscission zone. It inhibits IAA-induced straight growth of *Avena* coleoptiles, but has no gibberellin activity on dwarf maize.

Endogenous abscission-accelerating substances are now known to occur widely in the higher plants.[1] Recently, abscisin I was isolated from the mature fruit wall of cotton,[2] but little is yet known of its physiological properties. The abscission-accelerating substance that is presently best known physiologically occurs in the young fruit of cotton; its activity reaches a peak at the time of the onset of young fruit abscission.[1] This paper reports the isolation of this substance, here named abscisin II, and describes some of its chemical and physiological properties.

Abscission-accelerating activity was measured with explants (excised cotyledonary nodes) of 14-day-old cotton seedlings. Seedlings were grown at 32° ± 2°C with a 15-hour photoperiod of 2000 foot-candles provided by "warm-white" fluorescent lamps. The explants consisted of three-millimeter stumps of the cotyledonary petioles and of the stem and a ten-millimeter stump of the hypocotyl. Explants were placed upright in stainless steel holders in Petri dishes containing a five-millimeter layer of 1.5 per cent agar. Fractions to be tested were applied to the petiole stumps in five-microliter droplets of 1.0 per cent agar. Dishes with explants were kept in the dark at 30°C. Abscission was determined by applying a force of five grams to the end of the petiole stumps at daily or more frequent intervals.

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* These investigations were supported in part by funds from the Foundation for Cotton Research Education administered through the U.S. Department of Agriculture and a contract with the U.S. Army Biological Laboratories. Cotton fruit for extraction were obtained with the cooperation of the personnel at the U.S. Cotton Field Station, Shafter, California. We thank Margaret Deasper and Nancy Beck for their excellent assistance; Mr. Paul Allen of the Department of Agricultural Toxicology, University of California, Davis, for determinations of IR spectrum and UV absorption; Department of Chemistry, Stanford University, for mass spectrometry determinations and elemental analysis; and Dr. R. Cleland for providing facilities for Avena straight-growth tests.

** Department of Agronomy and Cotton Research Branch, A.R.S., U.S.D.A., University of California, Davis, California.

Four- to seven-day-old fruit were collected in the field and quick-frozen with dry ice. After lyophilization, approximately 78 kilograms (225 kilograms fresh weight) were extracted overnight in an ambient temperature of 20° to 25°C with 520 liters of 80 per cent acetone. After filtering and concentrating, the residual rich water was adjusted to pH 2.0 with dilute hydrochloric acid and extracted twice with equal volumes of ethyl acetate. The ethyl acetate phase was extracted three times with 2.0 per cent aqueous sodium bicarbonate. The sodium bicarbonate phase was acidified to pH 2.0 and extracted twice with equal volumes of ethyl acetate. The remaining acid fraction, weighing 147 grams, was separated by adsorption chromatography. Carbon (Darco G-60) and celite (Hyflo Super-cel) (1:2) were thoroughly mixed in water and packed in a column by stirring. The acid fraction was applied in a ratio of approximately 1:10 (acids to carbon) and eluted with increasing concentrations of acetone in water. Each of the ten fractions (10 to 100 per cent) contained three liters of solvent per 100 grams of carbon. A total of 4.15 grams of oily material having abscission-accelerating activity was found in the 50 per cent and 60 per cent acetone eluates. This was applied to a silicic acid:celite (1:2) column packed in chloroform, in a ratio of one gram of sample per 20 grams of silicic acid. Fractions were eluted successively with increasing concentrations of ethyl acetate in chloroform (one liter of solvent per 50 grams of silicic acid) starting with five per cent ethyl acetate. Peaks of abscission-accelerating activity were found in the 10 to 30 per cent ethyl acetate, and in the 50 to 60 per cent ethyl acetate eluates. Further purification of abscisin II involved only the most active peak, 10 to 30 per cent ethyl acetate. Eluates were combined and evaporated to dryness *in vacuo*. The residue was treated with a small amount of chloroform from which an insoluble crystalline material having no abscission activity was eliminated by filtration. The remaining 238 milligrams of chloroform-soluble material were streaked on four sheets of Whatman three-millimeter chromatographic paper (56.3 by 45.6 cm) and developed to 35.0 centimeters with iso-propanol:ammonia:water (10:1:1 v/v). Each paper was divided into ten portions according to Rf and eluted with methanol by macerating in a Waring blendor. Eluates were evaporated to dryness, dissolved in water at pH 7.0, and filtered. The filtrates were acidified to pH 2.0, extracted twice with ethyl acetate, and evaporated to dryness. Eluates from Rf 0.4 to 0.8 were combined (66 milligrams) and applied to a silicic acid:celite column, as described above. The 10 per cent ethyl acetate eluate was reduced *in vacuo* to 23 milligrams of semi-solid oily material. After recrystallizing twice from chloroform-petroleum ether, nine milligrams of highly active crystals were obtained. The substance was named abscisin II.

Purity of the crystals was tested by thin-layer and paper chromatography. Nine different solvent systems were used; in each case only one spot was detected with potassium permanganate spray.

Abscisin II has a melting point of 160 to 161°C and sublimes at 120°C. It is an acidic, colorless compound soluble in aqueous sodium bicarbonate, chloroform, acetone, ethyl acetate, diethyl ether, methanol, and ethanol, slightly soluble in benzene and water, and sparingly soluble in petroleum ether. Its ultraviolet absorption maximum in methanol is 252 millimicrons (ε 25,200); its infrared absorption spectrum in KBr pellets is shown in Figure 1. The molecular weight of abscisin II is 264 (determined from mass spectrometry) and it contains 68.76 per cent carbon and 7.96 per cent hydrogen. Tests for nitrogen, sulfur, and halogens were negative. Therefore, $C_{15}H_{20}O_4$ was assigned to abscisin II as a tentative molecular formula. These data show that abscisin II is chemically distinct from abscisin I isolated from mature fruit walls of cotton by Liu and Carns.[2]

Abscisin II is a very effective abscission accelerator. Figure 2 shows the results of applying three amounts of abscisin II to the petiole stumps of explants. Acceleration resulting from the application of 1.0 and 0.1 microgram is greater than can be obtained from maximum accelerating amounts of gibberellin A_3. Evidence indicating that abscisin II is an abscission-accelerating plant hormone is presented elsewhere.[1]

Abscisin II was also tested for its growth-inhibiting and gibberellin activities. In the presence of 0.1 microgram per milliliter of IAA, it completely inhibited Avena coleoptile straight growth at concentrations of 3.0, 10.0, and 30.0 micrograms per milliliter; at 0.1 microgram per milliliter it reduced growth at 60 per cent of that induced by IAA alone. No gibberellin activity was found when applications of 0.25 to 50.0 micrograms per plant were made to dwarf maize mutants d_1, d_3, and d_5.

Further research on the physiology and chemical characterization of abscisin II is in progress and will be reported shortly.

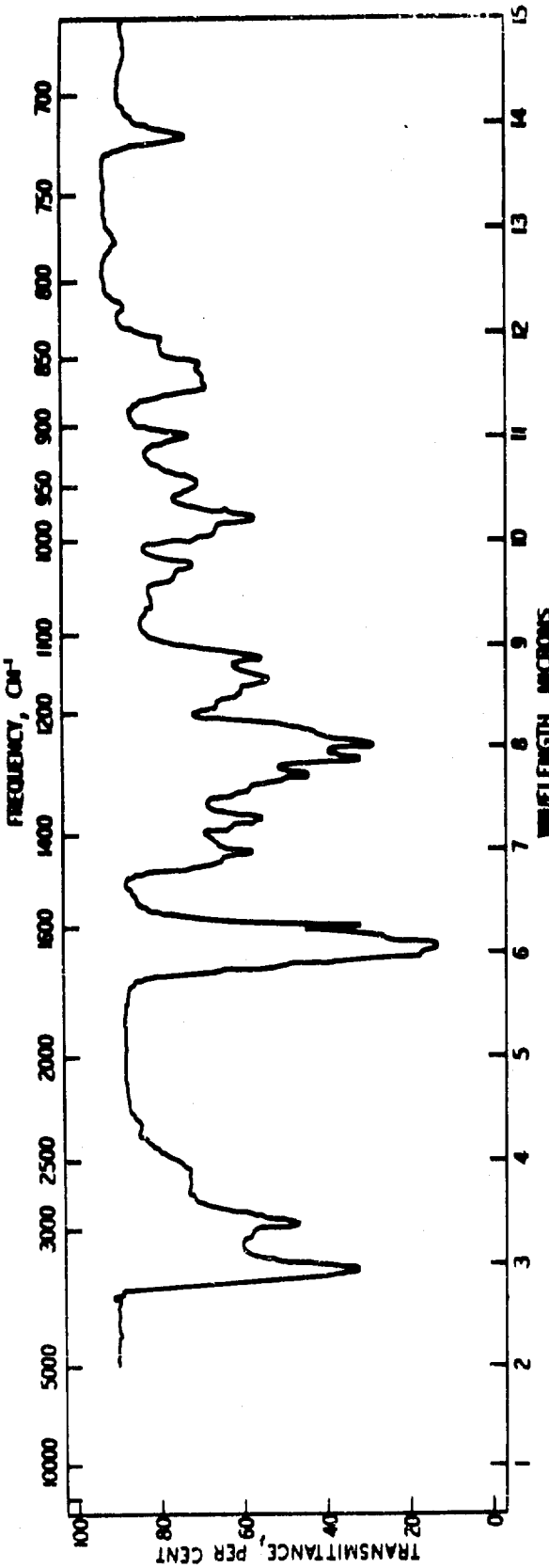

Figure 1. Infrared Spectrum of Abscisin II.

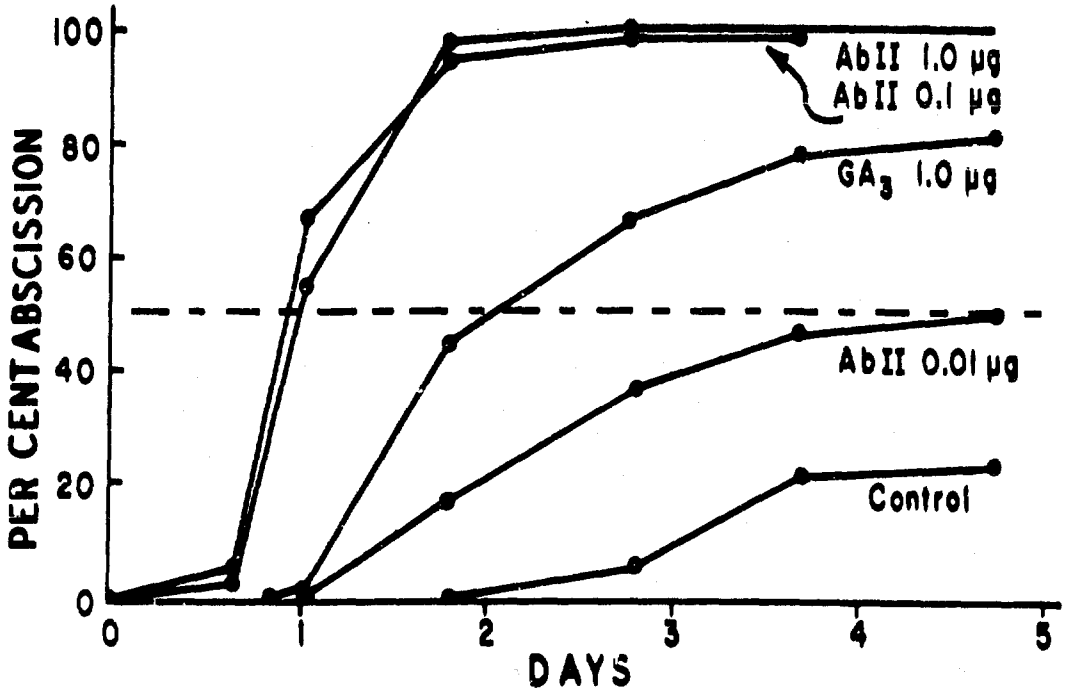

Figure 2. Abscission Acceleration Induced by Applications of Abscisin II to Petiole Stumps of Explants of Cotton Seedlings. Each treatment included 30 explants (60 abscission zones). For comparison, treatment with an optimum concentration of Gibberellin A_3 is included.

LITERATURE CITED

1. Addicott, F.T.; Carns, H.R.; Lyon, J.L.; Smith, O.E.; and McMeans, J.L. "Proc. fifth international conference on natural plant growth regulators" (in press).

2. Liu, W.C., and Carns, H.R. "Isolation of abscisin, an abscission-accelerating substance," Science, 134:3476:384-385, 1961.

VII. PREPARATION OF FLUOROAROMATIC COMPOUNDS AS PLANT GROWTH REGULATORS

Glenn C. Finger*

A. INTRODUCTION

Interest in fluoroaromatic compounds as plant growth regulators first appeared in the 1940's. A post-World War II report[1] indicated that the Army Chemical Corps at Camp Detrick (now Fort Detrick) was already interested in fluorophenoxyacids in 1944. A patent issued to Jones[2] in 1945 mentioned the use of fluorophenoxyacetic acids as herbicides. In 1947 the preparation of the compounds furnished to the Chemical Warfare Service for its initial studies was reported.[3] This latter article is an excellent summary of the various synthetic methods of phenoxyacetic acids. The first data on the herbicidal properties of fluorinated benzoic acids appeared in 1951, a study also pioneered at Ft. Detrick.[4] These references summarize the early background of the fluorobenzenoid herbicides.

B. SCOPE OF THE PROJECT

Since its inception in 1952, the general scope of our project with the Army Chemical Corps has been the synthesis of plant growth regulators containing an aryl or heterocyclic ring with fluorine as the chief substituent or in combination with such common substituents as -Cl, -Br, -I, -OH, $-OCH_3$, $-NO_2$, $-NH_2$, and others. To a limited extent, compounds with fluorine substitution on an atom attached directly to a ring nucleus are also included such as $-CF_3$, $-SO_2F$, etc.

C. GENERAL PLAN OF THE INVESTIGATION

The general plan of investigation is to prepare a representative group of compounds of various types of plant growth regulators. As a continuing objective, additions are to be made to the various types as suitable starting materials and intermediates become available.

The selection of the synthetic methods was governed mostly by the available starting materials and the shortest route. The route was not necessarily the most efficient method of preparation. If a compound gave promising results in screening, then more practical syntheses were in order.

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* Illinois State Geological Survey, Urbana, Illinois.

D. SUMMARY OF COMPOUNDS SYNTHESIZED

The synthesis program to date covers four well-known classes of compounds with plant growth regulator properties, namely the phenoxyacetic acids, the benzoic acids, the phenyl carbamates, and the N-phenyl-glycine hydrazides. Besides the individual compounds and their derivatives, many intermediates in the synthetic scheme were also submitted for screening.

1. Phenoxyacetic Acids

In general, the fluorinated phenoxyalkanoic acids were prepared by the alkaline condensation of a fluorinated phenol with chloroacetic or chloropropionic acid. With the exception of some recently synthesized compounds, most of the compounds have been reported in a 1959 paper[5] from the Survey laboratories.

$$FC_6H_4OH(1,4) \xrightarrow[NaOH]{ClCH_2COOH} FC_6H_4OCH_2COOH$$

4-Fluorophenoxyacetic Acid

More than 40 fluorophenoxyacetic acids and a number of fluorophenoxypropionic acids have been synthesized. Emphasis has been primarily on polyfluoro substitution and combinations of -F with -Cl and -Br. Some other group combinations were included as the intermediates were at hand. Table I lists the compounds that have been synthesized.

2. Benzoic Acids

The majority of the fluorinated benzoic acids were prepared by the following sequence: (a) the introduction of -F, -Cl, -Br, and -I groups in the benzene nucleus of key starting materials via an $-NH_2$ group, followed by (b) the generation of a -COOH group, through oxidation of a $-CH_3$ group (or, in a few cases, $-COCH_3$ and $-CH_2OH$ group) and carbonation of a Grignard reagent.

About 24 halogenated benzoic acids were prepared, of which 20 contained fluorine. Table II lists the di- and tri-substituted benzoic acids that have been prepared.

3. Phenyl Carbamates

The fluorinated N-phenyl carbamates, also known as urethanes or carbanilates, were conveniently prepared by reacting the appropriate fluorinated aniline with ethyl or isopropyl chlorocarbonate in an alkaline medium.[9] The yields varied considerably but 60 per cent was considered average.

$$FC_6H_4NH_2(1,4) \xrightarrow{ClCOOC_2H_5} FC_6H_4NHCOOC_2H_5$$

Ethyl N-(4-fluorophenyl) carbamate

TABLE I. FLUOROPHENOXYACETIC ACIDS

RING POSITIONS	SUBSTITUENTS
2-3	F-Cl CH_3-F
2-4	F-F[a] F-Cl F-Br F-I F-CH_3 F-NO_2 Cl-F Br-F I-F NO_2-F
2-5	F-F Cl-F NO_2-F
2-6	F-F
3-4	F-F F-Cl CH_3-F Cl-F
3-5	F-F
2-3-4	F-F-F F-Cl-F
2-3-5	F-F-F
2-4-5	F-F-F[a] F-Cl-Cl F-Cl-F F-F-Cl Cl-Cl-F[a] Cl-F-F NO_2-NO_2-F Cl-F-Cl
2-4-6	F-F-F F-Cl-Cl Cl-F-F F-Br-Br F-NO_2-NO_2 Cl-F-Cl Br-F-F Br-F-Br NO_2-F-NO_2
3-4-5	F-Cl-F Cl-F-Cl
2-3-4-5	Br-Cl-F-Cl
2-3-4-5-6	Cl-Cl-F-Cl-Cl

a. Also the β-propionic acid derivative.

TABLE II. FLUOROBENZOIC ACIDS

RING POSITIONS	SUBSTITUENTS
2-3	Cl-F Br-F CH_3-F F-Cl Br-NO_2
2-4	F-F F-Cl F-NO_2 F-NH_2 F-Br F-NHAc
2-5	F-F Cl-F
2-6	F-F Cl-F Cl-Cl Br-F Br-Cl
3-4	F-F
3-5	F-F
2-3-4	F-F-F
2-3-5	F-F-F
2-3-6	Cl-F-Cl F-NO_2-F

Thirty fluorinated N-phenyl carbamates have been synthesized, i.e., the ethyl and isopropyl N-phenyl derivatives resulting from 15 fluorinated anilines. Among the mono-substituted compounds, all of the ortho, meta, and para fluoro derivatives and a 3-trifluoromethyl ($-CF_3$) compound have been prepared. The polysubstituted compounds are listed in Table III.

4. N-Phenylglycine Esters and Hydrazides

The first fluorine-containing compound of the phenylglycine type was reported in 1958, namely N-(4-fluorophenyl) glycine hydrazide.[7] A year later N-(3-trifluoromethylphenyl) glycine[8,9] and its amide were reported in the literature.

The N-phenylglycine esters and hydrazides were conveniently prepared by a two-step synthesis. With aniline as a model, the reactions involved are illustrated as follows.

$$C_6H_5NH_2 \xrightarrow{ClCH_2COOC_2H_5} \underset{\text{N-Phenylglycine ester}}{C_6H_5NHCH_2COOC_2H_5} \xrightarrow{H_2NNH_2} \underset{\text{N-Phenylglycine hydrazide}}{C_6H_5NHCH_2CONHNH_2}$$

TABLE III. FLUOROPHENYL CARBAMATES

RING POSITIONS	SUBSTITUENTS
2-3	F-Cl CH_3-F
2-4	F-F Ch_3-F
2-5	F-F F-CF_3 OCH_3-F
3-4	F-F CF_3-F
3-5	CF_3-CF_3
2-3-5	F-CF_3-F
2-4-5	F-F-F

By using a substituted aniline, a wide variation in substituents is possible in the benzenoid nucleus. The anilines were readily converted to glycine esters in 25 to 50 per cent yields. By heating the esters with hydrazine in ethanol, the glycine hydrazides were formed. The hydrazides react quite readily with acetone to give isopropylidine derivatives. Some of these derivatives resulted quite accidentally, but were nevertheless submitted for screening. Table IV shows the compounds prepared.

$$C_6H_5NHCOCH_2CONHNH_2 \xrightarrow{\text{acetone}} C_6H_5NHCOCH_2CONHN{=}C(CH_3)_2$$

N-Phenylglycine hydrazide — Isopropylidine deriv.

5. Miscellaneous Compounds

Among the miscellaneous compounds that have been synthesized are a number of fluorinated indoles, a fluorophenyl phosphinic acid, (2-fluoroethyl) trimethyl ammonium bromide, and some fluorinated pyridine derivatives. The pyridine compounds were made available from a separate Survey project that was initiated in the mid-1950's.

TABLE IV. FLUORINATED N-PHENYLGLYCINE ESTERS AND HYDRAZIDES

RING POSITIONS	SUBSTITUENTS
A. Mono-Substituted Compounds	
2	F Cl Br
3	F Cl Br I CH_3 CF_3[a/]
4	F Cl Br CH_3 CF_3(ester)
B. Di-Substituted Compounds	
2-3	F-Cl Cl-Cl CH_3-F CH_3-CH_3
2-4	F-F Cl-F F-Br Br-F
2-5	F-CF_3 CH_3-CH_3
2-6	CH_3-CH_3
3-4	F-F Cl-F F-CH_3 CH_3-Cl CH3-CH_3 CF_3-F CF_3-Cl[a/] CF_3-Br[a/]
3-5	Cl-Cl

a. Ester only; acetone derivative of hydrazide prepared.

E. SYNTHESES UNDER WAY AND FUTURE PLANS

Plans for the future include (a) the synthesis of new fluorinated types of plant growth regulators, and (b) the occasional preparation of other group combinations in the types of compounds previously discussed.

Attention is being directed to the fluorinated derivatives of the following types of growth regulators: 2-methoxybenzoic acids, 2-methoxyphenylacetic acids, and α-methoxyphenylacetic acids.

LITERATURE CITED

1. Thompson, H.E.; Swanson, C.P.; and Norman, A.G. "New growth-regulating compounds. I. Summary of growth inhibitory activities of some organic compounds as determined by three tests," Bot. Gaz. 107:476-634, June 1946.

2. Jones, F.D. (to American Chemical Paint Company). U.S. Patent 2,390,941, 1945.

3. Newman, M.S.; Fones, W.; and Renoll, M. "New compounds as plant growth regulators," J. Am. Chem. Soc. 69:718-723, March 1947.

4. Minarik, C.E.; Ready, D.; Norman, A.G.; Thompson, H.E.; and Owings, J.R., Jr. "New growth regulating compounds. II. Substituted benzoic acids," Bot. Gaz. 113:2:135-147, December 1951.

5. Finger, G.C.; Gortatowski, M.J.; Shiley, R.H.; and White, R.H. "Aromatic fluorine compounds. VIII. Plant growth regulators and intermediates," J. Am. Chem. Soc. 81:94-101, 1959.

6. Oliverio, Vincent T., and Sawicki, Eugene "Some fluorine derivatives of urethan," J. Org. Chem. 20:363-367, January-June 1955.

7. Tien, N.B.; Buu-Hoi, N.P.; and Xuong, N.D. "Tuberculostatic N-aryl-glycines and derivatives," J. Org. Chem. 23:186-188, 1958.

8. Takeda, Akira "m-Nitro- and m-trifluoromethyl-aryl acids as plant growth regulators," Contrib. Boyce Thompson Inst. 20:191-196, 1959.

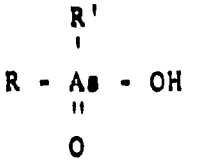
9. Takeda, Akira "α-Substituted aryl-acetic acids as plant growth regulators," Contrib. Boyce Thompson Inst. 20:197-203, 1959.

VIII. ANSUL SYNTHESIS PROGRAM

P. J. Ehman*

A. INTRODUCTION

Our primary job at Ansul is to make a series of pentavalent organic arsenicals called arsinic acids. The general structural formula is:



The two organic groups in these arsenicals, which can be the same or different, are attached directly to arsenic. Because the element arsenic is considered a metal, and the organic groups are combined to arsenic by a carbon-to-arsenic bond, these compounds are called organic-metallics.

In the series of arsinic acids that we are making, the R group is as shown in Table I:

Table I.

R	=	Methyl	=	CH_3 -
	=	Ethyl	=	C_2H_5 -
	=	Propyl	=	C_3H_7 -
	=	Butyl	=	C_4H_9 -
	=	Isopropyl	=	CH_3
		(1-methylethyl)		$CH_3 - CH$ -
	=	Isobutyl	=	CH_3
		(2-methylpropyl)		$CH_3\ CH - CH_2$ -

In this same series of arsinic acids the R' group is as shown in Table II:

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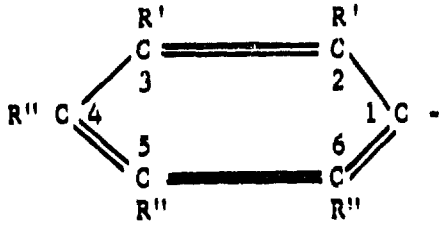
* Ansul Chemicals Company.

Table II.

Same alkyl groups as R
Long-chain alkyls, such as amyl, hexyl, heptyl, octyl, tetradecyl, octadecyl
Unsaturated straight chain, such as vinyl, propenyl, butenyl, allyl, etc.
Alicyclic, such as cyclohexyl and cyclopentyl
Phenyl-substituted alkyl, such as
$PhCH_2$ - , $PhCH_2CH_2$ - , $Ph_2CH_2CH_2CH_2$ -
Phenyl and phenyl-substituted



in which R" can be hydrogen, alkyl such as methyl, amino, substituted amino, nitro, hydroxyl, methoxyl, chloro, fluoro and others, and combinations of these.
Pyridyl and substituted pyridyl
Naphthyl and substituted naphthyl
Miscellaneous

With six R groups and fifty to one hundred R' groups, the combinations that we can produce are from 300 to 600. Unfortunately, many of the R' groups will be extremely difficult to add to arsenic. Our plans call for selecting the easier preparations and spending not more than about one man-week on a preparation that gives difficulties. Therefore, our actual production for the entire contract is expected to be about 80 arsinic acids and about 50 intermediates. We expect that ten to twenty preparations will be dropped because of difficulties.

In the preparation of these arsinic acids we will encounter at least two classes of intermediates. One class, the arsonic acids, have the following structural formula:

Arsonic Acids

in which R' is the series of organic groups shown in Table II. We will submit a sample of any arsonic acid that we prepare or purchase, that Fort Detrick has not already tested.

The other class of intermediates that we will encounter is a series of trivalent organic arsenicals, of which the following are examples:

$R' As = O$	or	$R' As Cl_2$
Arsine Oxide		Arsine Dichloride

In these intermediates R' is again the series of organic groups shown in Table II. Most of the intermediates are volatile, highly toxic, and not satisfactory as herbicides or defoliants. Therefore, only in cases where the volatility is low will we submit samples for screening.

Our contract began in June 1962 and runs through September, 1964. Through June 1963, 42 preparations were submitted, two preparations were dropped because of difficulties, and nine preparations were in process. Our manpower through June 1963 was two chemists and one technician. On 27 June we added a third chemist and the first week in August we will add a second technician.

B. PAST WORK

One of the available methods for making these acids is by application of what has become known as the Meyer Reaction.[1] This is the formation of alkanearsonates from sodium arsenite and haloalkanes. The original work has been much extended by other workers. In particular, Auger[2] has shown, initially, how to use this reaction to prepare arsinic acids.

We have successfully adapted these procedures to our needs and have been able to prepare all of the reported alkylarsonic and arsinic acids that we have tried. In addition, we have prepared a number of new compounds by this method. The reactions involved in this method are shown in the following equations:

$$As_2O_3 + 6NaOH \longrightarrow 2(NaO)_3As \qquad (1)$$

$$RX + NaOAs(ONa)_2 \longrightarrow RAsO.(ONa)_2 + NaX \qquad (2)$$

$$RAsO.(ONa)_2 + 2HA \longrightarrow \underset{\text{alkane-arsonic acid}}{RAsO.(OH)_2} + 2\,NaA \qquad (3)$$

$$RAsO.(OH)_2 + SO_2 \longrightarrow RAsO + H_2SO_4 \qquad (4)$$

$$RAsO + 2NaOH \longrightarrow RAs(ONa)_2 \qquad (5)$$

$$R'X + NaO.RAsONa \longrightarrow R.R'AsO.ONa + Na\,X \qquad (6)$$

$$R.R'AsO.ONa + HA \longrightarrow \underset{\text{dialkyl arsinic acid}}{R.R'AsO.OH} + Na\,A \qquad (7)$$

Using the above approach, we initially synthesized three arsonic acids and purchased three others. From these, we have completed 20 arsinic acid preparations with various combinations of R groups shown in Table I.

C. CURRENT WORK

We are now in the process of completing another group of acids having a variety of other moieties, these include:

Table III.

$CH_2{=}CHCH_2$ -	$C_6H_5.CH_3CH$ -
n-C_7H_{15} -	$C_6H_5CH_2CH_2CH_2$ -
n-C_8H_{17} -	2-$CH_3C_6H_4CH_2$ -
$C_6H_5CH_2$ -	3-$CH_3C_6H_4CH_2$ -
$C_6H_5CH_2CH_2$ -	4-$CH_3C_6H_4CH_2$ -

The Meyer Reaction does not usually work when using haloaromatic compounds if the halogen is attached to the ring.[3] Haloalkanes will, however, react with arsenosoaromatic compounds. We are now also completing a group of arsinic acids in which one organic group is an aromatic ring. The arsenoso materials are obtained by sulfur dioxide reduction of the numerous arsonic acids that are available commercially. The aromatic groups include:

Table IV.

4-H_2N,2-HOC_6H_3 -	4-HO C_6H_4
2-$H_2NC_6H_4$ -	4-HO, 3-$O_2NC_6H_3$
4-$H_2NC_6H_4$ -	2-O_2N C_6H_4
C_6H_5 -	3-$O_2NC_6H_4$
4-ClC_6H_5 -	4-$O_2NC_6H_4$
4-Cl,3-$O_2NC_6H_3$ -	3-$CH_3CO.NH$,4-HOC_6H_3

In addition to these preparations there are others that are not amenable to the above methods. A very valuable method of synthesizing aromatic arsonic and arsinic acids has been developed by Bart.[4] This involves the reaction of a diazonium salt with an alkali arsenite, as shown in the following equation:

$$RN_2{}^+X^- + (NaO)_3As \longrightarrow RAsO_3H_2 + NaX + N2 \quad (8)$$

This process can be extended to form diarylarsinic acids by coupling the diazo compound with an aromatic-arsonite.

$$RN_2{}^+X^- + (NaO)_2AsR' \longrightarrow R.R'AsO_2Na + NaX + N_2 \quad (9)$$

The usefulness of these reactions has been shown by Bart and a number of other workers to be quite great. In our work, we are currently using this reaction to prepare a number of aromatic arsonic acids. These can then be reduced with sulfur dioxide to form the arsenosocompound. This arsenosoarene can then be treated with a haloalkane according to Meyer's Reaction to form an arylalkylarsinic acid. The aromatic moieties involved in this group of compounds include the following:

Table V.

1-Naphthyl	2,4,6-trichlorophenyl
2-Naphthyl	2,3,5,6-tetrachlorophenyl
2-fluorophenyl	2-methyl-4-fluorophenyl
4-fluorophenyl	2,4-dichlorophenyl
	2,4-dichlorobenzyl
	4,methoxyphenyl
vinyl	
pyridyl	

D. FUTURE WORK

We expect soon to complete all of the preparations outlined above. We are now in the process of getting approval for beginning the synthesis of another hundred or so compounds. Our new proposal includes many homologues and isomers of the above compounds. The vast majority of the arsinic acids contemplated will contain one R alkylgroupfrom Table I.

In addition, some pentyl- and hexyl-containing compounds are proposed as well as some containing substituted alkyl groups.

E. PROBLEMS ENCOUNTERED

1. Meyer's Reaction - Arsonic Acids

This reaction is quite straightforward. Bromoalkanes seem to be the most practical for routine laboratory use. Chlorides react usually only slowly and with iodides there is trouble in isolation because of the following reaction.

$$RAsO_3H_2 + 2HI \longrightarrow RAsO + I_2 + 2H_2O \qquad (10)$$

This means that iodides must be removed prior to acidification. However, when the arsonic acid is insoluble in water, it is possible to isolate it by precipitation with a calculated amount of acid.

2. Meyer's Reaction - Arsinic Acids

This reaction is also straightforward. It is attended by many by-products. If the yield is high, there is not much trouble. If the yields are low, isolation and purification present great problems. The by-products are apparently very similar to the product. We have unsuccessfully tried to apply this procedure using bromocyclopentane and bromocyclohexane.

3. Bart Reaction - Arsonic Acids

This reaction, although straightforward on paper, presents all of the usual troubles of ordinary diazonium reactions. It may be thought of as an adaptation of the long-familiar Sandemeyer Reaction. In addition to this, some trouble is encountered with dearsonation during isolation.

4. Sulfur Dioxide Reduction

This procedure works well with alkanearsonic acids and a number of aromatic arsonic acids. Some of these latter appear especially sensitive and either reduce further than the arsenoso compound or dearsonate.

5. General

We are, of course, running into the age-old problem of literature reactions that do not work for us. In addition to this, we give ourselves a problem by examining each compound by acid-base titrimetry using an automatic recording titrator and for organic and inorganic trivalent arsenic. Sometimes we find a preparation that agrees with literature characteristics and has the correct elemental analysis, but proves to contain more acids than we bargained for or a considerable amount of the arsenic content is present in reduced form.

LITERATURE CITED

1. Meyer, G. Title not available, Ber. 16:1439-1441, 1883.

2. Auger, V. Title not available, Compt. rend. 137:925-927, 1903.

3. Rosenmund, Karl W. "Halogen bound to the ring carbon and its replacement by other substituents. III. Preparation of arsonic and sulfonic acids," University of Berlin, Ber. 54B:438-440, 1921.

4. Bart, H. "Ger. pat., 250,264, January 8, 1910," Chem. Abstr. 6:3312, 1912.

IX. PENNSALT SYNTHESIS PROGRAM

Harold J. Miller*

When, a little over a year ago, the Chemical Corps invited proposals for programs to synthesize and screen candidate defoliants and desiccants, Pennsalt submitted a quite detailed proposal. The first proposal was used (and to a somewhat smaller extent is still being used) as a working document for the selection of compounds. It goes without saying, therefore, that a good deal of planning went into the proposal.

Work was started early in July 1962. A stock of Black Valentine beans had been built up just previously, so that synthesis, analytical control, and primary screening could be begun nearly simultaneously. Some range-finding experiments were carried out in the greenhouses until such time as the analytical pipe line had been filled and an assured flow of chemical compounds was established. By range-finding experiments, I mean the evaluation on beans of a number of commercial defoliants and herbicides in various formulations. Results of the extraneous testing have been supplied to Fort Detrick as unnumbered compounds, since we anticipated that the number would be so small that no special numbering would be required.

Analytical confirmation of structures has been, in terms of manpower, a substantial part of the program. In all but a very few cases, we have been able to supply compounds of firmly established identity. In a very few instances we have supplied samples with some uncertainty as to structure with the provision that more detailed analytical work would be carried out if the products proved to have a degree of activity warranting continued interest. Analytical control introduced a necessary lag of one to two weeks, rarely more.

The synthesis program has been under the direction of Dr. Popoff and Dr. Smith, who are here with me today. The selection of compounds for synthesis, both in our planning phase and subsequently, has been to a very large extent the responsibility of these gentlemen with the assistance of Mr. William Lee and myself.

It has been our practice to transmit samples as nearly as possible simultaneously to Fort Detrick and to our own greenhouse for primary screening, and we have shipped samples in weekly lots of 20 to 25 or 30.

Reporting has been accomplished primarily by the use of three forms. A New Product Data Sheet forwarded to cover each compound along with weekly shipments; a Plant Response Data Sheet for each compound, provided with the monthly report; and a Synthesis Report Form describing the method of synthesis for each compound, submitted with the quarterly reports. A somewhat differently laid out Plant Response Data Sheet will be used for reporting supplementary screening on four woody species.

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* Pennsalt Chemicals Corporation.

In order to keep Chemical Corps aware of our program planning and particularly to enable them to avoid duplications between contractors, we have periodically supplied candidate compound lists, indicating the syntheses on which we proposed to work over the succeeding month or so. In a few instances they have called us off on proposed compounds that they had already received or expected from other sources, but there appears to have been surprisingly little overlap.

In no case have we completely fulfilled the plan shown on a candidate compound list, nor should we. Feedback of early screening data is, in our view, of utmost importance. The directors of the synthesis program see results in the greenhouse constantly and have been able to take advantage of these early observations to amend their synthesis programs. In addition, we meet at frequent intervals for detailed formal and informal discussions.

During the first contract year we have synthesized, screened, and submitted 1021 compounds, representing many chemical categories. In addition to screening at 0.1 pound and 1.0 pound per acre equivalent as requested by Chemical Corps, we have also screened at 10.0 pound per acre equivalent. We recognized, of course, that activity disclosed only at this heavy dosage would be of no direct interest but felt that such low-level activities should serve to channel further syntheses. This has proved to be the case. In several instances we have detected activities at this level and have been able to prepare analogs active at 1.0 or 0.1 pound per acre equivalent.

Taking into account this high dosage, 35 per cent of the compounds prepared have shown some activity, 13.3 per cent were active as defoliants at the 0.1-pound dose and an additional 13 per cent at the 1.0-pound dose. A substantial number of the first 750 compounds submitted have been selected by Chemical Corps for woody plant screening. Of the first 250 compounds we have supplied additional quantities of 21 for further evaluation.

For our own use in organizing plant response data, we have adopted a rating system that enables us to separate compounds of 50 per cent or more defoliant activity at 0.1 pound, 70 per cent at 1.0, and 90 per cent at 10 pounds. This gives us compact and consolidated information that is readily usable in the analysis of structure-activity relationships.

Periodically we tabulate for study the compounds in the higher activity categories, further subdivided by structural categories. Although computer equipment is available when needed, we have not to date found it necessary or desirable to employ it in this program. With a much larger number of compounds we would undoubtedly go to computer techniques.

We have welcomed and found most useful the feedback of data from Dr. DeRose and Mr. Ray Frank. In general our findings have corresponded very well in spite of the fact that we are employing quite different procedures. Correspondence has been particularly good on desiccant effects, as should be expected.

The selection of compounds for screening has been made on a number of bases including:

(a) Compounds related to endothall and to butynediol, defoliants that were originated by Pennsalt and that, in Chemical Corps tests preceding the program, had shown promising results.

(b) Compounds more or less related to substances having recognized activity of one sort or another in plant systems.

(c) Leads from Pennsalt screening (on cotton).

(d) Compounds for which a hypothetical mode of activity could be imagined. I emphasize the word hypothetical.

(e) Some compounds representing novel structures whose possible activity in biological systems, insofar as revealed by the literature, was unknown.

Compounds selected for synthesis are searched through the literature (Chemical Abstracts and Beilstein) before work is begun. If the compound has been reported, duplication of several given physical properties is considered adequate to confirm the identity. If the compound is not known, sufficient elemental and spectral analysis to establish identity is carried out. Analytical data are also obtained on known compounds where the literature is vague or contradictory, and where new synthetic approaches are employed.

It may be of interest to note that roughly one-half of the compounds synthesized during the contact year were new compositions of matter; that is, they were unrecorded in the literature.

Pennsalt had, of course, some years earlier, synthesized a number of analogs and derivatives of endothall for the purpose of establishing the areas for patent coverage. These compounds had been evaluated by different techniques from those employed in the present program. Several of the more active of these were resynthesized for screening on Black Valentine beans. In addition, particularly during the first several months of the program, a number of additional endothall analogs were prepared. Some of these have shown significant differences from endothall itself, presumably, however, as the result of the differences in penetration and translocation, the mode of action at the site appearing to be very similar. A few compounds showed promise of providing faster defoliation than disodium endothall.

A few endothall analogs named in our original proposal, or in subsequent candidate compounds lists, have proved very difficult to synthesize and some additional effort will be expended on these.

Several years ago Pennsalt also explored the analogs and derivatives of butynediol, using cotton as the screening plant. A collection of these compounds was offered to Fort Detrick at that time and these were more broadly screened. The structural simplicity of the prototype compound nevertheless made this an area of interest and one that we felt had probably not been thoroughly investigated in other unpublished programs. We anticipated that many of the structures proposed would present synthesis difficulties, as was indeed the case.

A number of butynediol derivatives and analogs have shown activity at 0.1 pound per acre equivalent. Some of these accomplish complete defoliation with no desiccation whatever. Indeed, the leaves may appear more turgid than normal. Their rate of action is, however, slow.

I cannot and should not discuss other structural categories, but it is worthwhile to mention that a number of phosphorus compounds have been included, and that all personnel who may come in contact with these unknowns are checked once a month for cholinesterase level. Our fourth quarterly report classifies compounds for discussion into 39 structural categories including a small miscellaneous section that covers those compounds each of which is a single representative of its class. Since many compounds are polyfunctional, a considerable number require consideration under two or even three categories.

I should add that in addition to the thousand-odd compounds synthesized for the program, we purchased or obtained through private channels about 130 structurally interesting products that were considered to warrant screening in their own right, for the sake of throwing additional light on structure-function relationships, if not with the hope of discovering a significantly active compound. These, therefore, were also screened and reported and samples were numbered and sent to Fort Detrick. These were designated as purchased compounds and were not considered to fulfill any part of our contractual obligations.

X. ETHYL SYNTHESIS PROGRAM

R. D. Closson*

In discussing our synthesis operations under the Army Defoliant Program, I propose to briefly mention pertinent Ethyl background, review the organization of our defoliant program, discuss our general approaches to the synthesis problems, and summarize the work done to date.

From 1947 to 1954, Ethyl Corporation manufactured and marketed BHC. In 1949, we initiated an exploratory program designed to discover new agricultural chemicals. In this program, the synthesis work was done at Detroit and the evaluation work at Boyce Thompson Institute. During the program, we found a number of compounds having high activity, particularly in the areas of defoliants and other plant-growth effects, and also as fungicides.

However, a decision was made in 1954 to stop activity in the agricultural-chemical field, and the exploratory program was terminated. The discoveries resulting from the program were sold to Pittsburgh Coke and Chemical Company, which later transferred them to Chemagro. Two commercial chemicals discovered in our program are today being very successfully marketed by Chemagro. These are DEF, which is extensively used as a cotton defoliant, and Dyrene, which is marketed as a fungicide.

Since most people do not connect Ethyl Corporation with agricultural chemicals, I wanted to point out that we have successful experience in this area of research.

A. ORGANIZATION OF OUR PRESENT PROGRAM

Figure 1 shows the formal organization of our present program.

The program is under the over-all direction of Dr. E.B. Rifkin. The synthesis portion is conducted at our Detroit Laboratories under the supervision of Dr. R. D. Closson, with Dr. J. C. Wollensak reporting to him as Project Leader. In addition, the synthesis program is staffed with three PhD chemists who have had considerable synthesis experience, and they have five assistants. The evaluation portion of the program is conducted at Boyce Thompson Institute under the direction of Dr. P. W. Plaisted. As consultants to the program, we have Dr. W. C. Hall, Dean of the Graduate School of Texas A and M, and Dr. G. T. McNew, Director of Boyce Thompson Institute. Both of these men are well known for their outstanding work on defoliants and other plant-growth effects.

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* Ethyl Corporation.

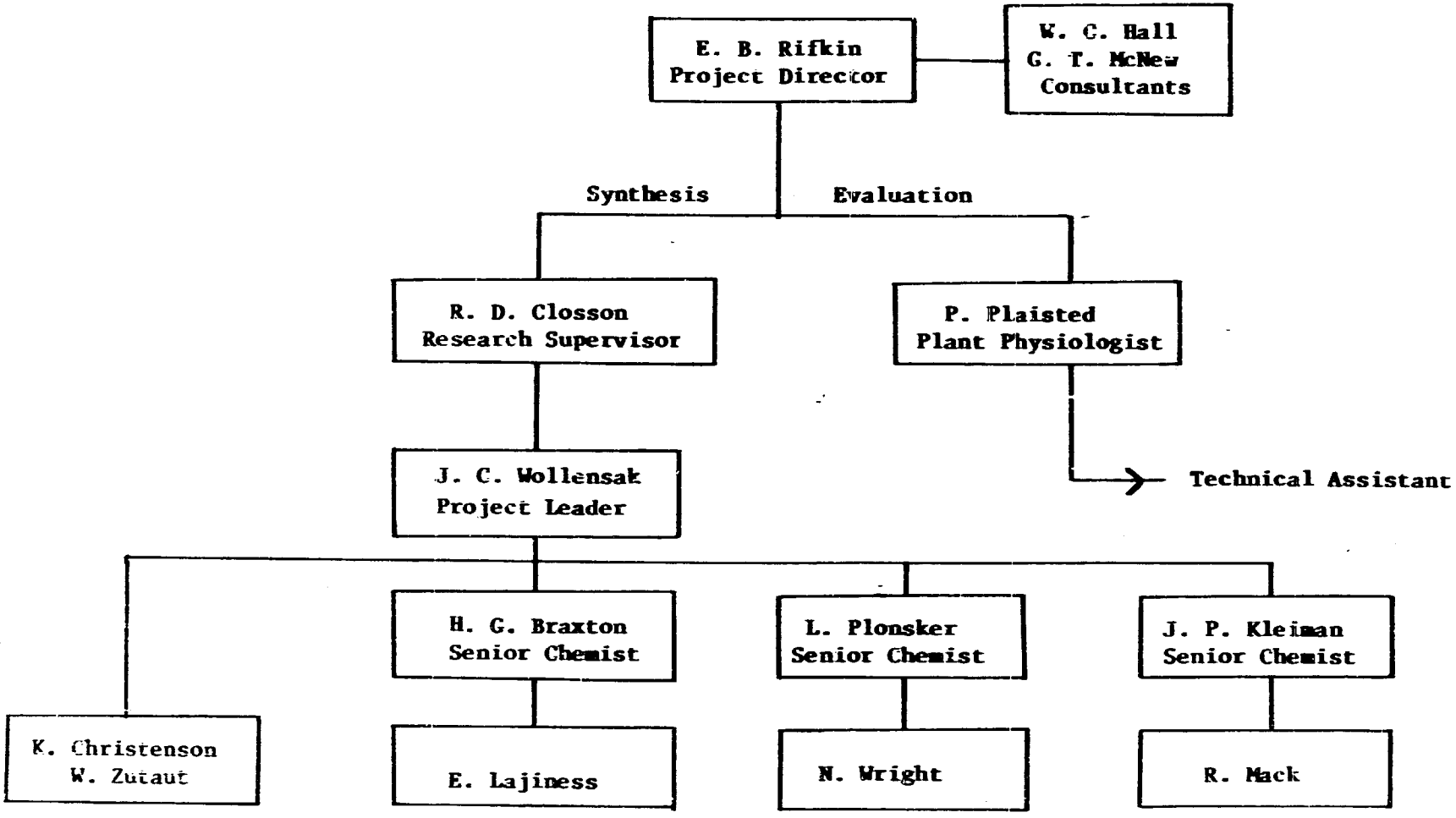

Figure 1. Defoliant Program

Selection of compounds for synthesis is a cooperative operation involving everyone connected with the program.

As a means of discussing our research approach, let's look at Figure 2, which outlines in a very simplified manner the steps leading to the discovery of a new defoliant.

1. SELECT COMPOUND FOR STUDY

2. SYNTHESIZE OR OTHERWISE PROCURE A SAMPLE OF THE COMPOUND

3. SCREEN THE COMPOUND TO ESTABLISH THAT IT IS A DEFOLIANT

4. DEVELOP THE COMPOUND THROUGH FORMULATION STUDIES, FIELD TESTS, PROCESS DEVELOPMENT, DISSEMINATION STUDIES, AND PRODUCTION

Figure 2. Steps Leading to Discovery of an Improved Defoliant for Military Application

Considering briefly each of the four steps outlined in Figure 2, we can ignore Step 4 at present, since it is outside the scope of this conference. Step 3, screening of the compound to establish its effectiveness, is certainly of major importance and is a very difficult problem. However, screening and screening procedures are on the program for discussion tomorrow. Step 2, synthesis or procurement of the compound, although requiring appreciable effort, usually does not present a major problem to a staff of competent synthesis chemists. However, Step 1, selecting compounds that will be effective defoliant compounds, represents the major challenge of the program.

Figure 3 outlines the general approaches we use in selecting compounds for the defoliant program. As I mentioned earlier, the compounds selected represent the combined thinking of our consultants and project personnel.

I. FOLLOW-UP ON LEADS FROM:

(a) PREVIOUS AGRICULTURAL CHEMICAL PROGRAM

(b) RECENT SCREENING RESULTS

(c) LITERATURE

(d) IDEAS BASED ON HYPOTHETICAL MECHANISMS

II. NEW AREA DEVELOPMENT - EXPLORATORY

Figure 3. Basis for Selecting Compounds for Screening

Over the past year, we have supplied 529 compounds for screening. A broad range of compound types has been represented. Figure 4 lists a number of these compound classes.

ACETYLENES	SULFIDES
ALKYLATED AROMATICS	SULFONES AND SULFOXIDES
AMIDES AND THIOAMIDES	SULFONATES
AMINES AND AMINE OXIDES	THIADIAZOLES
BUNTE SALTS	THIAZOLES
CARBAMATES	THIOCARBAMATES
CARBOXYLIC ACID ESTERS	THIOCARBONATES
DIOXALANES	THIOLS
ORGANOMETALLICS	TRIAZINES
ORGANOPHOSPHORUS COMPOUNDS	UREAS AND THIOUREAS

Figure 4. Classes of Compounds Screened

Each of these classes could be broken down further into subclasses, as has been done in Figure 5.

CLASS	ITES	ATES	THIONATES
R_3P	3		
$R_2P(OR)$		3	
$RP(OR)_2$	1	44	7
$RP(SR)_2$	8	4	3
$RP(NR)_2$	6	6	2
RP(SR)(OR)		6	
RP(NR)(OR)		2	
$P(OR)_3$	9	32	4
$P(OR)_2SR$		2	1
$P(OR)_2NR$	3	15	9
$P(SR)_2OR$	2	1	
$P(NR)_2OR$	1		1
$P(SR)_3$	1	2	5
$P(SR)_2NR$		1	1
$P(SR)(NR)_2$		1	
$P(NR)_3$	2	2	10
PHOSPHAZINES	6		
R_4P^+	1		
$(RO)_2PS_2^-$	12		
$RO_2PO_2^=$	3		

Figure 5. Organophosphorus Compounds Screened

In Figure 5, the 222 organophosphorus compounds screened to date are broken down on the basis of structural formula. The numbers of compounds of each structure type screened are shown there.

In terms of numbers of compounds screened, the organophosphorus group is our largest. This is mainly because, at the beginning of the program, we had on hand samples of a large number of organophosphorus compounds that could be supplied to the program.

Arranging our screening results by compound types and studying the activity results in each group frequently permits us to determine what structural characteristics are associated with observed defoliant or herbicidal activity.

However, I do not mean to imply that progress results solely, or perhaps even usually, from the systematic approach. This might be illustrated by the phosphoramidates, of which we had tested 22 compounds, all having no activity. One of the chemists still felt that a particular phosphoramidate was worth testing, since it was spatially related to another active compound. It was tested and found to be active. We have since prepared three more compounds in this class, two of which have shown activity.

B. GENERAL SUMMARY OF PROGRAM

During the past year, we have submitted 529 compounds for screening. The sources of these compounds were:

(a) 393 compounds were synthesized for the project.
(b) 82 organophosphorus compounds available from other laboratory programs were supplied to the program at no cost.
(c) 54 compounds from miscellaneous sources were obtained as samples, purchased, etc.

Although I doubt that we have yet uncovered the compound or compounds that will find application, I think the screening results obtained to date are generally encouraging. Approximately 35 of the compounds submitted to primary screening have shown an appreciable degree of activity on bean seedlings. These data have indicated a number of chemical areas for further investigation. We also were encouraged by some of the results of secondary screening, in which the more active compounds were screened on four tree species. Two of our compounds caused total defoliation of two of the tree species at five pounds per acre and very extensive defoliation at one pound per acre.

In conclusion I want to thank Dr. Minarik, Dr. Brown, Dr. Darrow, Dr. Robinson, and Dr. DeRose for their cooperation and guidance.

XI. MONSANTO SYNTHESIS PROGRAM

Stanley D. Koch*

In picking compounds as candidate defoliants, we have chosen from three main groups: (a) relatives of known defoliants and herbicides, (b) Monsanto Chemical Company hot leads, and (c) a rational screen of novel compounds not likely to have been tested previously for defoliant activity.

We have not put much effort on the first group. We have felt that the industry has exhaustively examined esters and analogs of 2, 4-D and the other well-known commercial products, and that our chances of success in this area were small. Where we have chosen relatives of the known compounds, their activity has not exceeded that of the commercial product.

Monsanto Chemical Company hot leads have provided us with one excellent group, the quaternary ammonium iodides. Since these compounds are disclosed by issued patents, I feel able to mention them by name to this group. I will discuss the activity of some of these compounds in detail in tomorrow's talk. In addition to these compounds, there are three other hot leads that have already been approved for synthesis by the Army. These compounds are now being made and we expect to have the results from some of them in the coming quarter (ending 30 September).

Most of the compounds we have suggested have fallen into the category of our rational screen. We feel that this emphasis is proper, in yielding the greatest chance of making a breakthrough to a new type of effective compound. To date, this screen has given us three leads. Two are metalorganics, with different metal atoms. The third is an organic sulfur compound. Homologs of these leads are being put into the system, and we expect to be able to test many more relatives in the coming quarter.

It is hard to know what the word "rational" means in the phrase "rational screen." Our list of candidate compounds is made up of the contributions from all of our chemists, and I edit their suggestions, sometimes tactfully trying to convince them not to offer some banal chemical function. The case of the three leads that have emerged from our rational screen is instructive: The first metalorganic was one of a large group suggested by one of our less experienced chemists. I felt that he was overdoing this particular group, and after he had suggested 30 or 40 with this particular metal central atom, I instructed him to stop, and move on to some other field of organic chemistry. The following day the screening results on the very active member arrived. This compound is the most active compound we have had all year in the primary screen, and shows high activity against several woody species as well.

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* Monsanto Research Corporation.

Another chemist, this one with long experience, decided to suggest a group of compounds chosen from a cabinet of chemicals he had in his lab. Since he exercised "rational" choice in picking them, I reluctantly decided to let them go through. There was immediate adverse comment from the other experienced professionals, who felt that the list had been cheapened and that this nonsense was sure to make us look foolish to the project monitor. Soon the information came back that one member of that group, another metal-organic, had been previously screened by Monsanto Chemical Company, and was an active defoliant (75 to 100 per cent at six pounds per acre). This class has not been reported active in the public literature.

The third active lead, the sulfur compound, was the one example that had sneaked through when other homologs had been consciously abandoned for fear they would be unstable to hydrolysis. It is quite possible, of course, that this active compound is also hydrolyzed, and that the activity comes from the products of the hydrolysis. In any event, editing out the homologs in an effort to make our list more rational was again the wrong thing to do.

In spite of all this, we still attempt rationally to choose compounds to suggest as candidate defoliants.

Our procedure for making our lists of candidates is as follows: Compounds are suggested by all senior professionals, and occasionally by the younger men. They are instructed to examine their candidates and reject those that are unsuitable because of expected toxicity to mammals, volatility, or instability. After editing in Boston, the list is sent to Monsanto's Sample Record and Control, in St. Louis. Sample Record and Control is an operation that controls and records (as the name suggests) almost all of the samples prepared in the entire Monsanto organization, including subsidiaries. The central feature is a computer system that is also able to handle conventional organic structural formulas. At Sample Record and Control, our candidate list is checked against the large five-digit number of samples Monsanto has already screened for herbicide and defoliant action. If a compound is found to have been tested on Monsanto's proprietary screen, it is dropped from our test list, so as to avoid needless duplication, but if active, its structure is noted, and homologs are submitted so as to introduce this activity into the contract program. About three per cent of the compounds on our lists have been rejected because of this type of duplication.

The culled list is then sent to the Army for approval. Our experience is that the Army has rejected about one per cent of the compounds we have suggested, presumably because of duplication with compounds they have tested, or which were suggested by other contractors.

Synthesis is carried out in our laboratories in Boston and Dayton, Ohio. About 20 per cent of the work has been done in Dayton. My authority is delegated to three senior professionals in Boston and one in Dayton who do the day-to-day bench supervision of the synthesis. Each chemist usually has the full-time use of a laboratory assistant, and the exclusive use of one hood.

Syntheses are carried out at the quality level required for work reported in the Journal of Organic Chemistry. Elemental analyses and structure proof are required for new compounds, and no compound is submitted unless it has been unequivocally characterized. Although high purity is sought, it is recognized that this is a screening program. Where further purification is not justified or practical, impure samples have been submitted with the degree of purity carefully noted.

We ship samples to the Army weekly, and have shipped 757 in about seven months. This extrapolates to a rate of 1335 per year. Of this number, about ten per cent were purchased, or acquired without synthesis. Ironically, so far these have proved to be more active, because they usually represent feedback from screening results or Monsanto Chemical Company data.

We hope to be able to maintain this quantitative level of production, and that the number of active compounds will rapidly increase as we get more feedback from screening results.

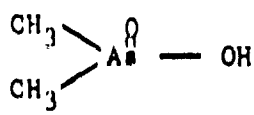
XII. PREPARATION OF NEW ARSINIC ACIDS AND ESTERS

M.E. Chiddix*

General Aniline has been screening new organic compounds for use as herbicides for a number of years. Our interest in the present contract work was twofold. First, we had found a number of biologically active groups in the course of our herbicidal screening, and, secondly, we had in the company a man with an unusual background of experience in arsenic chemistry, Dr. J.F. Morgan. Dr. Morgan worked with Prof. Hamilton at Nebraska during the war on organic arsenicals, and is co-author of the chapter in "Organic Reactions" on the Bart Reaction for the Preparation of Aromatic Arsonic and Arsinic Acids.

In Figure 1, there are listed two very effective defoliants, cacodylic acid (dimethyl arsinic acid) and butynediol. The first, as you know, has been found to be an effective defoliant, and the gentlemen from Ansul have described some variations on its structure, which they are under contract to prepare. The second compound, butynediol, is manufactured by GAF. Unfortunately, its excellent defoliant properties were discovered by Pennsalt and not by GAF.

Introduction



Cacodylic Acid

Butynediol

Proposed

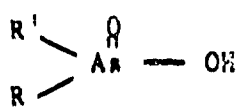


Figure 1. Preparation of New Arsinic Acids and Esters.

In our contract work, we propose to vary the alkyl groups of the arsinic acids through the incorporation of biologically active groups, particularly those containing triple bonds and related to butynediol. Other active substituents to be used will be illustrated later.

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* General Aniline & Film Corporation.

The methods for the preparation of these arsinic acids will be described. In Figure 2 are shown the reactions for the preparation of alkyl arsinic acids and their intermediates. In the first step, sodium arsenite is treated with an alkyl halide, preferably a bromide in the Meyer Reaction, to produce alkyl arsonic acid salts. In the second step, these salts are reduced with sulfur dioxide in hydrochloric acid solution to produce alkyl dichloroarsines. This intermediate is then converted with caustic to the disodium alkyl arsonite. If this compound is then alkylated again with another alkyl halide, dialkylarsinic acids are produced. This is an extension of the Meyer Reaction.

Alkyl Intermediates

1) $As(ONa)_3 + RX \longrightarrow RAsO(ONa)_2$ (Meyer Reaction)

2) $RAsO(ONa)_2 + HCl + SO_2 \longrightarrow RAsCl_2$

3) $RAsCl_2 + NaOH \longrightarrow RAs(ONa)_2$

Alkyl Arsinic Acids

4) $RAs(ONa)_2 + R'X \longrightarrow \begin{matrix} R' \\ R \end{matrix}\!\!>\!\! AsO_2Na$ (Meyer Reaction)

Figure 2. Preparation of Arsinic Acids

In Figure 3 are shown the reactions for the preparation of aromatic arsinic acids. In the first reaction, sodium arsenite is treated with benzene diazonium chloride to produce a phenyl arsonic acid salt by the Bart Reaction. This can then be reduced in the same way as the alkyl derivatives to form phenyl dichloroarsine and neutralized to form the disodium phenyl arsonite. There are two ways to prepare arsinic acids containing one aryl group. In Reaction 4, an alkyl halide can be used to alkylate the phenyl arsonite producing an alkyl phenyl arsinic acid. Another method is illustrated in Reaction 5, in which an alkyl arsonite is treated with an aromatic diazonium chloride (Bart Reaction). If R is aromatic, a mixed diaromatic arsinic acid can be produced.

In Figure 4, a number of the proposed variations on dialkyl arsinic acids are illustrated. R_1 is a lower alkyl such as methyl, ethyl, propyl, or butyl. R_2 is a more active substituent such as propargyl, butynyl, hydroxylbutynyl, or chlorobutynyl. R_2 can also be allyl, hydroxyethyl, or 2-(2-pyrrolidinon-1-yl)ethyl.

Aryl Intermediates

1) $As(ONa)_3 + C_6H_5N_2Cl \longrightarrow C_6H_5AsO(ONa)_2$ (Bart Reaction)

2) $C_6H_5AsO(ONa)_2 + HCl + SO_2 \longrightarrow C_6H_5AsCl_2$

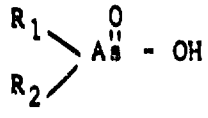
3) $C_6H_5AsCl_2 + NaOH \longrightarrow C_6H_5As(ONa)_2$

Aryl Arsinic Acids

4) $C_6H_5As(ONa)_2 + RX \longrightarrow C_6H_5(R)AsO_2Na$

5) $RAs(ONa)_2 + O_2N\text{-}C_6H_4N_2Cl \longrightarrow O_2N\text{-}C_6H_4\text{-}AsO_2Na$ (R above the ring) (Bart Reaction)

Figure 3. Preparation of Arsinic Acids

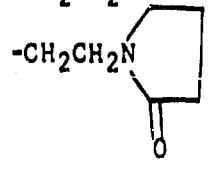


R_1 = Lower Alkyl

R_2 = $-CH_2C \equiv C - R_3$

$-CH_2CH = CH_2$

$-CH_2CH_2OH$



R_3 = H, CH_3, CH_2Cl, CH_2OH

Figure 4. Proposed New Aliphatic Arsinic Acids

In Figure 5, some specific examples of these variations are given. No. 1 is the propargyl methyl arsinic acid, which can be prepared through the Meyer Reaction with propargyl bromide. No. 2 is the hydroxybutynyl chloroethyl arsinic acid prepared from the bromohydrin shown. No. 3 is a hydroxypropylbutyl arsinic acid from propylene oxide. No. 4 is allyl methyl arsinic acid from allyl bromide, and No. 5 is a difunctional arsinic acid from the reaction of two moles of sodium methyl arsonite and one mole of the dibromobutene. Some additional examples are shown in Figure 6.

1) $HC \equiv CCH_2(CH_3)AsO_2H$ from $HC \equiv CCH_2Br$

2) $HOCH_2C \equiv CCH_2(ClCH_2CH_2)AsO_2H$ from $HOCH_2C \equiv CCH_2Br$

3) $CH_3CHOHCH_2(C_4H_9)AsO_2H$ from $CH_3\underset{\underset{O}{\diagup\diagdown}}{CH}CH_2$

4) $CH_2 = CHCH_2(CH_3)AsO_2H$ from $CH_2 = CHCH_2Br$

5) $HO_2As(CH_3)CH_2CH = CHCH_2(CH_3)AsO_2H$ from $BrCH_2CH = CHCH_2Br$

Figure 5. Examples of Aliphatic Arsinic Acids

1) $HOCH_2CH_2OCH_2CH_2(C_4H_9)AsO_2H$ from $HOCH_2CH_2OCH_2CH_2Br$

2) $HO_2As\begin{matrix}CH_3 \\ CH_2CH \\ | \\ OH\end{matrix} - \begin{matrix}CH_3 \\ CHCH_2 \\ | \\ OH\end{matrix}AsO_2H$ from $(ClCH_2\underset{\underset{OH}{|}}{CH}\rightarrow_2$

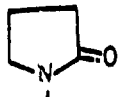
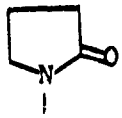
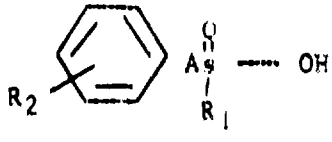
3)  $CH_2CH_2(C_2H_5)AsO_2H$ from  CH_2CH_2Cl

Figure 6. Examples of Aliphatic Arsinic Acids

The variations proposed with aromatic arsinic acids are shown in Figure 7. R_1 will be a lower alkyl group and the other substituent a substituted benzene where R_2 may be chloro, nitro, methoxy, or sulfonamide. The work on chloro and nitro substituents will be emphasized.



R_1 = Lower Alkyl

$-CH_2C \equiv C - R_3$

$-CH_2CH = CH_2$

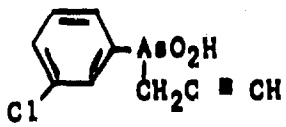
$-CH_2CH_2OH$

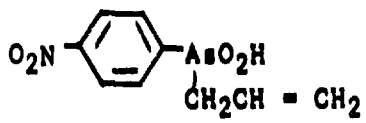
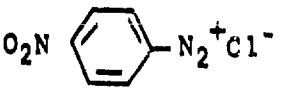
R_2 = Cl, NO_2, CH_3O-, $-SO_2NH_2$

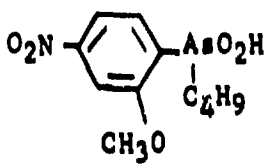
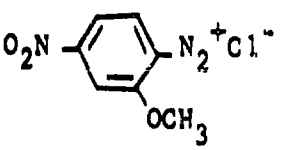
R_3 = H, CH_3, CH_2Cl, CH_2OH

Figure 7. Proposed New Aromatic Arsinic Acids

Some specific examples of aromatic arsinic acids are shown in Figure 8. Chlorophenyl propargyl arsinic acid is to be prepared from propargyl bromide. Allyl p-nitrophenyl arsinic acid will be prepared from the allyl arsonite through the Bart Reaction. No. 3, 2-methoxy-4-nitrophenyl arsinic acid, can be prepared from the butyl arsonite, and No. 4, 2-hydroxyethyl-o-chlorophenyl arsinic acid, from ethylenechlorohydrin using the Meyer Reaction.

1)  from $HC \equiv CCH_2Br$

2)  from 

3)  from 

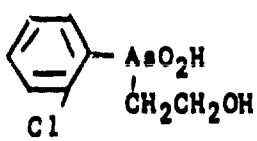
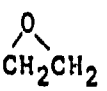
4)  from $ClCH_2CH_2OH$ or 

Figure 8. Examples of Aromatic Arsinic Acids.

Since our contract work just started on July 1, 1963, very few compounds have been prepared thus far, but Figure 9 shows five intermediates that are being prepared at the present time. They are the ethyl, phenyl, allyl, butyl, and 2-chloroethyl arsonic acids.

1) $C_2H_5AsO_3H_2$

2) $C_6H_5AsO_3H_2$

3) $CH_2 = CHCH_2AsO_3H_2$

4) $C_4H_9AsO_3H_2$

5) $ClCH_2CH_2AsO_3H_2$

Figure 9. Intermediates Being Prepared.

XIII. CROPS DIVISION SCREENING PROGRAM

Edward L. Robinson*

The basic goal of our program here at Fort Detrick is a rapid-acting defoliant capable of use in vegetational types throughout the world. Such a defoliant may have the capability of rapid defoliation of tropical or temperate vegetation with subsequent recovery, or it may cause the plants to die following initial defoliation. True defoliants or desiccants would fit the first category; systemic or growth regulant herbicides would be examples of the second type.

My discussion of the defoliation program will be a general summary of research at Fort Detrick. Details of test procedures and results will be explained by other speakers, Mr. Ray Frank and Mr. Demaree. We hope that our presentation will stimulate discussion on how to improve our program. It is possible that changes in screening procedures such as the use of different plant species or techniques may be desirable. Your comments and questions on the program will be appreciated in the interests of improvement.

As most of you know, I am relatively new in the program here. As a newcomer, I have been greatly impressed at the interest shown in the defoliation program at this meeting. We feel that, with the level of research effort given to defoliation in the program at Fort Detrick, a substantial contribution can be made not only from the standpoint of military use, but in agricultural applications. Through the synthesis and screening program, new potential herbicides and chemicals of specific value in agriculture may be discovered.

The initial step in the research program in defoliation at Fort Detrick consists of synthesis of new chemicals -- candidate defoliants, desiccants, or herbicides. Synthesis effort is conducted under contract with several major chemical companies. In the larger contracts, a screening program is carried out by the contractor on 14-day-old Black Valentine beans and on selected woody species in close liaison with the Chemical Branch personnel at Fort Detrick. Under other contracts, the synthesized chemicals are submitted directly to Fort Detrick for initial screening.

It should be emphasized that full consideration is given to sources other than government synthesis contracts for candidate defoliants. Close scrutiny is given lists of new chemicals received by the Industrial Liaison Office (ILO) at Edgewood Arsenal for a chemical with possible defoliant activity. Large numbers of these chemicals from many industrial sources are evaluated for herbicidal and defoliant activity in our screening program.

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* U.S. Army Biological Laboratories.

All chemicals that are received by the Crops Division from synthesis, ILO, or other sources are subjected to a primary screening program under the direction of Dr. DeRose. This screening is conducted on one-week-old plants using six plant species, four broadleaf species and two grasses. The activity of each chemical is rated on a scale of one to four on each species for a maximum rating of twenty-four points. Chemicals are applied in acetone solutions at rates of 0.1 and 1.0 pound per acre. Ratings are made over a period of two weeks.

The next step in the defoliation screening program involves tests on 14-day-old Black Valentine beans at similar application rates. Chemicals tested in this program include those with an activity rating of ten or more in the primary screening program on six plant species. All chemicals showing activity in screening programs conducted by contractors are tested at Fort Detrick. Details and results of these tests will be described by the next speaker, Mr. Roy Frank.

Chemicals that exhibit moderate or extreme activity in defoliant or contact action on 14-day-old Black Valentine beans are then subjected to secondary screening on six or eight woody species in greenhouse tests. Plants used include two- to three-year-old transplants of conifers and hardwoods. Defoliation or contact activity is evaluated on all woody species over a period of 21 days. Plants are normally retained for longer periods to determine ultimate lethal or recovery effects of treatment. This type of secondary screening has been conducted in-house on newly synthesized chemicals. Six hundred chemicals that showed defoliant or herbicidal activity in earlier research at Fort Detrick were evaluated on woody species under contract.

Chemicals selected on the basis of activity in the greenhouse tests on woody species are then carried forward to field screening trials. A subsequent speaker, Mr. Demaree, will report on this phase of the 1963 program. For this phase, we are currently developing a nursery of selected woody species.

The final step in the testing program would involve application on a field-scale program on native vegetation using airplane dissemination equipment. We are hopeful that this program can be carried out at several locations representative of major vegetational types of the world. Furthermore, formulations and mixtures of chemicals will be evaluated at various rates, volumes, and seasons of application as a basis for selection and standardization of a defoliant.

XIV. DEFOLIATION SCREENING PROGRAM

J. Ray Frank*

The compounds reviewed in our defoliation screening program come from many sources. One of the main sources in the last two years is from the companies represented here today in the chemical synthesis program. The Industrial Liaison Program provides an additional source of compounds originating from many chemical companies. We also receive compounds from many private companies as parts of unsolicited proposals, from individuals who are working in other areas of chemical synthesis, and from organizations with many different types of screening programs. The combined results of all these programs should have the potential to produce effective candidate defoliants, herbicides, and/or target markers.

A. INTRODUCTORY SCREENING

Compounds from all sources are assigned a Fort Detrick accession number and are given an introductory screening by Dr. DeRose. He evaluates the compounds on seven-day-old plants on six crop species. Compounds are rated on a numerical basis, with 24 points equaling a perfect score. Results are submitted to the defoliation screening group, and all active compounds with a numerical rating of ten or above are then tested by this group. Compounds that indicate activity in the screening programs of individual contractors are also tested. Thus, any differences in the results of the two programs due to formulation or greenhouse technique may be resolved. We have noticed in our tests that formulation has been a factor in some situations; therefore, all compounds showing activity in preliminary tests are further evaluated.

B. PRIMARY DEFOLIATION SCREENING

In our primary defoliant program we use 14-day-old Black Valentine bean seedlings. At this age, plants have primary leaves, with one fully expanded trifoliolate leaf and the second trifoliolate well-formed. The plants are placed in a clear plastic spray cabinet in an area measuring one-third of a yard square. A glass spray atomizer is held above the plants and the compound is sprayed uniformly over the area at rates of 0.1 and 1.0 pound per acre.

We are searching for a rapid-acting defoliant that will cause abscission of all leaves on any woody or herbaceous plant. The goal of our program is to find that defoliant, but we also are investigating desiccants, herbicides, and target-marking chemicals. Earlier, it was mentioned that tributyl phosphate could serve as a target marker; 3-amino-1,2,4-triazole

* U.S. Army Biological Laboratories.

could also serve in this capacity because of the unusual foliage color exhibited after treatment.

Defoliant activity is measured as slight, moderate, and extreme. On this basis, slight is 1 to 25 per cent moderate is 26 to 75 per cent, and extreme 76 to 100 per cent.

Because we are investigating compounds that cause an extreme response, we evaluate only those compounds in the secondary screening program that indicate a degree of defoliation or kill the plants within 14 days. This brings up the question, "What is a defoliant?" Commercially, many compounds known as defoliants are not defoliants, but desiccants. In my tests, a compound is an active defoliant if the leaves abscise from a plant without the aid of any mechanical force such as hitting.

Approximately 1600 compounds have been examined since July 1961, and the results entered in a Remington-Rand computer system. Of these 1600 compounds, 100 showed defoliant activity and 300 exhibited herbicidal effects in the primary defoliation screening.

C. SECONDARY SCREENING

All compounds showing defoliant or herbicidal activity in the primary defoliation screening are evaluated in a secondary screening program involving woody plants. Well-established two- to three-year-old seedlings of Norway spruce, Canadian hemlock, Chinese elm, black locust, Norway maple, pin oak, Scotch pine, and California privet are treated under greenhouse conditions at one, five, and ten pounds per acre. With this selection of species and rates we feel that we are able to evaluate the activity of the compound. Some species are easily damaged, for example, black locust and Chinese elm. Frequently, oak and California privet show little damage from chemical sprays, but the one species that is least affected is the Scotch pine.

We are in the elementary stages of the secondary screening program, but to date we have tested about 300 of the 400 compounds that are active as defoliants and/or herbicides. We do not have any final results to give you today, but this information should be forthcoming in the near future. Our general observations at this time indicate that very few compounds will defoliate all eight species and inhibit future leaf growth. Because of the possibility of regrowth, we keep our trees as long after treatment as greenhouse space allows, approximately 90 days.

Perhaps I should mention a few of the main groups of compounds that we have investigated. These groups are not in any particular order and the list does not cover all of them. As you know, the inorganics have been used as defoliants and desiccants in the past. In this group we find the chlorates and thiocyanates, many of which have been included in our screening program. Dr. Finger mentioned the fluorine compounds; we have screened a large number of this group. Benzoic acids have always found a place in the group of effective growth regulators. Many urea

compounds have been investigated. The organometallic compounds that were discussed here yesterday, such as tin, lead, mercury, silver, selenium, and quite a few others, have been tested. The carbamates have been checked extensively.

Acetic acids of various types, such as trichloroacetic acid (TCA) and various phenoxy compounds, have been tested. The phenoxy compounds have been screened from herbicidal, anticrop, and defoliant standpoints. We have tested butoxy ethyl, n-butyl, and iso-butyl esters of 2,4-D and 2,4,5-T on seedling trees with 32 of the most active defoliants that produced defoliation in previous screening programs. In this particular test, the results indicated the butyl esters were more active than the butoxy ethyl esters.

Compounds containing phosphorus have been investigated extensively. Interest in this group was initiated because of the presence of a number of commercial compounds such as DEF, Folex, and tributyl phosphate. The arsenical group has been investigated in connection with cacodylic acid. The diols have been checked extensively because of the activity of butyne 1,4-diol. The endothal group is being investigated extensively; some of the new forms are of interest to us.

I should point out that we are indebted to Mr. Morneweck, Mr. Hall, and Mr. Buschmann, who deserve much credit for their work in this program.

XV. PENNSALT SCREENING PROGRAM

Harold J. Miller*

To begin with, our contract and, I think, the other industrial contracts as well, called for synthesis and primary screening only. Initially the screening was to be carried out on Black Valentine beans; currently the program includes supplementary screening on a variety of woody plants. The methods to be used in the primary screening were rather clearly indicated in the original invitation for proposals, that is, as to age of plants and duration of observations. We have followed these procedures very closely, with the addition of a ten-pound dosage rate.

In view of the Chemical Corps goal, we were at first inclined to question the desirability of a three-week observation period, but we have found that, with some structural categories, this extended observation period is most significant. The continuous use of control plants assures us that, under our greenhouse conditions, little or no normal leaf drop occurs over the three-week period and that it can be differentiated from induced defoliation. There are, of course, some questionable cases. We do now feel, however, that as in the high dosage, a third week of observation is desirable, to disclose minor effects that may be turned to advantage through additional syntheses.

We feel that the primary screening, particularly as supplemented by the six-crop screening, the confirmatory screening on beans, and the supplementary screening on woody species, provides an excellent characterization of the compounds tested; it does, that is, what a screening is expected to do. For the purpose of saying "no further interest" or "continuing interest," we believe that the holes in the screen are about the right size, but we must not forget the inherent limitations of a screening program.

Our contracts, then, have stipulated screening, and we could have complied with their legal requirements by conducting this screening in the simplest possible yes-or-no terms. We should not, however, have been doing the best possible job for the Chemical Corps without extending the terms of our evaluation somewhat.

First, we have felt that it was essential to adopt a number of type formulations selected on the basis of the structure, solubilities, and occasionally the reactivity of the particular compounds to be tested. These formulations include:

(a) Solutions in water including, when appropriate, the addition of acid or base to form water-soluble salts.

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* Pennsalt Chemicals Corporation.

(b) Dispersions in water made by ball-milling, sometimes including the addition of a surfactant to aid dispersion, or a colloidal suspension stabilizer.

(c) Solutions in water and water-miscible solvent, e.g. alcohol, acetone, or diacetone alcohol.

(d) Solutions in xylene, subsequently emulsified in water with the aid of a non-ionic surfactant.

(e) Solutions in active solvent with subsequent dilutions in the same non-phytotoxic solvent. (A variety of solvents including butyl Cellosolve, dimethylformamide and cyclohexanone can be used as solvents in quantities as high as 90 pounds per acre equivalent with no significant phytotoxic effects.

(f) Solutions in active solvent subsequently diluted with non-phytotoxic oil.

One can offer objection to the use of a variety of formulations, since compounds are thus evaluated in variable terms. We think that this is not very important in a screening program. In particular, when it is desired to make immediate use of the response data for further synthesis planning, we feel it is important to disclose by formulating expedients whatever activity the compound under test may possess.

In addition to the thoughtful selection of an initial formulation, we very often screen in two or more formulations, reporting these to the Chemical Corps by means of additional plant response data sheets bearing the code number followed by A, B, etc. It is usual to observe differences in plant responses evoked by different formulations, and on occasion differences of an order or more are observed.

In general we prefer the use of solution formulations to emulsion formulations, although the latter are undeniably convenient. We employ ball-milled suspensions in water only when it is unfeasible to use another type of formulation. We like to avoid the use of straight acetone formulations because under our conditions much of the solvent may be lost in the spray application and some of the dosage may reach the leaves in dry particulate form, which does not even make good contact. We avoid in general the use of dispersions made by dissolving the compound in water-miscible solvent and precipitating by dilution in water, since we feel that these may tend to give irregular dosage.

We may sometimes use other formulating expedients such as addition of an antioxidant or adjustment of pH.

A few additional screenings have been run on combinations of active agents where there was reason to hope for a synergistic effect.

Other than the variables that have been introduced through formulation, we have carried out a limited number of extra tests differing from the standard screening.

To preclude any possibility of traces of material being deposited on the soil and made available for root absorption, we have sometimes shielded the soil. In a few cases we have elevated temperatures above the normal greenhouse environment. In some cases we have shielded terminal growth during spraying to observe possible upward translocation.

All observed plant responses are noted and reported, including abscission, desiccation, chlorosis, deformation, plant kill, and effects on trifoliate growth.

Supplementary screening has now been begun on two broad-leafed deciduous trees, Chinese elm and Norway maple, and on two broad-leafed evergreens, English laurel and Euonymous. Two dosages only are used, one pound and five pounds per acre equivalent, and observations are continued for four weeks. As above, all responses are recorded. Plant specimens are discarded after a single use.

About ten per cent of the compounds that we have submitted appear to warrant this broader spectrum re-evaluation.

As is to be expected, performance on these test plants is quite different from that on beans; the maple and, in particular, the elm respond to many of the same compounds, whereas the broad-leafed evergreens are quite resistant to defoliation.

All of us here who have been connected in any way with screening programs are well aware of their limitations, and the comments that I am about to make have no great originality.

A screening program that depends on random compounds such as might be selected from a collection of chemical catalogs in a purely hit-or-miss fashion must depend on luck to be successful. A somewhat higher percentage of active compounds can be expected from a screening program that can impose thoughtful selection on a large enough collection of random compounds, even if those making the selection must admit to a considerable uncertainty as to mechanism of the desired action. Further, an important increase in the number of hits is to be expected from collections of compounds made for the screening program, for here the selector is not limited to immediately available compounds and can draw on whatever knowledge he may have as to mechanisms, or more or less remote analogies, or on God-given intuition.

Now, if there can be a feedback of screening data followed by further directed synthesis, another gain should be scored and we have far more than luck to rely on.

Chemical Corps has depended on its contractors to bring some measure of sophistication to bear on the selection of compounds, if not specific knowledge of the mechanism of defoliation, at least broad familiarity with the chemistry of plant-growth modifying compounds.

The principal limitation of a screening program like ours is that it gives a measure of final, grossly observable effect, but no insight whatever into the nature of the separate physiological and biochemical events that culminated in that effect. We cannot avoid the view that a successful defoliant must do three things. In simplest terms it must get into the leaf, it must move some distance, it must cause defoliation. The final effect may presumably take place directly or indirectly. That is, the compound may serve in its own right to activate the abscission area or it may trigger a chain of events on the molecular level that ultimately results in damage to this cell community, or alternatively perhaps, accelerates normal events to a normal conclusion. I am, of course, speculating.

We have all observed that leaf desiccation by chemicals is often a very rapid process. It can sometimes be observed within an hour of application. One can guess that in such cases the effect is direct rather than via a triggered chain. Translocation is presumably not necessary and is probably more or less inhibited by the desiccation. On the other hand, defoliation unaccompanied by desiccation usually involves a lag period, during which various subtle events must be taking place.

Much valuable work has been done and is being done to improve our understanding of the phenomena of natural and induced leaf abscission. The problem has not, however, attracted the massive effort that has been brought to bear on other plant-growth control problems. We sincerely hope that the current program may engender greater efforts to elucidate the nature of the processes involved, from the points of view of both the plant physiologist and the plant biochemist.

XVI. ETHYL SCREENING PROCEDURES

J.C. Wollensak*

Dr. Closson discussed the synthesis phase of Ethyl Corporation's defoliant program, and now I plan to tell you something of the screening procedures that are being used to evaluate the potential defoliants.

The screening portion of this contract is being handled by the Boyce Thompson Institute for Plant Research in Yonkers, New York.

Dr. George McNew has been Managing Director of Boyce Thompson Institute for more than 12 years and has been in over-all charge, during this time, of many screening programs for determining defoliant, herbicidal, growth-regulation, and other plant effects of chemical compounds.

The personnel at the Institute are currently evaluating agricultural chemicals for three industrial sponsors. These screening studies are backed up by the Institute's fundamental studies on the metabolism of herbicides and the mode of action of fungicides. In recent years, experimental studies have been conducted on the mechanism of abscission, both natural and induced, on maple trees and cotton plants. The object of this work has been to determine the difference in abscission processes between different plants. These defoliation studies were under the direction of Dr. Plaisted, who is also directing the screening activity under the present contract.

In the several-year period up to 1953, an extensive screening program on agricultural chemicals was conducted at Boyce Thompson Institute under the sponsorship of Ethyl Corporation. This program, which involved screening chemicals for defoliant action and other agricultural activity, included primary studies on bean plants, secondary tests on cotton, and field tests on cotton. Dr. Closson has already mentioned the thiophosphate defoliant, now commercially known as DEF, which was one of the results of this work. Dr. Plaisted was in charge of defoliant testing during the major portion of this agricultural chemical program. Dr. Plaisted has conducted research on the biochemical changes accompanying abscission, on free nucleotides in plants, and on relative phytotoxicity of triazine herbicides and plant metabolism of these compounds.

During the early phases of the present defoliant screening program being conducted under Army contract, a number of changes were made in the primary screening procedure. These changes were worked out jointly by Dr. Plaisted at Boyce Thompson Institute and by people at Ethyl and the Army Biological Laboratories. We were attempting to devise a convenient

* Ethyl Corporation.

and efficient screen that would identify the greatest number of compounds having defoliant activity and, at the same time, indicate the relative effectiveness of the compounds on a species less susceptible to chemical defoliation. We also desired a primary screening procedure different enough from that conducted under Dr. DeRose at the Army Biological Laboratories to provide a maximum of information on each compound, with some overlap to check uniformity of techniques.

Table I shows the primary screening procedure that evolved early in the program, which we are presently using. Two formulations of the compound to be screened are made up in water, one at a concentration of four milligrams per milliliter and the other at a concentration of 0.4 milligram per milliliter. To each of these solutions is added 0.04 per cent of the wetting agent Triton X-155. The compound also is formulated in acetone at a concentration of four milligrams per milliliter, with the same concentration of Triton X-155. Twelve milliliters of formulation sprayed on one-third of a square yard is equivalent to one pound per acre of the compound at the high concentration and one-tenth of a pound of the compound at the low concentration.

TABLE I. PRIMARY SCREENING PROCEDURE

SPECIES	FORMULATIONS	CONCENTRATION, pound per acre
PRIVET, 8 to 12 inches	WATER	1.0 AND 0.1
	ACETONE	1.0
BLACK VALENTINE BEAN, 14-Day	WATER	1.0 AND 0.1
	ACETONE	1.0

Four plants are placed in a plastic enclosure with a floor area of one-third of a square yard. Then they are sprayed by atomizing, through the top of the spray chamber, each concentration of the formulated chemical. The spray chamber and spray guns are essentially indentical to those used at the Army Biological Laboratories by Mr. Frank. The three formulations, two in water and one in acetone, are each sprayed on two 8- to 12-inch privet plants and on two 14-day-old Black Valentine bean seedlings. In all, a total of 12 plants are used in the primary screening of each compound. The choice of these two species lies partially in the fact that the bean seedling is relatively easy to defoliate chemically; defoliation of the privet is

considerably more difficult. Compounds that are phytotoxic to privet or cause foliar abscission of privet most often result in more severe damage to the bean seedlings.

The effect of the chemical treatment on the plants is observed in the greenhouse after about 3, 8, and 14 days. Abscission is recorded in the case of bean seedlings, as a percentage of both primary and secondary leaves abscised. Abscission of privet is recorded as none, light, medium, severe, or total. Those plants that show toxic symptoms caused by the chemical are rated on a scale ranging from zero for no damage to eleven for dead leaf. Each experiment is terminated at the end of 14 days, since the chances are slight of chemical defoliation or other effects occurring 14 days after application.

As mentioned, the primary screening procedure went through a number of stages of evolution early in the program, before the procedure depicted on the slide was used. At first, bean plants were treated only with a low and a high water formulation. Compounds insoluble in water were formulated in a variety of solvents, including acetone, acetone-ethanol, and N,N-dimethylformamide. It soon became evident, however, that the solvent had an effect on plant response. Therefore, screening in two different solvents, water and acetone, was undertaken. Nearly all of the compounds that have been screened are soluble in one or both of these solvents. The final change that was made in the screening procedure was substitution of privet plants for half of the bean plants in the primary screening of each compound.

The effect of solvent on plant response is shown in Table II.

TABLE II. PRIMARY SCREENING RESULTS ON O,O-BIS(2-ETHYLHEXYL)CADMIUM PHOSPHORODITHIOATE

$$\left[(CH_3CH_2CH_2CH_2\underset{CH_3CH_2}{\underset{|}{C}}HCH_2O)_2\overset{S}{\overset{\|}{P}}\text{-}S\right]\text{-}Cd$$

SOLVENT	AGE OF BEAN PLANT, days	WETTING AGENT	RESULT AFTER 14 DAYS	
			P[a]	A[b]
BTI				
WATER	14	Triton X-155	1	86
ACETONE	14	"	1	31
ACETONE	7	"	1	71.5
ARMY				
WATER	7	Triton X-155	Death	
WATER	7	Tween-20	No effect	

a. Phytotoxicity on a scale of 1 to 11.
b. Per cent of primary leaves abscised.

The compound under test, O,O-bis(2-ethylhexyl)cadmium phosphorodithioate, was prepared some years ago for a proprietary program of Ethyl Corporation. The compound was tested early in this program on bean seedlings.

It showed interesting activity in the primary screening program, but disappointing results in the secondary screening. This compound does illustrate, though, the effect some of the screening variables may have. In the last column of the table, "P" is phytotoxicity rated on a scale of 0 to 11, and "A" is per cent of primary leaves abscised. Formulation of the compound in water and application to 14-day-old bean plants at a rate of one pound per acre resulted in abscission of 86 per cent of the primary leaves as shown in the first line. The substitution of acetone for water in this test resulted in only 31 per cent abscission. The second and third lines indicate that the age of the bean seedling was an important factor in our primary screening procedure. The younger plant in this test is considerably more susceptible to chemically induced defoliation. The Army results shown in the last two lines indicate that the wetting agent also can have a considerable effect on screening results. It is generally recognized that the age of a plant is an important factor in its susceptibility to chemical defoliation. However, the reasons for variation in defoliant activity with solvent and wetting agent are not completely clear.

It appears that greenhouse conditions also can affect screening results. Figure 1 shows the results obtained with the commercial cotton defoliant DEF, which is used as a check during each series of tests. These abscission data are averages for each month, with three to eight determinations being made per month. The per cent of defoliation in this figure is a measure of the abscission of both primary and secondary leaves. Only the primary leaves are treated with the defoliant, and the secondary leaves emerge during the two-week observation period. The percentage of primary leaf abscission is greater than these figures by a factor of about two. Here, it can be seen that effectiveness of the compound decreases during the winter months. This effect may be due to any of a number of greenhouse conditions, including temperature, amount of light, or amount of moisture. A second control is now being used in addition to DEF for comparison with the other data. We cannot say that the solvent, the wetting agent, or the environment will effect all defoliants or herbicides in the same way, but they are factors that we have considered in the primary screening program.

The secondary screening procedure is shown in Table III.

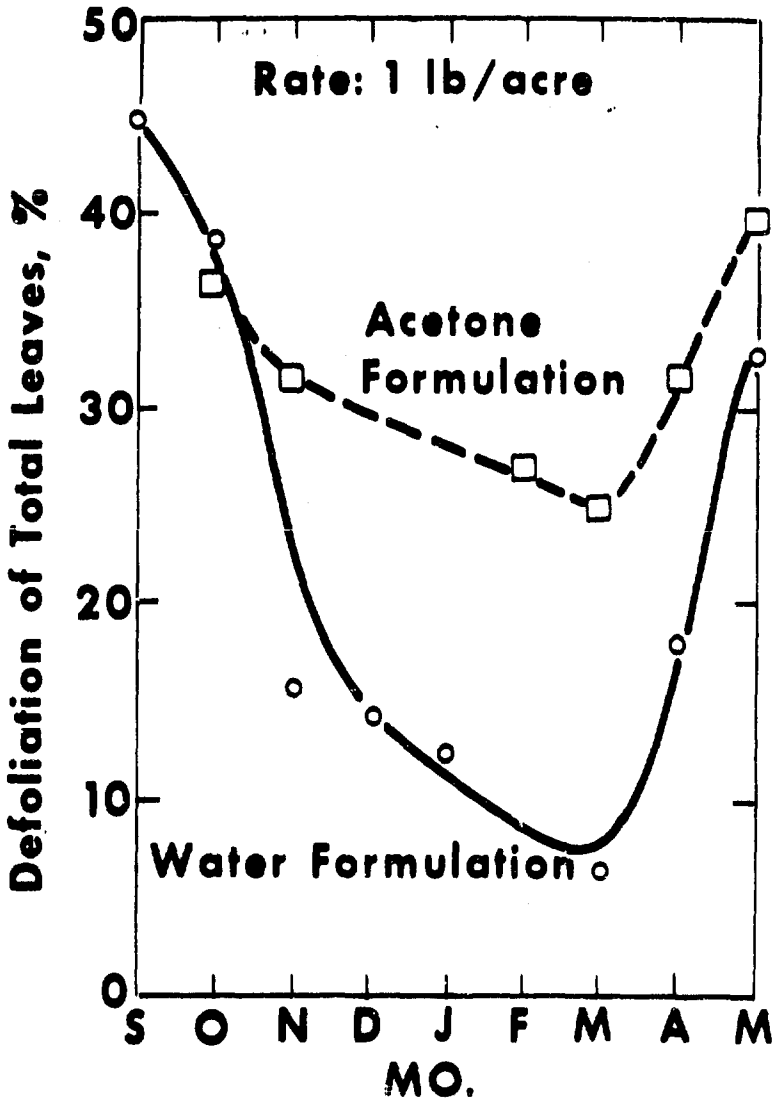

Figure 1. Variations in Plant Response to DEF.

TABLE III. SECONDARY SCREENING PROCEDURE

FORMULATIONS: WATER AND ACETONE

CONCENTRATIONS: 1 AND 5 LB/ACRE

SPECIES: PIN OAK
AMERICAN ELM
EUONYMUS
BOXWOOD

Forty compounds that showed high activity in the primary screening program and were representative of all the active classes of compounds yet discovered were placed in the secondary screening program. The same general procedure was used as in the primary screening. We have found that a number of the chemical compounds subjected to secondary screening effectively defoliate or desiccate at least one of the four species. A few compounds cause severe damage to the leaves of two or more species.

Finally, a summary of the over-all primary screening results is shown in Table IV.

TABLE IV. PRIMARY SCREENING RESULTS

COMPOUNDS WITH PHYTOTOXICITY RATINGS OF 8 TO 11 OR ABSCISSION RATINGS OF 25 PER CENT OR MORE	122
COMPOUNDS WITH PHYTOTOXICITY RATINGS OF 4 TO 7 OR ABSCISSION RATINGS OF 15 TO 24.9 PER CENT	71
REMAINING COMPOUNDS	297
TOTAL	490

Of the 529 compounds subjected to primary defoliant screening, results are available on 490. One hundred and twenty-two compounds showed a phytotoxicity rating of at least 8 or had an abscission rating of 25 per cent or more. Seventy-one additional compounds had a phytotoxicity rating of 4 to 7 or an abscission rating of 15 to 24.9 per cent. The remaining 297 compounds showed less activity. These percentages also must be multiplied by two to give the per cent abscission of the primary leaves that were actually sprayed.

We observe these results optimistically and believe that the leads uncovered may eventually lead to a defoliant or herbicide that will be useful for jungle application.

XVII. MONSANTO SCREENING PROGRAM

Stanley D. Koch*

Monsanto Research Corporation is a wholly owned subsidiary of Monsanto Chemical Company. Our greenhouse screening program on this contract is carried out under subcontract to our parent, in this case the Agricultural Research Laboratory of Monsanto Chemical Company in St. Louis.

Screening results are obtained and correlated automatically by Sample Record and Control, described in yesterday's talk.

The screening procedure on Black Valentine beans is as follows. Bean plants are grown in disposable pots, first four to a pot, then thinned to two. When the plants have one mature trifoliate and one partially opened trifoliate they are treated with the test compound, applied as a spray from a DeVilbis atomizer under controlled pressure. The compound is formulated either as a solvent-emulsified solution, a wettable powder formulation, or an aqueous solution with a wetting agent. The plants are placed in a greenhouse at 70° to 90°F and observed for defoliation, desiccation, or other abnormality, for two weeks. The environment in the greenhouse is kept free of insects and fungi.

Species represented in our primary and secondary screenings are shown in Table I.

TABLE I. PRIMARY SCREENING

Black Valentine Bean	0.1 and 1.0 lb/acre
Soybean	10.0
Apple Seedling	10.0

SECONDARY SCREENING

Maple	
Elm	
Pin Oak	all at 5, 3, 1, and
Privet	½ lb/acre
Euonymus	
Boxwood	
Ilex	

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* Monsanto Research Corporation.

A few more active compounds are also being tested on live oak in the secondary screening. Compounds in the secondary screening are initially tested at five pounds per acre. If active, they are tested successively at one, three, and one-fourth pound per acre.

Table II shows the form of reporting used for the data on our primary screening.

TABLE II

B 35005 (an organometallic)

CROPS		RATE lb/acre	ABSCISSION	CONTACT	KILLING	ACTIVITY RATING	REMARKS
SPECIES	VARIETY						
BEAN	BLACK VALENTINE	0.1	4	1		4	60% ABS. 10 DAYS 80% ABS. 14 DAYS
		1.0	1	4		4	SEVERE DES. 3 DAYS ABS. 10 DAYS
SOYBEAN	CLARK	10.0	1	4	4	4	DES. 1 DAY ABS. 6 DAYS KILL IN 8 DAYS
APPLE	SEEDLING	10.0		4	4	4	SEVERE DES. 6 DAYS KILL IN 10 DAYS

This example, B35005, is the first organometallic referred to in yesterday's talk.

The one group of compounds I wish to mention by name today is the quaternary ammonium iodides. Many of these compounds are extremely active against the woody species. So far, five have been found to be active against more than one woody species at the low rate of one pound per acre (Table III).

TABLE III. DEFOLIANTS ACTIVE AGAINST MORE THAN ONE WOODY SPECIES AT ONE POUND PER ACRE

B35175	phenyltriethylammonium iodide
B35188	(2-hydroxytrimethylene)bis(trimethylammonium iodide)
A35436	2-methylmercapto-4,5-dihydroimidazole hydroiodide
A35477	3-n-dodecylthiazolidine-2-thione hydroiodide
A35878	propionylcholine iodide

The results against elm and privet of these five iodides are shown in Figures 1 and 2.

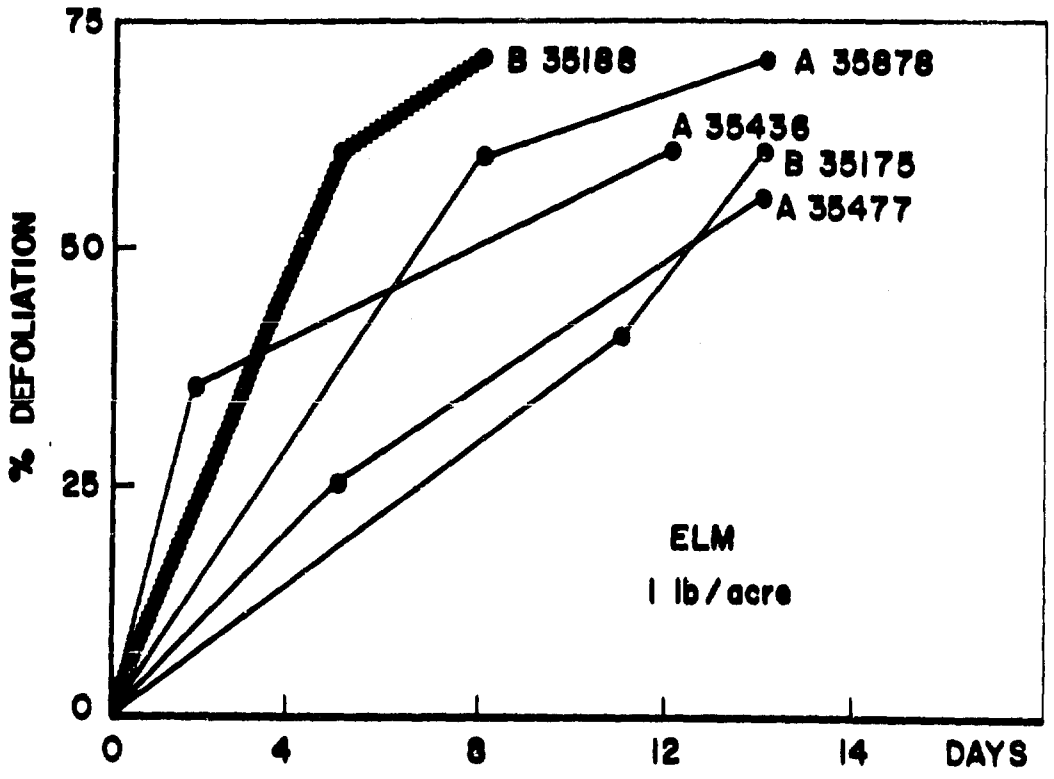

Figure 1. Reaction of Elm to Five Iodides.

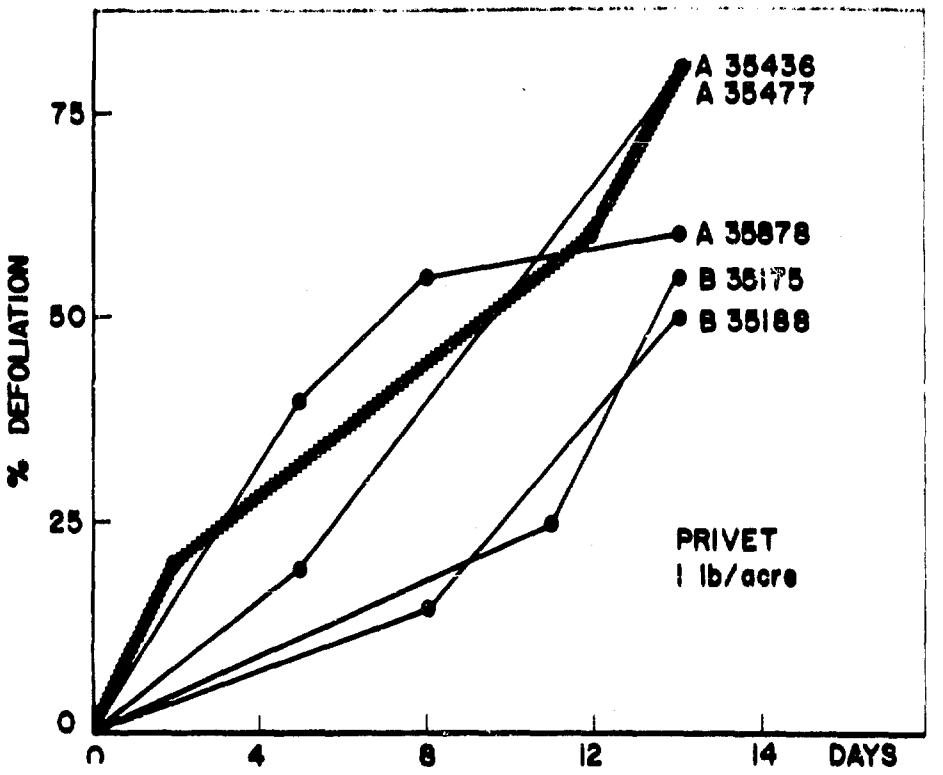

Figure 2. Reaction of Privet to Five Iodides.

It can be seen that the defoliation activity is roughly of the same order of magnitude for all five compounds. In those two figures the most active compound is shown as a heavier line.

At the higher rate of three pounds per acre, 1-methyl-4-picolinium iodide, number B35191, is active against four woody species, as is shown in Figure 3.

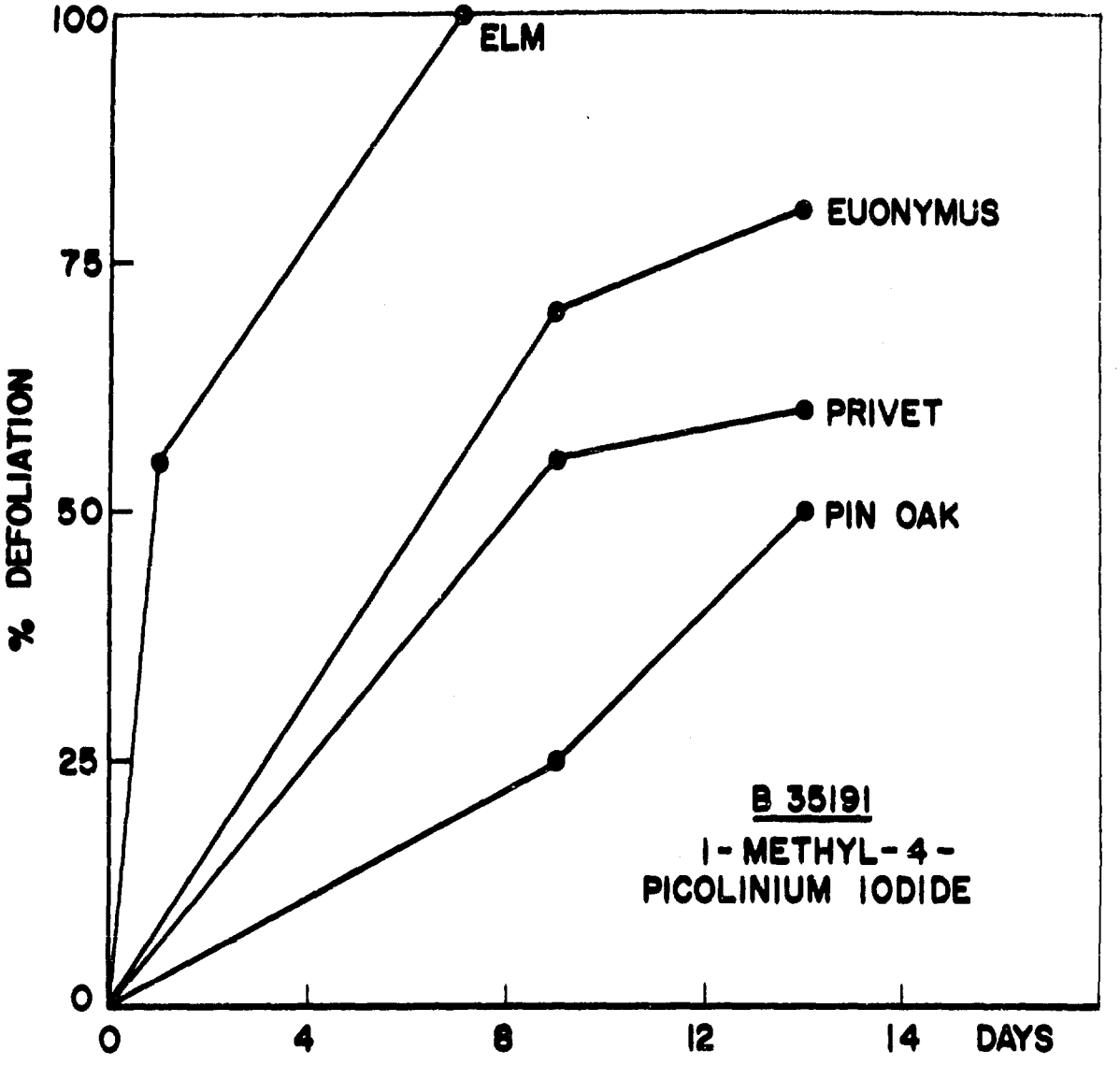

Figure 3. Defoliant Active Against Four Woody Species at Three Pounds Per Acre.

Four iodides are active against only three woody species at three pounds per acre. They are shown in Table IV.

TABLE IV. DEFOLIANTS ACTIVE AGAINST THREE WOODY SPECIES AT THREE POUNDS PER ACRE

		Elm	Pin Oak	Privet	Euonymus
B35150	diethyldimethylammonium iodide	X	X		X
B35175	phenyltriethylammonium iodide	X		X	X
B35477	3-n-dodecylthiazolidine-2-thione hydroiodide	55%, 1 day		X	X
B35878	propionylcholine iodide	60%, 1 day		X	X

Finally, I want to show another six quaternary ammonium iodides that are active against two woody species at three pounds per acre (Table V).

TABLE V. DEFOLIANTS ACTIVE AGAINST TWO WOODY SPECIES AT THREE POUNDS PER ACRE

		Elm	Privet	Euonymus
B35079	(2-furfurylcarbamoylethyl)diethylmethyl-ammonium iodide	X		X
B35171	benzyltriethylammonium iodide		X	X
B35193	4-[cyano(hydroximino)methyl]-1-methylpyridinium iodide	30%, 1 day	X	
A35436	2-methylmercapto-4,5-dihydroimidazole hydroiodide	X		X
A35479	3-methylthiazolidine-2-thione hydroiodide		45%, 1 day	X
A35879	valerylcholine iodide	35%, 1 day		X

Besides the quaternary ammonium iodides mentioned here, other active defoliants are being disclosed to the Army and we hope to have more classes in the coming quarter.

XVIII. FIELD TESTING PROGRAM

Kenneth Demaree*

The screening program is carried out in three phases. Dr. Robinson and Mr. Frank discussed the first two phases: primary screening of new chemicals sprayed on 14-day-old Black Valentine beans at 0.1 and 1.0 pound per acre, and secondary screening of the most promising chemicals sprayed on seedling trees in the greenhouse at one, five, and ten pounds per acre. The trees chosen for the experiments are maple, spruce, pine, locust, privet, pin oak, hemlock, and elm.

The third phase consists of field screening. Compounds that show the most activity are applied at rates of five and 10 pounds per acre to indigenous trees 10 to 15 feet tall in wooded areas. This year we sprayed 108 trees consisting of six genera - ash, elm, locust, red maple, northern red oak, and chestnut oak. A number of compounds used for this test were Tordon, 2, 4-dichlorophenoxyacetic acid (2,4-D), Diquat, and Endothal, and each compound was then combined with Tordon. Because the trees were spread out over a considerable area and the terrain was very rough in spots, we used three-gallon tank sprayers with a 20-foot hose and a 9-foot stainless steel wand. On the end of the wand is a 20-inch boom with three No. 2 Whirljet nozzles. The compounds were carefully weighed to the desired rates in the laboratory and then poured into the tank sprayer with just enough water to cover a tree. The sprayers were outfitted with pressure gauges so that each tree could be sprayed at 30 pounds' pressure. Spraying is done from a large tank truck so that the spray is directed down on the foliage to more closely simulate aerial spraying.

Compounds were applied on 11 and 12 July. Readings will be taken at weekly intervals until the end of September, then at six months and one year after spraying.

We also have another area at Fort Meade, Maryland. This area will be used to answer the question "At what rate are certain compounds effective if not effective at five or ten pounds per acre?" In this area, vegetation consists of scrub pine, maples, oaks, American chestnut, sweet gum, tulip poplar, quaking aspen, and vaccinium. It was marked off in plots and all vegetation was sprayed. Compounds used were butynediol, Folex, and cacodylic acid. These were chosen because of their availability in various staging areas around the world. They were applied at rates of 10, 25, 40, 55, 70, 85, and 100 pounds per acre. Data will be taken at weekly intervals up to ten weeks after spraying and again the following spring and summer.

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* U.S. Army Biological Laboratories.

We have a nursery at Fort Detrick consisting, at present, of about 1800 trees, and plans for another 18,000 to 20,000 trees to be planted this fall in blocks. The species now in the nursery are dogwood, maple, pine, spruce, hemlock, bald cypress, and oak. We will reserve some blocks so that we can spray trees that are 10 to 12 years old. A logarithmic sprayer will be used on some plots to determine the minimum amount necessary for complete kill.

Next year we plan to go into aerial application of sprays, using helicopters. We have seen some of the work of Tennessee Valley Authority along their power lines. We expect to write contracts with TVA, Georgia Power Company, or one of the larger brush control companies to use their equipment, their labor, and our newer promising compounds. We would like to find subtropical areas in the Everglades of Florida or bayous of Louisiana that are similar to vegetation found in the tropical areas of the world.

XIX. CONTROL AND DEFOLIATION OF TROPICAL AND SUBTROPICAL VEGETATION*

Dayton L. Klingman**

Date: Request for cooperative work was transmitted to us January 30, 1963. Work initiated April 1, 1963.

Place: Work will be done on tropical vegetation in Puerto Rico and on subtropical vegetation at College Station, Texas.

Species: Work will be done on species that are hard to kill by herbicides commonly used for brush control such as 2,4,5-T and 2,4-D. Species will also represent different families.

In Texas, major work will be done on:

Common name	Genus & species	Family
Yaupon	*Ilex vomitoria*	Holly
Winged elm	*Ulmus alata*	Elm
White brush	*Aloysia lycioides*	Verbena
Live oak	*Quercus virginiana*	Beech
Huisache	*Acacia farnesiana*	Legume
Greenbriar	*Smilax* sp.	Smilax
McCartney	*Rosa bracteata*	Rose

In Puerto Rico species that are hard to kill and that are important in tropical regions will be chosen for work.

Nature of Research:

1. Evaluate new chemicals and mixtures of chemicals for brush control and defoliation, utilizing:

   a. Natural stands of brush species
   b. Nursery plantings
   c. Greenhouse-grown plants

2. Develop equipment, techniques, and principles for improved application techniques.

   a. Study mechanism of spray formation in gas streams:

      Use small wind tunnel and close-up ultra-high-speed motion pictures to record spray droplet formation.

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* In cooperation with Advanced Research Projects Agency, DOD, the Texas Agricultural Experiment Station, College Station, Texas and the Federal Experiment Station, Mayaguez, Puerto Rico.

** U.S. Department of Agriculture.

b. Study effects of particle size and makeup on deposition of sprays on foliage. Use tracer techniques.
c. Develop equipment for applying herbicides to small plots in a manner that will simulate expected aerial application techniques.

3. Determine effects of environment on defoliation and killing activity of different herbicides.

4. Determine the most effective dosage, volume, formulation, and times of application.

5. Investigate methods of improving absorption, translocation, and activity of herbicides; study nature of action of selected herbicides in woody plants; related physiological studies.

Personnel:

In addition to Drs. Howard Morton and Robert Meyer, who were already conducting research on brush control at College Station, Texas, we will have three weed scientists and an agricultural engineer in Texas and two weed scientists and an agricultural engineer at Puerto Rico.

College Station, Texas:

1. Howard Morton and Robert Meyer assumed temporary responsibility for initiating the project.

2. Dr. Morris G. Merkle joined our staff on July 1, 1963.

3. Frank S. Davis will be transferred from Nebraska to Texas in January.

4. Vacancy - plant physiologist.

5. Vacancy - agricultural engineer.

Mayaguez, Puerto Rico:

1. Dr. Clyde C. Dowler, transferred from Whiteville, North Carolina to Puerto Rico, April 15.

2. Dr. Fred H. Tschirley, transferred from Tucson, Arizona to Puerto Rico, June 11.

3. Robert McCalmont, Agricultural Engineer, will transfer from Beltsville, Maryland to Puerto Rico, September 15.

Progress:

College Station, Texas:

1. Seventy five acres of land set aside for our use for a woody plant nursery by the Texas Agricultural Experiment Station.

   a. Irrigation water is available, and irrigation pipe was delivered June 10.
   b. Transplanting was done the week of June 17. Plans call for planting each species in a separate block. Each block will contain 168 plants. The plants will be spaced four feet apart in rows eight feet apart. There will be 42 rows per block and an eight-foot alley between blocks. The arrangement of plants provides a four-plant grouping that should facilitate treatment.

   Transplanted: 1320 mesquite plants
   504 live oak
   1008 winged elm
   336 McCartney rose

2. Eighty-acre tract near Carlos, Texas has been leased.

   a. Has good stand of yaupon, winged elm, and oak.
   b. Area was fenced and prepared for experimental treatments. Area was divided into 60- by 200-foot lands with 12-foot alleys. The outside 22 feet will be sprayed (22- by 200-foot plots), leaving 16 feet for buffer strip between treatments.

3. Forty-three acres near Victoria, Texas, has running live oak. Lease is being prepared. The site will be fenced and staked and treatments are scheduled for late July.

4. Forty-three acres at Llano, Texas, covered with white brush is being leased and also will be prepared for treatments.

5. Further exploration is being made for suitable sites for research on huisache, greenbriar, running live oak, white brush and other brush.

6. A 3/4-ton pickup to be used for mounting a 3-section roadside spray boom was delivered June 20. It should be ready to put treatments in the field by mid-July.

7. A plastic greenhouse was finished in June (on other funds). Plans are being readied for construction of a 30- by 40-foot temporary, insulated, air-conditioned metal building to be used as a field laboratory. In addition, two fiber-glass

greenhouses are to be erected adjacent to the plastic greenhouse.

8. Office and laboratory space is being provided for our personnel in the new Plant Science Building and in the Agricultural Engineering Building at Texas A & M. Also, the Texas Agricultural Experiment Station has provided warehouse area, about 50 by 50 feet, to be used for shop, machinery and storage.

Mayaguez, Puerto Rico:

1. We had no work underway in Puerto Rico, therefore, Dr. W. B. Ennis, Jr. and I spent the week of March 3 there and at St. Croix to establish cooperative relations with appropriate authorities.

2. Drs. Tschirley and Dowler of the Crops Protection Research Branch met Drs. Williams and Smith of the New Crops Research Branch in Puerto Rico during April 14-27, 1963. They spent most of the two weeks becoming familiar with possible sites for research and the species available and developing a classification grouping of important species for consideration in developing research plans. They met Mr. Frank Wadsworth and others of the U.S. Forest Service; Dr. Woodbury, botanist and Dr. Roque, Director of the Puerto Rico Experiment Station; Dr. Benjamin Seda, Director of Commonwealth Forests; and Jos Miguel Garcia, Assistant Secretary of Agriculture. All gave help and expressed willingness to cooperate.

3. One site on the Luquillo National Forest (rain forest) has been selected for experiments, others will be selected on the Maricao and Guanica Commonwealth Forests. Initially, soil-applied herbicides will be evaluated on these forests that differ in soil types and range in rainfall from 25 to more than 100 inches annually. Foliage spraying will have to be done initially on private lands. It is expected that necessary land can be leased. Finding sites where foliage spraying can be done is a difficult problem.

Botanical Investigations - Beltsville, Maryland:

An in-depth literature survey on the botany of Southeast Asia has been initiated. This is prerequisite to all present-day studies on the vegetation of that area and is not, to our knowledge, being undertaken elsewhere. In addition, a list has been compiled of all genera of Pteridophytes (ferns and their allies) and Spermatophytes (seed plants) known to occur in the Caribbean Islands. A similar list will be prepared for Southeast Asia. These lists will provide a starting point for consideration of elements common to the two regions in developing guidelines for the selection of plants and sites for herbicide testing.

Coordination with Research Activities Conducted by Ft. Detrick under ARPA Order:

Several meetings of ARS and Ft. Detrick personnel have been held to exchange information and to facilitate coordination of the cooperative work sponsored by ARPA. These meetings have been fruitful and will be continued.

Conclusion:

We believe good progress has been made on this project. We believe such concentrated research efforts will pay off in new findings in the area of brush control.

XX. TORDON HERBICIDE FOR VEGETATION CONTROL

Mark G. Wiltse*

A. INTRODUCTION

The Dow Chemical Company has been actively involved in evaluating chemicals for plant-growth control since the late 1930's. Research laboratories involved in plant-growth control projects have been located at Seal Beach and Pittsburg, California, Lake Jackson, Texas and Midland, Michigan. Projects involving synthesis and screening of chemicals are conducted at these locations. If a compound shows sufficient activity to be considered for further evaluations for plant-growth control in the field, plots are established at field research stations, which are located at Davis, California, Greenville, Mississippi, and Midland, Michigan. An extensive woody plant nursery is used in research evaluation of potential new products. When compounds continue to show promise in field research plots, experiments are conducted in many different locations to incorporate various species of weeds and woody plants growing under different soil and rainfall conditions. Only a few compounds survive this rigorous screening and are introduced to research agencies outside of our Company.

Tordon - Dow's trademark for 4-amino-3,5,6-trichloropicolinic acid - has undergone extensive research evaluation and has recently been released outside of our Company. It is a highly active herbicide and plant-growth regulator. It shows considerable promise for control of many woody plant species that up to now have been a problem to control. At relatively low rates of application, its growth-regulating effect is evident in stimulation of the growth of bluegrass, the growth of beans, and the rooting of hibiscus cuttings. Other growth-regulating properties have also been observed in limited studies with Tordon.

In screening tests, Tordon proved to be very active for the control of many broadleaved plants. Rates as low as one-half ounce per acre in foliage sprays have controlled such weeds as lambsquarter, wild buckwheat, and pigweed and have killed crop plants such as tomatoes, soybeans, and peanuts. Tordon is apparently rapidly absorbed through leaves of plants and translocated throughout the plants in a very short time. Tordon is also readily taken in by plant roots. Most broadleaved plants are susceptible to Tordon; however, the *Brassica* spp. have considerable tolerance to Tordon and some species tolerate applications of one to 2 pounds per acre. However, on many plants, Tordon herbicide is many times more active than phenoxy compounds such as 2,4-D and 2,4,5-T. Most grasses are tolerant of rates of Tordon that will kill broadleaved plants.

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* The Dow Chemical Company.

In early evaluation tests, Tordon herbicide appeared to be very active on several woody plants as leaf-stem sprays and soil treatments.

B. LEAF-STEM SPRAYS

A preliminary study was conducted on two-year-old plants growing in one-gallon cans. A wetting spray was applied to the leaves and stems of the plants, using approximately 200 gallons per acre of spray solution. Table I gives the per cent top-kill when observed 22 weeks after treatment.

TABLE I. PER CENT TOP-KILL 22 WEEKS FOLLOWING LEAF-STEM SPRAYS WITH TORDON AND 2,4,5-T

Plant Species	Tordon 3/4 lb. aehg.*	Tordon 1½ lb. aehg.	Tordon 3 lb. aehg.	2,4,5-T 1½ lb. aehg.
Prunus ilicifolia	100	100	100	98
Rhamnus californica	100	100	100	33
Ceanothus thyrsiflorus	100	100	100	100
Quercus agrifolia	78	100	100	20
Salix spp.	100	100	100	78
Rosa californica	100	100	100	48
Pinus coulteri	---	83	100	45
Pinus radiata	---	72	100	2
Pinus halepensis	---	100	100	10
Pinus canariensis	---	22	68	8

* Acid equivalent/100 gallons

Leaf-stem sprays were applied with sprayers in many experiments to evaluate the effect of Tordon on brush species in the United States and Caribbean area. All of these experiments were evaluated at the end of the first growing season and, with several species, results were determined at the end of the second growing season. In a test conducted in Michigan, Tordon at one-half pound per 100 gallons gave complete top-kill without resprouting of wild red cherry (Prunus pensylvanica L.), willow (Salix spp.), silver maple (Acer saccharinum L.), quaking aspen (Populus tremuloides Michx.), eastern cottonwood (Populus deltoides Bartr) and paper birch (Betula papyrifera Marsh.) when evaluated a year following treatment. White ash (Fraxinus americana L.) was not effectively controlled with Tordon at one-half pound per 100 gallons.

Experiments conducted in Mississippi indicated that Tordon at one pound per 100 gallons was more effective than 2,4,5-T and 2,4-D at four pounds per 100 gallons on a number of woody plants when evaluated the second growing season. The following species were effectively controlled with Tordon at one pound per 100 gallons in these experiments: red bud (Cercis canadensis L.), poison ivy (Rhus radicans L.), dewberry (Rubus spp.), red vine (Brunnichia cirrhosa Bank), sweet gum (Liquidamber Styraciflua L.), and sassafras (Sassafras albidum (Nutt.) Nees).

In other tests, Tordon controlled with sprays several species that normally are not effectively controlled with sprays of 2,4-D and 2,4,5-T. Most coniferous species were very susceptible. Table II gives the summary of control ratings made a minimum of five months after treating of some coniferous species included in these experiments.

TABLE II. SUMMARY OF RESPONSE OF SEVERAL CONIFERS SPRAYED WITH LEAF-STEM SPRAYS OF TORDON AND ESTERON 245 O.S.[a]

Common Name	Scientific Name	Average Control Rating (0-10)[b] Tordon 1/2 lb. aehg.	Tordon 1 lb. aehg.	Esteron 245 O.S. 4 lb. aehg.
Spruce	Picea glauca (Moench) Voss	10	10	5
Balsam Fir	Abies balsamea (L.) Mill.	10	9	4.5
White Cedar	Thuja occidentalis L.	10	-	3
Loblolly Pine	Pinus taeda L.	7.5	10	1.5
Short Leaf Pine	Pinus echinata Mill.	6.5	10	2
Slash Pine	Pinus caribaea Morelet	10	10	9.5
Red Cedar	Juniperus virginiana L.	-	10	-
Long Leaf Pine	Pinus australis Michx.	-	8	-
Virginia Pine	Pinus virginiana Mill.	10	-	-
Ground Juniper	Juniperus depressa Pursh.	8	10	1
White Pine	Pinus strobus L.	2	8.5	2

a. Esteron 245 O.S. contains four pounds per gallon of 2,4,5-trichlorophenoxy acetic acid as the propylene glycol butyl ether ester. Esteron is a registered trademark of The Dow Chemical Company.
b. Control Ratings (0 = no effect, 10 = complete kill).

Some species of conifers appear to be more tolerant than others; however, all conifers are more susceptible to Tordon than to 2,4,5-T.

The maple (Acer) species are very susceptible to Tordon. Control of regrowth of red maple has been outstanding. A summary of the response of several species of maple to Tordon and Esteron 245 O.S. herbicides observed a minimum of five months after treating is given in Table III.

TABLE III. SUMMARY OF RESPONSE OF MAPLE (ACER SPP.) SPRAYED WITH LEAF-STEM SPRAYS WITH TORDON AND WITH ESTERON 245 O.S.

		Average Control Rating (0-10)		
		Tordon		Esteron 245 O.S.
Common Name	Scientific Name	1/2 lb. aehg.	1 lb. aehg.	4 lb. aehg.
Hard Maple	Acer saccharum Marsh.	9	9	8
Red Maple	Acer rubrum L.	10	10	8.5
Silver Maple	Acer saccharinum L.	10	10	9

Tordon killed the stems of maples within three months of spraying and no regrowth occurred. Regrowth from the root collar was noticed during the season of treatment on similar plants treated with 2,4,5-T. It is well recognized that 2,4,5-T is one of the most effective phenoxy herbicides for the control of maple.

Several woody plants that have been a problem to control because they are prolific root sprouters have been susceptible to sprays with Tordon. A summary of the control ratings made on some of these species in experiments in the Eastern United States is presented in Table IV.

TABLE IV. SUMMARY OF RESPONSE OF BLACK LOCUST, SASSAFRAS, AND ASPEN TREATED WITH LEAF-STEM SPRAYS OF TORDON AND WITH ESTERON 245 O.S.

Common Name	Scientific Name	Tordon 1/2 lb. aehg.	Tordon 1 lb. aehg.	Esteron 245 O.S. 4 lbs. aehg.
Black Locust	Robinia pseudo-acacia L.	10	10	6
Sassafras	Sassafras albidum (Nutt.) Nees	10	10	8.5
Aspen	Populus alba L.	9.5	10	10

A list of the relative susceptibilities of some woody plants to Tordon is included in the Appendix.

C. SOIL TREATMENTS

Laboratory studies on mesquite (Prosopis juliflora (SW) DC.), honey locust (Gleditsia tricanthos L.), and ash (Fraxinus uhedii) growing in one-gallon cans have suggested that Tordon can be effective for the control of these species by soil treatment. The results in Table V were obtained in a preliminary test observed seven months after treatment.

TABLE V. PER CENT KILL FOLLOWING SOIL TREATMENT WITH TORDON AND FENURON

	Lb. Per Acre	Mesquite	H. Locust	Ash
Tordon	5	100	100	100
	10	100	100	100
	20	100	100	100
	40	100	100	100
Fenuron	5	10	43	0
	10	95	98	5
	20	95	70	10
	40	100	100	10

Field tests with soil applications of Tordon at five pounds per acre applied in the early spring controlled both sassafras and silver maple.

Additional tests were conducted in North America with the pelleted formulation of Tordon (Tordon 10K pellets) and evaluated the season of treatment. They suggest that many susceptible species such as black locust and sassafras can be controlled with rates between four and six pounds per acre of Tordon. Most woody plant species can be controlled with six to ten pounds per acre applied in the early part of the growing season when rainfall can be expected after treatment to carry the chemical into the root area of the plant. Additional experiments are being conducted at the present time on the possible use of soil applications of Tordon for the control of many different species of woody plants growing under various soil and rainfall conditions.

D. TOXICOLOGICAL INFORMATION

Toxicological studies indicate that Tordon herbicide is safe to handle and should present no hazard to men or animals when used as directed. It has a low acute oral toxicity with LD_{50} value for rabbits, mice, guinea pigs, chicks, and rats ranging from 2.0 grams (for rabbits) to 8.2 grams (for rats) per kilogram of body weight. It also is low in chronic toxicity and presents no serious hazard from eye and skin contact or skin absorption.

To better determine the effect of accidental ingestion of Tordon by large animals the following experiments were conducted:

Tordon was administered as the potassium salt to sheep at the rate of 100 milligrams per kilogram of body weight each day for 30 days, individual yearling calves were given single oral doses of 750 milligrams per kilogram of body weight, and sheep single oral doses of 1000 milligrams per kilogram of body weight. None of the animals showed any evidence of ill effects. Tordon was included in the rations of self-fed swine and chickens at the rate of 45 ppm. There was no evidence of ill effects as shown by weight gains or feed conversion in these experiments. These results indicate that no hazard exists for large animals accidentally consuming vegetation treated with Tordon.

Experiments were conducted to determine the effect of Tordon on fish. Untreated Lake Huron water at 50°F was used in the test which was run for 96 hours. The results from some of the experiments (Table VI) indicate that Tordon is relatively low in toxicity to fish.

TABLE VI. MEDIAN TOLERANCE LIMIT (TLM) AND MAXIMUM SAFE LIMIT CALCULATED IN PARTS PER MILLION OF TORDON (AS THE POTASSIUM SALT) FOR SEVERAL FISH SPECIES

	Concentration, ppm.	
	Maximum Safe Limit	TLM
Fat Head Minnow (Pimephales promelas Rafinesque)	21.6	29.2
Green Sunfish (Lepomis cyanellus Rafinesque)	38.9	90.7
Black Bullhead (Ictalurus melas Rafinesque)	69.1	90.7
Brook Trout (Salvelinue fontinalis Mitchell)	69.1	90.7
Brown Trout (Salmo trutta fario Linnaeus)	21.6	51.8
Rainbow Trout (Salmo gairdneri Richardson)	21.6	50.1

Ramshorn snails and daphnia were maintained in tap water containing various concentrations of Tordon for 22 hours at 72°F. Test organisms were not affected at concentrations of 30 ppm, but injury did occur at 40 ppm. Based upon these findings, it is believed that the accidental contamination of stream or pond water with Tordon when used for woody plant control would not be hazardous to the fish population.

E. SUMMARY

Most woody plant species in the United States are controlled with leaf-stem sprays using Tordon at one-half to 1 pound per 100 gallons. A combination formulation using Tordon at three-fourth pound and 2,4-D at two pounds per gallon (Tordon 101 Mixture) and used at one gallon per 100 gallons of water as a leaf-stem spray has effectively controlled a broad range of woody plant species in many experiments. A few species, including ash, have not been controlled effectively with this rate of application. In a limited number of trials, ash has been controlled with sprays containing Tordon at two pounds per 100 gallons per acre. Tordon herbicide is readily translocated from the roots to the above-ground parts of the plant. Hence, on ash and other species that are more tolerant, sprays should be applied to the soil around the root collar as well as on the stems and leaves. A combination of Tordon plus 2,4-D, which has given excellent control, should be considered for use where broad-spectrum control of woody plant growth is desired.

Soil applications of Tordon formulated as a pellet have been encouraging. Soil conditions and rainfall following treatment apparently will influence the control obtained. Highly susceptible plants such as sassafras and black locust have been controlled with rates as low as four pounds per acre of Tordon applied early in the growing season when rainfall occurred after treating. Higher rates may be required for some other species under less ideal soil and rainfall conditions. Toxicological information accumulated to date suggests that Tordon is a relatively safe material to use.

BIBLIOGRAPHY

1. Hamaker, J.W.; Johnston, H.; Martin, R.T.; Redemann, C.D. " A picolinic acid derivative: A plant growth regulator," Science 141:3578:363, July 1963.

2. Laning, E.R., Jr. "Tordon - for the control of deep-rooted perennial herbaceous weeds in the western United States," Down To Earth, Summer, 1963.

3. Watson, A.J., and Wiltse, M.G. "Tordon - for brush control on utility rights-of-way in the eastern United States," Down To Earth, Summer 1963.

APPENDIX

RELATIVE SUSCEPTIBILITY OF WOODY PLANTS TO TORDON

HIGHLY SUSCEPTIBLE

Botanical Name	Common Name in Area Tested
Abies balsamea (L.) Mill	balsam
Abrus precatorius L.	red bead vine
Acacia farnesiana (L.) Willd.	huisache
Acacia villosa Willd.	yellow tamarind
Acer macrophyllum Pursh.	big leaf maple
Acer rubrum L.	red maple
Acer saccharinum L.	silver maple
Acer saccharum Marsh.	hard maple
Alnus oregona Nutt.	red alder
Alnus rugosa DuRai Eprengl	black alder
Ampelopsis aborea (L.) Koehne	pepper vine
Arctostaphylos viscida Parry	manzanita
Betula lutea Michx.	gray birch
Betula papyrifera Marsh.	paper birch
Borreria laevis (Lam.) Griseb	buttonweed
Brunnichia cirrhosa Banks	red vine
Bryophyllum pinnatum (Lam.) Kurz.	leaf-of-life
Carya globra (Mill.) Sweet	hickory
Carya ovata Mill	shagbark hickory
Cassia occidentalis L.	John crow pea
Ceanothus cuneatus (Hook.) Nutt.	buckbrush
Celtis laevigata Willd.	hackberry
Celtis pallida Torr.	spiny hackberry
Cephalanthus pubescens Ref.	button bush
Condalia obovata Hook.	brazil
Cornus florida L.	flowering dogwood
Cornus stolonifera Michx.	red osier dogwood
Corylus americana Walt.	hazelnut
Crataegus sp.	hawthorn
Crotalaria verrucosa L.	rattle weed
Cytisus scoparius (L.) Link	Scotch broom
Diospyros virginiana L.	persimmon
Echites umbellata Jacq.	white nightshade
Flemingia strobilifera (L.) R. Br.	wild hops
Gleditsia triacanthos L.	honey locust
Juglans nigra L.	black walnut
Juniperus depressa Pursh.	ground juniper
Juniperus virginiana L.	red cedar
Larix laricina DuRoi	larch
Liriodendron tulipifera L.	tulip poplar
Lonicera japonica Thunb	Japanese honeysuckle
Lycium andersoni Gray	wolfberry
Mimosa sp.	mimosa

Momordica charantia L.	cerasee bush
Morus rubra L.	mulberry
Nyssa sylvatica Marsh	blackgum
Oxydendrun arboreum (L.) DC.	sourwood
Picea Abies (L.) Karst	Norway spruce
Picea glauca (Moench) Voss	white spruce
Pinus australis Michx.	long leaf pine
Pinus caribaea Morelet	slash pine
Pinus echinata Mill.	short leaf pine
Pinus Strobus L.	white pine
Pinus Taeda L.	loblolly pine
Pinus virginiana Mill.	Virginia pine
Pisonia aculeata L.	cockspur
Pithecellobium sp.	---
Populus deltoides Bartr.	eastern cottonwood
Populus grandidentata Michx.	large-tooth aspen
Populus tremuloides Michx.	quaking aspen
Prosopis juliflora (SW.) DC.	mesquite
Prunus emarginata (Dougl.) Walp.	bitter cherry
Prunus pennsylvanica L.	wild red cherry
Prunus serotina Ehrh.	wild black cherry
Quercus laurifolia Michx.	laurel oak
Quercus Phellos L.	willow oak
Quercus rubra L.	red oak
Rhamnus californica Esch.	California coffeeberry
Rhus diversiloba T. and G.	poison oak
Rhus glabra L.	smooth sumac
Rhus radicans L.	poison ivy
Rhus typhina Nutt.	sumac
Robinia pseudo-acacia L.	black locust
Rosa bracteata Wendl.	Macartney rose
Rosa rubiginosa L.	wild rose
Rubus spp.	wild blackberry
Rubus spp.	dewberry
Rubus procerus P. J. Muell.	Himalaya blackberry
Rubus spectabilis Pursh.	Salmonberry
Salix longifolia Merkl.	willow
Salix nigra Marsh	black willow
Sambucus canadensis L.	elderberry
Sambucus simpsonii Rehder	southern elder
Sassafra albidum (Nutt.) Nees	sassafras
Sida sp.	broomweed
Solanum ficifolium Ort.	gully bean
Solanum torvum Sw.	---

Taxodium ascendens Brongn.	pond cypress
Taxodium distichum (L.) Richard	bald cypress
Thuja occidentalis L.	northern white cedar
Tournefortia hirsutissima L.	chigger nut
Tournefortia volubilis L.	---
Tsuga canadensis L.	hemlock
Ulmus alata Michx.	winged elm
Ulmus americana L.	American elm
Urechites lutea (L.) Britton	yellow nightshade
Vitis sp.	grapevine
Waltheria americana L.	raichie
Zanthoxylum Fagara Sarg.	lime prickly-ash

MODERATELY SUSCEPTIBLE

Botanical Name	Common Name in Area Tested
Acacia tortuosa Willd.	wild poponax
Bourreria sp.	---
Bumelia lunuginosa (Michx.) Pers.	wooly camelia
Campsis radicans (L.) Seem.	trumpet creeper
Carya illinoensis (Wang) Koch	pecan
Cascaria sp.	---
Cascaria hirsuta Sw.	wild coffee
Cassia emarginata L.	---
Cercis canadensis L.	redbud
Chamaebatia foliolosa Benth.	Mt. Misery
Diospyros texana Scheele	black persimmon
Ehretia tinifolia L.	---
Ilex glabra (L.) Gray	gall berry
Liquidambar Styraciflua L.	sweetgum
Maclura pomifera (Raf.) Schneid.	osage orange
Malpighia glabra L.	---
Myrica heterophylla Raf.	wax myrtle
Opuntia sp.	prickly pear
Piscidia piscipula (L.) Sarg.	---
Psidium sp.	guava
Prosopis chilensis Stuntz.	cashaw
Pseudotsuga taxifolia (Poir.) Britton	Douglas fir
Quercus alba L.	white oak
Quercus Chapmanii Sarg.	Chapman oak
Quercus incana Bartr.	bluejack oak
Quercus laevis Walt.	turkey oak
Quercus marilandica Muenchh.	blackjack oak
Quercus myrtifolia Willd.	myrtle oak
Quercus nigra L.	black oak

Quercus Prinus L.	chestnut
Quercus stellata Wang.	post oak
Quercus virginiana Mill.	[illegible] liv
Schaefferia frutescens Jacq.	[illegible]
Sellos glutinosa Spreng.	[illegible]
Zanthoxylum flavum Vahl	[illegible]

RESISTANT

Cissus sicyoides L.	Mare [illegible]
Cornus Drummondii Meyer	rough dog
Croton linearis Jacq.	Spanish [illegible]
Eupatorium odoratum L.	Jack-in-t
Forestoria texana Cory.	elbow bus
Fraxinus americana L.	white ash
Fraxinus pennsylvanica Marsh.	greenash
Gaultheria shallon Pursh.	salal
Guaiacum officinale L.	[illegible]
Heliotropum indicum L.	scorpion [illegible]
Ilex opaca Ait.	American [illegible]
Lantana camara L.	white sage
Leucaena glauca (L.) Benth	lead tree
Ligustrum lucidum Ait.	glossy pr
Lithocarpus densiflorus (R.Br.) Abrams	tan oak
Magnolia virginiana L.	sweet bay
Mahonia trifoliolata (Moric.) Seale	agarita
Morinda royoc L.	duppy poi
Petitia domingensis Walp.	fiddlewoo
Porlieria angustifolia (Engelm.) Gray	guayacan
Quercus douglasii Hooker and Arnott	blue oak
Quercus wislizeni Engelm.	interior
Sabal minor (Jacq.) Pers.	scrub pal
Serenoa repens Bartr. Small	dwarf pal
Smilax spp.	[illegible]
Stachytarpheta jamaicensis (L.) Vahl	[illegible]
Vaccinium ovatum Pursh.	[illegible]
Yucca filamentosa L.	[illegible]

DEPARTMENT OF THE ARMY
US ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
EDGEWOOD CHEMICAL BIOLOGICAL CENTER
5183 BLACKHAWK ROAD
ABERDEEN PROVING GROUND, MD 21010-5424

REPLY TO
ATTENTION OF

RDCB-DPC-RS

03 MAR 2011

3 MAR 11

MEMORANDUM THRU Edgewood Chemical Biological Center, Technical Director, (RDCB-D/Mr. Wienand), 5183 Blackhawk Road, Aberdeen Proving Ground, MD 21010-5424

FOR RDECOM Office of Chief Counsel (AMSRD-CC/Mr. Brian May), 5183 Blackhawk Road, APG, MD 21010-5424

SUBJECT: RDECOM Freedom of Information (FOIA) Request

1. References:

a. Army Regulation 380-86, Classification of Former Chemical Warfare and Biological Defense, and Nuclear, Biological, and Chemical Contamination Survivability Information, dated 22 Jun 05.

b. Army Regulation 25-55, The Department of the Army Freedom of Information Act Program, dated 1 Nov 97.

2. The request from RDECOM asks for release of the following three documents pertaining to agent orange. ECBC subject matter experts have recommended allowing public release for these documents.

a. Technical Memorandum 46, Field Screening of Desiccants and Defoliants, Kenneth D. Demaree, April 1964.

b. Proceedings of the First Defoliation Conference, 29-30 July 1963, published January 1964.

c. Technical Report BWL 16, Defoliation and Desiccation, Preston, W.H., Downing C.R., and Hess, C.E., July 1959.

3. The ECBC point of contact is the undersigned at 410-436-7232 or june.sellers@us.army.mil.

JUNE K. SELLERS
ECBC Security Manager

Concur with ECBC's recommendation.
BRIAN A. MAY
FOIA Officer, HQ RDECOM

MAY.BRIAN.A.1037481463
Digitally signed by MAY.BRIAN.A.1037481463
DN: c=US, o=U.S. Government, ou=DoD, ou=PKI, ou=USA, cn=MAY.BRIAN.A.1037481463
Date: 2011.03.03 15:38:08 -05'00'