

ANATOMICAL CHANGES IN TREMBLING ASPEN (POPULUS
TREMULOIDES MICHX.) INDUCED BY THE APPLICATION
OF CHEMICAL AGENTS TO THE STEM

By

HAROLD JAMES RAPHAEL

A THESIS

Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Forest Products

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AN ABSTRACT

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A. J. Paoohui

The purpose of this study was to observe the anatomical changes occurring in the stems, roots, leaves, and twigs of trembling aspen (Populus tremuloides Michx.) induced by the application of various chemicals to the tree stem. It was hoped that these observations might help explain why some chemicals not only kill trees, but also cause the bark to loosen.

The following chemicals were chosen for the problem: propylene glycol butyl ether ester of 2,4-dichlorophenoxyacetic acid, propylene glycol butyl ether ester of 2,4,5-trichlorophenoxyacetic acid, and sodium monochloroacetate. The esters of 2,4-D and 2,4,5-T were applied as oil solutions by means of a basal spray. Sodium monochloroacetate was dissolved in water and sprayed on previously made, peeled girdles or frill girdles.

Seven different treating dates were used during the 1952 growing season. Stem, root, and twig samples, containing the cambial region and adjacent tissues, were removed from trees periodically during the summer, from the fifteenth day after treatment through the eightieth day. Leaf samples containing the midrib portion and adjacent blade tissues were also collected.

It was found that all three chemicals caused death and produced varying amounts of bark loosening. However, the degree of injury to the cambial region and effectiveness in

loosening the bark were quite different. Sodium monochloroacetate, applied to a frilled girdle, was found to be very effective as a tree-killer and as a debarking agent. The ester of 2,4,5-T was less effective, and the results with 2,4-D were inconsistent.

Stem samples taken from trees treated with sodium monochloroacetate showed severe injury to most parenchymatous tissues. Samples collected 71 days after treatment showed complete collapse of the cambial region. The last-formed cells of the xylem were in an advanced state of disintegration, forming many small lacunae between xylem and cambial region. The phloem contained collapsed and disintegrated parenchymatous tissue throughout the region. The only phloem cells left intact were fibers and sclereids. These cells were often left in isolated groups due to complete disintegration of surrounding parenchymatous tissue.

Stem samples taken 71 days after treatment from 2,4-D and 2,4,5-T treated trees showed complete collapse of the cambial region, but the degree of injury to the xylem and phloem was much less than in trees treated with sodium monochloroacetate.

It was concluded that bark loosening is caused by a combination of chemical and physical changes occurring in the cambial region of the stem. Collapse of newly-formed xylem cells and accompanying lacunae, in conjunction with

collapse and disintegration of cambial and adjacent phloem cells, evidently results in the formation of a belt of weakened material, consisting of remnants of cell walls and cell contents that can be ruptured easily. Collapse of cells apparently is caused, at least in part, by a contraction of the entire phloem region. This contraction is restrained by the xylem, causing long vertical splits to occur in the bark of many trees after treatment.

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I. PURPOSE AND SCOPE OF PROBLEM

The purpose of this study was to observe the anatomical changes occurring in the stems, roots, leaves, and twigs of trembling aspen (Populus tremuloides Michx.), after the application of various chemicals to the stems of the trees.

Observations were made on the cambial region and adjacent tissues of the stems, roots, and leaves. Examinations were also carried out on the midrib portion of the leaves.

Various investigators had observed that certain chemicals not only killed trees but also caused the bark to peel off readily, even after the normal sap-peeling season terminated. Accordingly, three chemicals which had been reported as having a loosening effect on bark were selected for this study. It was reasoned that the observation of anatomical changes occurring within a tree might help explain how these chemicals caused the separation of bark from the tree stem.

The following chemicals were chosen for the problem: propylene glycol butyl ether ester of 2,4-dichlorophenoxyacetic acid, propylene glycol butyl ether ester of 2,4,5-trichlorophenoxyacetic acid, and sodium monochloroacetate. The first two chemicals are commonly referred to as 2,4-D ester and 2,4,5-T ester, respectively. For the sake of brevity, the abbreviations are used in this paper.

In view of the fact that bark-free wood is very important to the pulp and paper industry, and because the trunk of the tree is usually the only portion used in the manufacture of pulp, the major emphasis of this study was placed on the anatomy of the cambial region and adjacent tissues of the stem. Observations on the anatomy of leaves, twigs, and roots were made in order to obtain additional information on the effects of the chemicals.

In addition to collecting data on the mechanics of bark separation, it was hoped to determine which of the chemicals used was the most effective in loosening the bark of aspen. Different concentrations of chemicals were employed in order to study the effect of strength of solution. Methods and dates of treating were varied in an attempt to establish optimum conditions for treating aspen.

Field observations were made in an attempt to correlate external changes in the tree with internal anatomical changes. These observations were made on leaves, branches, stems, and roots of treated trees. Periodic inspections of all treated trees were made to check the effectiveness of the various chemical treatments.

The problem was limited to trembling aspen because of the abundance of this species in the Lake States and because of the recent increased use of this tree by the pulp and paper industry as a source of raw material.

In 1942 the most important pulpwood species in the Lake States from the standpoint of amount cut was spruce; in that year approximately 426,000 standard cords, rough wood basis, were cut. The amount of aspen cut was approximately 322,000 cords. The amount of aspen harvested in 1951 was 935,000 cords. The next highest species in 1951 was pine with a total of 544,000 cords being cut (38). In 1953, aspen continued to lead other species with a total of 938,902 cords harvested. This represented about 45 percent of the total pulpwood harvest in the Lake States (39).

II. INTRODUCTION

In the manufacture of pulp and paper, it is imperative that all bark be removed from the pulpwood stick before the latter is processed. Particles of bark are objectionable because they are not reduced to pulp and show up in the finished paper as specks. Bark of most trees not only possesses little or no fiber value, but contains dirt and other impurities which must be removed before the final product is formed. When chemical pulping methods are used, bark consumes chemicals and steam to no useful purpose. In addition, it occupies valuable digester space that could be utilized by additional wood chips. For these reasons pulpwood must be completely barked either in the woods or just prior to the pulping operation at the mill.

Many mills prefer pulpwood that **has** been hand-peeled in the woods because shipping such wood usually results in greatly reduced transportation charges. More actual wood substance can be transported for less money because of the utilization of space that otherwise would be occupied by the bark, and because of the saving in weight due to removal of the bark.

In the upper peninsula of Michigan pulpwood is shipped by rail according to a weight agreement between railroad and

shipper. For aspen the agreed weight for a standard cord of rough (unpeeled) wood is 4,300 pounds; the agreed weight for a standard cord of peeled wood is 3,000 pounds. All freight charges are based on these weights.

Hand-peeled wood is also less wasteful of raw material than mechanically barked wood. Most mechanical barkers remove a certain amount of wood in the process of removing the bark. Hand peeling, on the other hand, removes little, if any, wood.

Pulpwood peeled in the woods eliminates the bark disposal problem that confronts mills which peel with some type of mechanical barker at the mill. Although much research has been conducted to find uses for the bark, in most instances it can only be used for fuel or must be disposed of by other means. Even when used as fuel it must be pressed, dried, stored, and burned in special ovens if maximum efficiency is to be obtained(64). This process involves a considerable outlay of money for equipment and demands additional space for large storage bins.

In order to remove bark by hand-peeling methods it is necessary to cut and peel trees during the so-called sap-peeling season. For aspen, in the Lake States, this season extends from about the middle of May until the end of August, depending on location and temperature.

During the sap-peeling season, for reasons to be explained later, there exists in the cambial zone of the tree

trunk, a plane of weakness which allows the bark to be peeled off easily. Before and after this season the bark is held very tightly to the stem of the tree and is difficult to remove.

One of the most serious problems connected with hand peeling today is the scarcity of woods labor. It is virtually impossible for pulp mills to obtain enough hand-peeled pulpwood during the sap-peeling season to satisfy their needs for the year. It is also impossible to hire woods labor outside the sap-peeling season if they are to be paid on a piece basis because of the low production resulting from the difficulties encountered in removing tight bark. Likewise, it would not be economical to hire labor by the hour for this type of work.

Mechanical barking is preferred by some despite the advantages cited for hand-peeled pulpwood. Pulpwood peeled in the woods frequently becomes imbedded with dirt and grit enroute to the mill and must be thoroughly cleaned before using. If transportation distances are short, or if the wood can be driven down rivers or streams, the savings effected by peeling in the woods may not be very great. If an adequate supply of hand-peeled wood is not available, mechanical barkers will have to be maintained to meet the balance of the requirements.

Even when mechanical barkers are used, they are most efficient when the pulpwood has been cut and shipped during the sap-peeling season. Wood that has been cut before or after the sap-peeling season, or wood that has been cut during this season and not peeled quickly, will have tight bark. These sticks frequently must be sent through typical drum barkers three or four times in order to remove all the bark. If cut and barked during the sap-peeling season, most sticks will bark cleanly with one or at the most two trips through the barker.

In order to eliminate some of these problems, much research has been conducted in an effort to find methods of treating trees with chemicals which would kill the trees during the sap-peeling season and maintain the bark in a loosened condition for an indefinite period of time. In this way pulpwood could be harvested and peeled either by hand or by mechanical barkers at any season of the year, eliminating the problems of scarcity of woods labor and excessive trips through barkers. This would help insure an adequate, continuous supply of pulpwood which could be peeled at low cost due to the ease of peeling.

III. REVIEW OF LITERATURE

A. Historical Development of Tree Poisoning

The use of chemicals for the purpose of killing undesirable trees and shrubs is not a new idea. Australian foresters employed various solutions containing arsenic as a silvicultural tool as early as 1917 (45).

Early experiments in the United States concerning the treatment of trees with chemicals were made by Rudolfs in 1919 (59). He conducted experiments to determine the physiological changes that occurred in living trees when they were treated with sodium chloride. One of his most interesting observations was the great variation in reaction among different species and individuals within a species. He found that certain species showed vigorous growth after an application of 7 pounds of salt per tree while others showed extensive injury with an application of only 4 pounds. The degree of growth and injury also varied widely among individuals within a species.

Herbert (37) reported the use of arsenic as a tree killer in 1923. A solution of 1 pound arsenic and 1 pound washing soda, dissolved in 4 gallons of water gave very good results in killing certain broadleaved trees and prevented sprouting.

The United States Department of Agriculture conducted large scale tests with sodium arsenite as a tree poison and published the results in 1928 (12). The purpose of these tests was to determine if tree poisoning was an effective method of clearing land and hastening decay of stumps and logs. It was reported that within one year after treatment, seventy-seven of 208 poisoned trees had fallen. The wood in these trees was discolored, soft and spongy and had no value for lumber. Most of the remaining trees were dead, although still standing. A very concentrated solution of 1 pound white powdered arsenic and 2 pounds of lye, mixed in 2 gallons of water, was used to treat 30 trees having an average diameter of 15 inches at breast height. The solution was poured into frill girdles which had been cut into the sapwood.

During November 1929 a study was conducted in southeastern Virginia on a stand of timber composed of loblolly pine and mixed hardwoods. One phase of this study dealt with a comparison of methods of removing undesirable hardwoods and of preventing subsequent sprouting. Some trees were felled, some were girdled and received no treatment, and others were frilled and poisoned with sodium arsenite. One year after treatment, 51 percent of the girdled trees had dead crowns and 85 percent of the frilled-treated trees had dead crowns. It was further reported that 68 percent of

the felled hardwood stumps had vigorous sprouts, 55 percent of the girdled trees were sprouting and only 36 percent of the frilled-treated trees were sprouting. It was also noticed that the sprouts of the frilled-treated trees were much shorter and less vigorous than the sprouts of either the stumps or the girdled trees (45).

Stowasser (65), working with potassium dichromate and other chemicals in 1930, reported that when holes were bored into the trunks of lombardy poplar trees and chemicals poured into the holes, only the vessels directly above and below the holes were affected. His conclusions were that this method was not satisfactory for killing the trees.

Cope and Spaeth made similar observations in 1931 using sodium arsenite to kill thorn-apple (20). They made axe cuts around the stems and left a space between cuts. They found that the sodium arsenite did not diffuse laterally more than one-half inch beyond the edges of the cuts. They treated during the months of February, May, August and November and reported the largest percentage of killing and the largest number of sprouts followed the May treatments. One phase of their experiments compared the effects of poisoning using a frill with the effects produced by boring holes three-quarters of an inch in diameter with an auger bit. They found the frill and auger-hole methods to be equally effective for small-sized trees, but reported that the auger-hole method decreased in effectiveness with larger

tree diameters. From these investigations they developed the "Cornell Tool" which has been used extensively for tree poisoning.

Pearson (54) reported on experiments conducted during 1934, 1935, and 1936 by the Southwestern Forest and Range Experiment Station. These investigations were carried out to determine if tree poisoning could be used as a means of stand improvement for ponderosa pine. Trees poisoned were over 6 inches in diameter at breast height, and the auger-hole method of poisoning was employed, using sodium arsenite. On most trees the holes were refilled a second time and a kill of almost 100 percent was obtained. His conclusions were that undesirable trees poisoned in this manner released seedlings and, by carrying part of the snow load on their dead branches, gave them protection for a number of years. There was also strong evidence that trees killed by this method did not attract the Ips beetle which readily infests untreated felled trees in the Southwest.

Further observations of the same experiments were published in 1939. This paper reported that holes bored more or less parallel to the circumference of the tree produced quicker kills than holes bored straight into the tree towards the pith. It was also stated that higher strength solutions increased the number of trees killed, other factors remaining unchanged (44).

Cook (18) described the results of poisoning 13 different species with sodium arsenite in 1944. He found great diversity among the various species as to resistance to the poison and to the amount of sprouting after treating. Complete killing of aspen clumps, originating from root suckers of an extensive system, was found to be practically impossible. The above-ground portions were completely killed but root suckers came up in abundance, often within a few inches of completely killed stems. He concluded that because of the hazard to man and beast, and because of the variation in effectiveness, sodium arsenite had only limited usefulness in forest and wildlife management.

Towards the end of World War II tree poisoning was given a further impetus with the appearance of new chemicals, which were being marketed as selective herbicides. These chemicals were non-toxic to men and animals in the quantities and concentrations used; early experiments indicated that they might be very effective as tree-killers.

One of these chemicals, ammonium sulfamate (trade name "Ammate"), received considerable attention, especially in the south. The use of "Ammate" was strongly advocated by Peevy in 1947 and 1949 for killing undesirable hardwoods in the south, either by placing the crystals in frills or pouring a water solution in them (55 and 56).

Various formulations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) ap-

peared on the market about the same time as "Ammate." These chemicals, especially the former, became very popular as weed-killers. They were also employed successfully as brush-killers and some of the earliest applications for this purpose were for the control of transmission rights-of-way (61).

In 1950 the results of extensive tests with these two chemicals indicated that 2,4,5-T was much more effective than 2,4-D as a brush-killer (2).

The Dow Chemical Company reported in 1950 that after five years of research and field tests, they found most woody species could be killed with basal bark sprays of 2,4-D and 2,4,5-T applied during any season of the year. Even many species which were resistant to foliage sprays appeared to be susceptible to basal bark sprays (3).

A comprehensive paper by Chaiken (17) in 1951 described the results obtained with many different chemicals and different treating methods. He noted that 2,4,5-T was much superior to 2,4-D in killing undesirable hardwoods in the Southeast. "Ammate" also proved to be very potent but cost two to three times as much as 2,4,5-T. He also noted that some types of treatment were more effective than others when applied to the same species. For example, sweetgum was readily killed by foliage sprays of 2,4,5-T but slow to respond to basal sprays. Some of the oaks were easily killed

by basal sprays but sprouted vigorously after foliage spraying. An emulsion of 2,4,5-T in frills was found to be very slow in killing trees but also very thorough. No sprouting was detected on any tree that had been properly treated in this manner with a 2,4,5-T emulsion. A basal spray of 2,4,5-T in oil also gave good results but cost much more than the frill method because larger volumes and higher concentrations were needed. It also appeared to be most efficacious on thin-barked species.

In 1953, Arend (6,7) reported on investigations carried out in Michigan with esters of 2,4-D and 2,4,5-T. He found that a 1 percent, by weight, solution of 2,4,5-T in diesel oil, applied in frills was very effective in killing scrub oak, aspen and red maple.

This same investigator also reported in 1953 on the results of basal spray treatments of scrub aspen with the same two chemicals (8). He observed that basal spray treatments were most effective when applied during the growing season, from the time of full leaf development through the end of September. Trees sprayed during this period showed no signs of suckering when examined 3 years after treating. Trees treated in a similar manner but during the dormant and early growing seasons sprouted prolifically beginning with the second year after treating. Trees frilled and treated with with the same solutions sprouted the first year after treat-

ing. It was also observed that spraying the tree trunk from the ground to a point 4 feet above the ground was more effective than spraying to a height of 2 feet above the ground, even when the strength of solution applied to the 2 foot spray was doubled.

In 1953 Morrow (52) reported on investigations conducted near Ithaca, New York. Aspen was treated by the basal spray method using oil solutions of 2,4-D and 2,4,5-T during the growing and dormant seasons. The results of this experiment were not in agreement with those reported by Arend (8). Morrow found no apparent differences due to height of spray or season of treating on subsequent suckering. No sprouting of any kind was found on any of his test plots. His explanation of the disagreement in results between his work and that of Arend was the possible influence of tree vigor on subsequent suckering. His trees were of slow growth, 60 years old, whereas Arend had conducted his research on vigorous young aspen.

B. Development of Chemical Debarking in Canada

The first experiments with chemicals to facilitate bark removal were conducted by Alexander R. White of Ontario, Canada, in June, 1941 (71). White based his investigations on the theory that if a "vegetable toxicant" could be injected into the sap stream of a tree, death might result quickly and prevent the tree from completing normal, annual growth. He further reasoned that if this was accomplished and the tree did not lose moisture too rapidly, the bark might remain in a peelable condition for perhaps 30 days beyond the normal sap-peeling season.

At first holes were bored into the trunks of spruce and poplar trees at ground level. The poisoning agent was a water solution of calcium chloride and sodium chlorate. The results of the first attempts were negative because it was impossible to get enough solution into the holes to kill the trees. It was observed that there was very little lateral movement of the solution from the holes.

In the late summer of 1941, the method of applying the chemical was changed and the trees were girdled. A 5 percent, by weight, water solution of calcium chloride and sodium chlorate was applied to the girdled area and held in contact by means of a water-tight dam at the base of the girdle; details of this dam were lacking in the literature. The chemicals were used in a ratio of 3 parts calcium chlo-

ride to 1 part sodium chlorate. This method appeared to be partially successful on both spruce and aspen. The crowns of the trees were killed by the chemicals but the expense of the method and the difficulties encountered in fashioning a water-tight dam around the girdle led to the use of a thick paste towards the end of August. The paste contained the same chemicals and was smeared on the girdled area. The entire girdle was wrapped with cheesecloth. Before applying the chemical, the girdle was scored to a depth of about one-quarter of an inch. Poplar was used with this method and all trees were killed within 7 days after treating. It was, however, too late in the season to influence the peelability of the trees during the current growing season, but the trees peeled fairly well the following spring (71).

In 1942 the process of applying chemicals to trees for the purpose of debarking was patented by White (53). During the same year many pulp and lumber companies conducted tests to determine the effectiveness of the chemical. Burk (15) reported the experiments carried out on spruce which had been treated with Cambicide A (this was a commercial product which consisted of prepared rolls of paper bandage, coated with a mixture of sodium chlorate and calcium chloride). It was noted that some spruce trees treated during the sap-peeling season did not peel effectively after the sap-peeling season was over. | Some sticks peeled readily but some did

not peel at all. It was concluded that the chemical did not give satisfactory results over the entire length of the tree, the sticks from the upper portions of the trunk being much more difficult to peel than those from the lower regions. Similar conclusions were drawn after observing material which had been run through a drum barker.

In 1943 tests were undertaken by the Forest Products Laboratories of Canada at the Dominion Forest Experiment Station at Petawawa. A variety of chemicals and tree species were employed in these experiments and treatments were made during June, July and August.

It was observed that the various chemicals reacted in two distinct ways. Some of them reacted in a very rapid manner, causing defoliation and death within a week. The action of others was much slower, the trees retaining their leaves for weeks, and in some cases up until the time of normal leaf fall. It was determined that the fast-acting chemicals were the most effective in facilitating bark removal. One of the best chemicals observed was a combination of arsenic trioxide and caustic soda in a paste form. Treatments applied during the early part of the normal sap-peeling season gave better results than those made towards the end of summer (32).

By 1946 it had definitely been established that solutions and pastes containing either sodium arsenite or ar-

senic trioxide, when applied to girdles, were the most effective chemicals for loosening bark. However, because of the hazards involved in using arsenic compounds, the search for a "perfect" chemical continued. Therefore, it was proposed to test the recently developed week-killers (1).

In 1947 Hale (33) summarized the work of the Forest Products Laboratories in connection with the chemical treatment of trees to facilitate bark removal. New chemicals had been tested but arsenic paste mixtures still appeared to be the best. It was observed, however, that while arsenic apparently had some toxic effects towards insects, "Ammate" had none, with the result that trees treated with this chemical were subject to serious bark insect attack. This caused the bark to break up into small pieces along the numerous galleries making hand peeling very difficult. Conifers peeled readily in the drum barker, but it was found that the sticks from the upper parts of aspen trees did not peel cleanly, necessitating a second trip through.

The sodium salt of 2,4-D applied as a water paste showed promise of being an effective chemical for debarking when used during June. Trees treated after June did not give favorable results. No evidence of stain or bark insects was found on 2,4-D treated trees.

Experiments conducted at Stevens, Ontario during 1947 and 1948 were reported by McIntosh (46,47). Among other

things, weight reduction of treated trees was studied and comparisons were made between treated and untreated trees, as well as between different chemicals. Weight reduction was determined by means of test sections cut from standing, treated trees and compared with similar sections taken from untreated trees. It was found that while weight reductions of up to 10 pounds per cubic foot were obtained with some conifers, hardwoods in general showed little or no weight reduction. There was no significant difference of one chemical over another in reducing weight.

A subsequent report concerning weight reduction was published in 1949 (48). This report covered experiments in which some trees were girdled and treated, some were girdled only, and others were used untreated as controls. Two methods were used to check weight reduction. One method was to fell trees about four months after they had been girdled and treated. Sections were taken from all portions of the trunks and compared with similar sections taken from the normal trees felled at the same time. The second method employed the same technique but was not conducted until a year after treating and included the trees which had been girdled but not treated. It was found that jack pine and balsam fir trees were not much lighter in weight than trees which had been girdled without a chemical treatment. Aspen and balsam poplar showed no significant reduction in weight when com-

pared to untreated trees four months after treating. Treated and girdled white birch actually were heavier than untreated trees when compared four months after treating. The wood of hardwood trees which had been girdled and treated was in some instances heavier than that of hardwoods which had been girdled only, when checked one year after treating.

McIntosh and Hale (50) presented additional information pertaining to the effects of different chemicals and time of treatment on the debarking of numerous hardwood and softwood species. They concluded that a paste of one part arsenic trioxide and one part caustic soda was the best for debarking both conifers and hardwoods. A combination of arsenic and ammonium sulfamate was next best. Ammonium sulfamate alone was effective in loosening the bark of some species but not all. The sodium salt of 2,4-D was erratic in performance and was not recommended as a bark-peeling chemical. It appeared to be partially successful on the lower portions of the trunk, but sticks from the upper portion peeled with difficulty. All chemicals were applied as pastes, which were spread on a band of crinkled paper. The treated paper was pulled tightly around the girdled portion of the tree trunk and held fast with an upholstery tack. It was observed that in most instances conifers peeled better than hardwoods regardless of the chemical used.

Trees treated during June and July, with the more effective chemicals, peeled easily when they were felled the following October. They were also easily peeled when tested the following year.

The effectiveness of August treatments seemed to depend, to a large extent, on the species treated. Balsam fir was readily peeled when felled the following October but aspen, balsam poplar, jack pine, and spruce peeled only slightly better than normal, untreated trees felled at the same time. Treated white birch was just as difficult to peel as untreated trees felled at the same time. Most trees treated in August and tested the following June peeled as easily or more easily than untreated control trees.

With the exception of balsam fir, all species which had been treated in September and felled in October were as difficult to peel as untreated trees felled at the same time. Trees treated in September and tested the following year were observed to peel more easily as the summer progressed. Jack pine and black spruce gave the poorest results when tested the following year.

The same two investigators (34) reported in December, 1949, the development of a gasoline-powered girdling tool which was reported to speed up greatly the treating process. They also noted that recent tests had indicated the use of pastes without the protective paper band appeared to be as

successful in loosening bark as the commonly accepted method of using a band with the paste.

By 1951 it had definitely been established that applying a thin water paste of arsenic to the girdled area of a tree with a paint brush and not wrapping it was the most economical method yet tried. It was also just as effective as any other method tried. A report summarizing the results of tests using 25 different chemicals showed that various forms of arsenic were still the most effective in loosening the bark of trees and controlling bark insects. It was also pointed out that when chemicals were not applied evenly around the girdle, the ease of peeling was not uniform. The chemical appeared to affect only the bark directly above the point of application. The bark remained green above areas of the girdle which had not received sufficient chemical. It was also observed that with trees which contained normal resin canals, it was necessary to apply the chemical immediately after girdling, otherwise a film of resin coated the surface of the girdled area and impeded absorption of the chemical into the sapwood (49).

C. Chemical Debarking in the United States

Experiments with chemicals, for the purpose of debarking trees, were started in the United States shortly after

A. R. White announced his process in Canada. However, it was not until 1948 that large scale tests were undertaken.

The Armstrong Forest Company of Johnsonburg, Pennsylvania, was the pioneer testing agency in this country. On the basis of their extensive tests, they published a report containing recommended methods of treating trees, organizing treating crews and planning large scale operations (10). They advocated the use of a water solution of sodium arsenite applied to girdled trees by means of a paint brush and bucket or a tank-fed outfit. It was reported that a five-man crew, consisting of a foreman, three girdlers, and one painter could treat enough timber to produce about 100 cords per 8-hour day. A seasoning period of 8 to 14 months was suggested to aid in the completion of bark separation. This period was said to be sufficient to eliminate almost all hand peeling; most of the bark sloughing off either on the standing tree or through subsequent handling.

In 1952 the Armstrong Forest Company purchased U. S. Patent No. 2,324,968 originally granted to A. R. White, covering the use of chemicals for debarking trees, and made it available for public use in this country (4).

Bennett (11) described other work undertaken by the Armstrong Forest Company and reported some interesting observations. It was noted that a clean girdle with absolutely no cuts made into the sapwood was the best method of pre-

paring the trees for treatment. Scoring or cutting into the sapwood was regarded as preventing the upward translocation of the poison and causing green streaks in the bark above such incisions. It was also found that almost all hardwoods sprouted after treating with sodium arsenite, thus insuring regeneration of the stand. Tests made with the bark of arsenite-treated trees indicated that the poison did not affect the use of the bark for tannin extraction.

Among other chemicals tested as substitutes for arsenic, Dow Defoliant (Sodium monochloroacetate) and various formulations of 2,4-D and 2,4,5-T were tried by many investigators. Cook and Hamilton (19) and Wilcox (73) reported that these chemicals were not as effective as sodium arsenite in facilitating bark removal. Wilcox reported that Dow Defoliant applied to girdled trees in rubber reservoirs produced peeling comparable to arsenic but when applied by the conventional brush method gave very poor results. He also observed that 2,4-D and 2,4,5-T applied to girdles in rubber reservoirs were less effective than Dow Defoliant.

Cook and Hamilton (19) agreed with Bennett that scoring of the sapwood retarded the upward flow of the chemicals. Wilcox (73), however, found no difference existed between scored and unscored trees as to peelability.

Arend (5) worked with 2,4-D, 2,4,5-T and "Armate" in Michigan. He tested the three chemicals on aspen, red

maple, white oak, and northern pin oak. His results showed that none of the chemicals was effective on any of the species except red maple. The bark of red maple could be stripped off at the end of the second growing season but the bark of all the others remained tight. The "Ammate" crystals were applied in notches spaced every 4 to 6 inches around the base of the tree. Esters of 2,4-D and 2,4,5-T were applied as basal sprays in a concentration of 8 pounds a. h. g.* in diesel oil. Trees were sprayed from the ground to heights of 2 or 4 feet above ground. Treatments were applied during the dormant season, spring, sap-peeling season (after full leaf development), and in the fall.

Day et al. (23) also investigated the use of 2,4-D and 2,4,5-T in Michigan. Treatments were made on trembling aspen and large tooth aspen. Water and diesel oil were used as carriers and treatments included basal spraying, frill girdle plus chemical, and peeled girdle plus chemical. All tests using water solutions produced negative results. It was concluded that the waxy bark of aspen allowed only a small amount of solution to be absorbed, or that the solution was washed off before the chemical could be taken into the sap stream.

Diesel oil used alone produced negative results. The amine salts and an ester of 2,4-D, as well as an ester of

*Acid equivalent per 100 gallons of solution.

2,4,5-T, in diesel oil (8 pounds a. h. g.) produced variable results. The ester of 2,4,5-T was observed to be the best, followed by an ester of 2,4-D while the amine salt of 2,4-D produced inconsistent results. Stem spraying was reported to be more effective than girdling followed by spraying. No bark loosening was observed in trees that died during the first growing season, but pulpwood sticks cut from these trees the following November appeared to peel more easily than untreated trees cut at the same time.

Investigations conducted by the State University of New York, College of Forestry, were described by Jahn (41). These experiments were performed on hardwoods and softwoods using sodium arsenite applied to a peeled girdle. It was observed that the best results were obtained when the trees were treated in June, although the treatment appeared to be effective at any time during the sap-peeling season. Treatments applied before and after the sap-peeling season gave poor results. Dominant and co-dominant trees of good vigor responded somewhat better than trees of other classes and of poor vigor. Trees between 8 and 17 inches diameter at breast height all gave good results, while those above and below that limit sometimes gave poorer results.

By means of radio-active arsenic, it was determined that the chemical left the unscored, girdled areas very rapidly. Approximately 95 percent of the arsenic was gone at

the end of 48 hours when applied to spruce. The movement away from the girdle was slower and more gradual when the girdle had been scored. However, it was observed that scoring had no effect on the peeling characteristics of the bark; treated trees with scored girdles peeled just as easily as those which were unscored.

It was concluded that because the chemical does leave the girdled face so rapidly, the damage to wildlife should be at a minimum.

It was recommended that conifers be allowed to stand for three months after treatment and hardwoods not be felled until after freezing and thawing had taken place. These periods were determined to be sufficient to give good bark peeling.

Sarles (60) reported that sodium arsenite applied to girdled white pine as late as September 24 gave excellent results in loosening the bark. One-half gallon of the chemical was applied to 58 girdled trees by means of a paint brush. Twenty-three of the trees were felled the first week of November and all peeled satisfactorily. The other 35 were cut by the end of the following January and they too peeled very well; the bark was reported to have come off in long, large pieces.

D. The Periodicity of Secondary Growth and the Related Changes Occurring in the Cambium and Adjacent Tissues of Woody Plants

The exact manner in which chemicals facilitate bark loosening has not been investigated to any great extent. Numerous theories on the mechanics of chemical bark loosening have been presented, based on the condition of the cambial region during the normal sap-peeling season. Therefore, it was thought that a brief summary of these theories, and a review of some of the more pertinent studies conducted on the cambium and adjacent tissues during different seasons of the year, would be appropriate before describing the effects of chemicals on plant tissues.

Although the theories advanced by various investigators concerning chemical debarking of trees vary as to detail, most agree on certain fundamentals. They state that during the sap-peeling season, the cambial region possesses extremely thin walls which represent a plane of weakness, allowing the bark to be peeled readily. At the close of the sap-peeling season, the walls become relatively thick and the region of cleavability disappears. They further reason that if a tree can be killed quickly during the sap-peeling season, so that the cambial region is fixed in a thin-walled condition, it should be possible to peel off the bark long after the normal sap-peeling season has terminated (27,33,41, 49,71).

The initiation of cambial activity in the spring has been studied by many investigators, who have observed that it starts in the apical portions of the twigs and spreads basipetally through the trunk to the roots. It has been agreed by many observers that the stimulus for this initiation is probably a growth hormone which originates in the terminal portions of the branches when the buds start to swell in the spring.

Brown (14) noted that cell division began in the upper portions of the trunks of white pine before it began in the lower portions. Swarbrick (66) observed a swelling of the cambial region at the same time that the buds were beginning to increase in size. Likewise, he found that xylem differentiation began in the terminal regions of all shoots and spread downwards towards the roots. Priestley (58) reported cambial activity as being initiated in the developing buds; Brown (13) and Esau (30) observed it starting just below the foliar buds. They all reported a basipetal spread of activity. Most of these investigators also noticed that cessation of activity in the fall was in exactly the same order as initiation in the spring. Activity terminated first in the twigs and spread downward through the trunk with the roots being the last part of the plant to go into a dormant condition (13,14,58,66). According to Esau (30) the xylem cells observed in Vitis had thick, lignified walls, while

the cambium and adjacent phloem could not be distinguished on the basis of wall thickness. She reported both cambium and phloem mother cells as having thick, un lignified walls. Brown (14) observed that an initiating layer could not be identified in white pine because the meristematic cells on both sides had the same size, form, and protoplasmic contents as the true cambium. The cambial region has been observed to be 7 to 10 cells wide in the stem of white pine (14) and 4 to 6 cells wide in the branches of apple trees (66). In many species this region apparently contains undifferentiated xylem and phloem cells as well as initiating cambial cells (14,43).

The first indication of spring activity is a swelling of the cambial cells in a radial direction. The cells, at this time, may become almost double their dormant width (14). This swelling has been reported to occur several weeks before actual cell division takes place (14,30,43,66). The radial walls become very thin during the swelling phase, especially in those regions where the new tangential walls will eventually be attached (30). Cell contents change from a gel-like state to a sol-like state, becoming semi-fluid and less densely granular (43,58,66).

During the swelling of the cambial region some undifferentiated xylem and phloem cells, from the previous year's growth, start to differentiate (43). Cell division begins

several weeks after the cambial region starts to swell. Xylem and phloem cells apparently are formed at about the same time with more xylem than phloem being produced (14,30). Cell formation is very rapid at first and the cambium produces cells faster than they can differentiate, causing a rather wide band of undifferentiated cells with thin walls on both sides of the initiating layer (14,66,72).

Towards the end of the growing season, cambial activity slows down and eventually terminates. Brown (14) observed that xylem formation in white pine had ceased by September 26. Ladefoged (43) reported that cessation of wood formation varied greatly among different species, some forming wood until September, but many forming very little or none after July.

Maturation of previously formed cells continues for about a month after cambial division ceases (72). Eventually only a few cells on either side of the initiating layer remain undifferentiated. As the tree goes into the dormant condition, the walls of this layer of cambial initials and undifferentiated cells become firm and relatively thick (30, 43).

The relation between cambial activity and the slipping of bark in the spring has been reported by many investigators (16,30,58,72). Both cambial activity and bark slipping occur with a rise in temperature. Wilcox (72) determined

that bark slipping occurred on the trunks of eight species, in New York State, after the mean weekly temperature had passed 40 degrees Fahrenheit. This bark slippage preceded actual cell division by about one month. He also reported that peeling resistance was at a minimum during the period of greatest cambial cell division. He concluded from this, that although cambial division was not a requirement for peeling, it did help materially. Peeling was observed to continue for about one month after cell division had stopped but with a gradual increase in resistance. Esau (30) observing the thin-walled condition of the cambium before and during cell division concluded as follows:

The obvious result of this weakness of the radial walls, during the earlier and later stages of cambial activity, is the slipping of the stem bark that is easily induced by mechanical means when spring growth begins.

According to Wilcox (72), at the close of the growing season the cambial cells harden into the dormant condition, and the cambium becomes resistant to rupturing. As a result the resistance of the tree to peeling increases.

E. Various Responses of Some Plants When Treated With Chemicals

Some of the first things noted about the 2,4-D compounds were the selectivity of the chemical for different species of plants, and the variation in types of response

brought about in different plants and different parts of the same plant. Hamner and Tukey (35) observed that in some plants the growing tips were killed while in other plants they were merely arrested. Some plants showed a distinct upward curvature of the various organs (hyponasty) while others demonstrated a downward curvature (epinasty). Some stems were found to stiffen and split whereas some underground parts assumed a typical water-soaked appearance. Various tissues often showed a tendency towards proliferation.

The translocation of 2,4-D in plants has been investigated by many workers. Dhillon and Lucas (24) observed that 2,4-D applied to the soil was absorbed by the roots and translocated upwards, probably by the xylem. When the chemical was applied to the leaves, translocation was downward and was thought to take place in the phloem or other living tissues, with some possibly moving through xylem cells. The chemical was found in various organs of the tomato plant 26 days after being applied to decapitated stems.

Mitchell and Brown (51) found that when 2,4-D was applied to the leaves of bean plants, translocation of the chemical was closely associated with the translocation of food materials and was confined to living cells. When the acid was applied to the roots, it was found to move through the non-living cells of the stem. Sunlight appeared to hasten the translocation of the stimulus. Weaver and De Rose

(70) also found a relationship between the movement of the chemical and the movement of synthesized carbohydrates. There were indications that more ultimate damage resulted with very rapid translocation.

According to Tukey et al. (67) the leaves of bindweed yellowed within five days after the application of 2,4-D. They observed that seven days after treating, the cells of the upper and lower palisade layers, and the mesophyll had lost their dense contents and had become contracted, narrow, and irregular in shape. The response of the stem was varied; cambium and phloem, when observed seven days after treating, appeared to be greatly stimulated. The phloem was reported as having a 66 percent increase in width in the radial direction, and the cambium as being doubled in radial width. There was little or no response noted in the xylem.

Watson (68,69) reported that when very young bean plants and velvet bent grass plants were subjected to 2,4-D treatments, the immature, developing leaves became filled with "replacement tissue." This tissue had thicker-walled cells than were normally found in the palisade layer or spongy mesophyll; the chloroplasts were either absent or abortive and the cells had assumed odd shapes, not at all resembling normal tissue. It was found that cells which had already differentiated into palisade or mesophyll cells did not revert to "replacement tissue" but those cells which had not

developed prior to the application of the acid did. Similar tissue was reported by Eames (25) in nut grass. Besides an increased thickness in the leaf blade, caused by "replacement tissue," he observed a proliferation and splitting of the outer tissues of the corm.

Hamner and Tukey (36) observed the effects of 2,4-D on several shrubs, vines, and trees and found a variety of responses to the chemical among the different plants. They reported that warmer weather appeared to aid the chemical in its toxic effects. Plants treated when the temperature was 81 degrees Fahrenheit were injured much more quickly than those treated when the temperature was 55 degrees. They observed that the bark of a 40-foot poplar tree, which had been treated by applying a salve of 2,4-D to hatchet cuts in the trunk, began splitting above the point of application one month after treating. They further noted that the splitting was caused by a proliferation in the region outside the cambium. This region developed a formation of soft, spongy, new tissue five-eighths of an inch thick. Peach trees treated in a similar manner developed small, discolored spots on the limbs, from which gum exuded freely. This exudation was thought to be caused by an increased activity of the cells outside the cambium, which caused a flow of gum. It was noted that trees in shaded areas responded much more slowly than trees in direct sunlight.

According to Arend (5) the trunks of oak trees cracked and ruptured, forming callus-like growths due to the hormone action of 2,4,5-T. However, the bark, in general, remained tight.

Ehrhardt (27) working with sodium arsenite reported that the length of time elapsing between treatment and the first effects on the tree, varied greatly among different species. He noted that with the withering of the foliage, the bark became tight, regardless of the normal conditions at the time, and remained that way for varying periods of time, depending on the species. Oaks and evergreens showed bark separation by the end of the first growing season, but bark on many other species remained in a tight condition until the following spring.

Anatomical modifications occurring in the stems of red spruce and yellow birch, which had been treated with either sodium arsenite or sodium monochloroacetate, have been reported by Girolami (31). His observations were based on samples collected eight days after treatment and taken just above the point of chemical application. Treatments had been made before the sap-peeling season, during the sap-peeling, and after the sap-peeling season. His observations made on birch samples which had been treated during the sap-peeling season were as follows:

1. The cambium of sodium arsenite and sodium mono-

chloroacetate-treated samples had collapsed.

2. Most recently formed xylem cells of arsenite-treated samples had collapsed, causing the formation of large lacunae between the xylem and cambium. The same effects were noted in the samples which had been treated with sodium monochloroacetate but damage was not as extensive.
3. A partial collapse of immature phloem cells had taken place in the arsenite-treated samples. Some distortion was evident in the functioning phloem. All sieve tubes showed an excessive deposition of callus (which is usually considered to be a sign of decreased function). The results were the same for the samples treated with sodium monochloroacetate but again were not as pronounced.

Popp et al. (57) also conducted anatomical studies on arsenite-treated trees. Their report was confined to the results obtained on black cherry trees. Treatments were made during July and samples collected six days later from the trunk. They reported that partially differentiated cells of the xylem, cambium, and phloem appeared to be plasmolyzed and showed collapse. The contents of the sieve tubes of treated trees were observed to be more darkly stained than the contents of sieve tubes from normal trees.

They interpreted this darkening as being caused by disorganization of the sieve tube cytoplasm.

IV. EXPERIMENTAL PROCEDURE

Location and marking of trees. Most of the field work for this study was carried out at the Dunbar Forest Experiment Station, Sault Ste. Marie, Michigan. A total of 361 trembling aspen, Populus tremuloides Michx., were treated on this area during the summer of 1952. Fifteen additional trees were treated in the spring of 1953 at the Jennison Forest, located about 14 miles west of Lansing, Michigan.

Tree sizes ranged from 4 to 12 inches in diameter at breast height, the majority being from 6 to 8 inches. Metal tags, bearing letters and numbers, fastened with zinc-coated nails, were used to identify trees selected for treating. Each tree was then blazed with an axe to facilitate relocation.

Methods used to apply chemicals. All chemicals were applied to the stems of the trees by one of three methods: (1) a frill girdle followed by spraying directly into the frill; (2) a peeled girdle followed by spraying on the peeled surface; and (3) a basal spray applied directly to the bark.

The frill girdle was formed by making a series of overlapping axe cuts around the entire circumference of the tree, approximately 18 inches above the ground. The cuts

were made through the bark and into the sapwood for a depth of about one-half inch. The axe-head was pressed downward, while in the tree, causing the bark and cut portion of the sapwood to be pried away, and forming a crude reservoir around the tree stem. The chemical solution was then sprayed into the frill until the reservoir overflowed, saturating the lower portion of the tree trunk.

The peeled girdle was made by removing a strip of bark, 8 to 10 inches wide, from around the tree trunk, with the lower edge of the girdle about 18 inches from the ground. In making this girdle, an axe and bark spud were employed in such a manner that the sapwood was not injured. The chemical was then sprayed directly on the peeled area until the solution ran down the lower portion of the trunk.

The third method of treating was the basal spray method. In this type of treatment, the chemical was sprayed on the basal portion of the tree trunk without any previous preparation having been made. The trees were sprayed from the ground to a point approximately 30 inches high on the trunk. The bark was saturated with solution, and spraying was continued until the solution ran down the bark and started to moisten the soil.

Hand sprayers, with a capacity of 2 gallons each, were used to apply the chemicals. Separate, marked sprayers were employed for each chemical to prevent contamination. The solutions were mixed in the sprayers.

The solutions were not applied in accurately measured amounts because it was desired to approach actual treating conditions, such as might be encountered in commercial practice. However, an effort was made to treat 10 trees per gallon of solution, regardless of the type of treatment which had been used.

Modifications of the treating methods described above were employed on two occasions. One group of trees was treated by pouring very small quantities of a sodium arsenite solution into the frilled girdles, which had been made in the usual manner. Another group of trees was treated with sodium monochloroacetate, which had been sprayed into frill girdles that extended only half way around the stem.

Chemical solutions used. As stated previously, three chemicals were used in these experiments: propylene glycol butyl ether ester of 2,4-dichlorophenoxyacetic acid (2,4-D ester), propylene glycol butyl ether ester of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T ester) and sodium monochloroacetate.

The 2,4-D ester was always applied as an oil solution; number 2 diesel oil being used as the carrier. The 2,4,5-T ester was also used as a diesel oil solution in every instance except one, in which it was applied as a water emulsion. These two chemicals were used in different concentrations: 8 pounds a. h. g.; 16 pounds a. h. g.; and 24 pounds

a. h. g. (acid equivalent per 100 gallons of treating solution).

Sodium monochloroacetate was dissolved in water and used in strengths of approximately 20 and 24 percent, by weight. One group of trees was treated with an emulsion made up by dissolving sodium monochloroacetate in one-half water and one-half diesel oil; the strength of this emulsion was approximately 27 percent, by weight.

As a matter of curiosity, three groups of trees were treated with other chemicals or combinations of chemicals: one group was treated with a 5 percent, by weight, solution of pentachlorophenol in diesel oil; a second group was treated with a solution containing equal parts of a 5 percent, by weight, pentachlorophenol-diesel oil solution, and an 8 pounds a. h. g. solution of 2,4,5-T ester in diesel oil; a third group was treated with a 20 percent solution, by weight, of sodium arsenite in water. /

Treating schedule. Trees were treated on seven different dates at Dunbar; the earliest date was June 4 and the latest August 11. The trees, treated on each of these days, were considered to constitute a treating series; each series was made up of a varying number of tree groups; and each group averaged 10 trees. Each tree group of a particular series represented a different combination of treating method and chemical, according to a previously conceived plan.

One gallon of chemical solution was made up for each group, and as was stated previously, an effort was made to treat 10 trees with it. However, when an unusual number of large trees occurred within a group, it sometimes was not possible to treat 10 trees per gallon and the group then contained a lesser number of treated trees. Groups seldom contained more than 10 trees because, if any solution was left over after the tenth tree had been treated, the group was usually sprayed lightly a second time to use up the excess solution in the sprayer. Complete information relative to treating methods and dates of application for each tree treated at Dunbar can be found in Table I.

Three treating dates were employed during the spring of 1953 at the Jennison Forest; the earliest was April 24 and the latest May 15. Complete information regarding these treatments can be found in Table II.

The chemicals used at Dunbar were also employed at Jennison. The same concentrations and quantities were used except in two instances, when rates of 20 trees per gallon and 40 trees per gallon were used. Treating methods were also the same, except that no peeled girdles were utilized.

All treatments, at both areas, were made on clear days and no rain fell for at least 24 hours after treating except on two occasions: on August 11 a heavy rain, of approximately 3 hours duration, fell shortly after treating was

TABLE I

SCHEDULE OF TREATMENTS MADE AT DUNBAR DURING 1952

(S means basal spray, F means frilled girdle,
and P means peeled girdle.)

Tree numbers within group	Chemical used	Strength	Method	Number trees per group
<u>June 4 treatments</u>				
DL-DL3	2,4,5-T ester	8# a.h.g. ^a	S	13 ^b
<u>June 24 treatments</u>				
DK1-DK10	Sod. monochloro- acetate	20%, wt.	P	10
DK11-DK20	Sod. monochloro- acetate	20%, wt.	F	10
DK21-DK25	Sod. monochloro- acetate	20%, wt.	S	5
DK26-DK35	2,4,5-T ester	8# a.h.g.	S	10
DK36-DK45	2,4,5-T ester	8# a.h.g.	F	10
DK46-DK55	2,4,5-T ester	16# a.h.g.	S	10
DK56-DK64	2,4-D ester	8# a.h.g.	S	9
<u>June 25 treatments</u>				
DK66-DK74	Pentachlorophenol	5%, wt.	S	9
DK75-DK85	Pentachlorophenol and 2,4,5-T ester	8# a.h.g.	S	11
DK86-DK91	NONE	--	F	6
DK92-DK95	NONE	--	P	4
<u>July 3 treatments</u>				
DK96-DK104	Sod. monochloro- acetate	20%, wt.	P	9
DK105-DK114	Sod. monochloro- acetate	20%, wt.	F	10
DK115-DK124	2,4,5-T ester	8# a.h.g.	F	10
DK125-DK134	2,4,5-T ester	8# a.h.g.	S	10
DK135-DK144	2,4,5-T ester	16# a.h.g.	S	10
DK145-DK153	2,4-D ester	8# a.h.g.	S	9

TABLE I (continued)

Tree numbers within group	Chemical used	Strength	Method	Number trees per group
<u>July 9 treatments</u>				
DH1-DH10	Sod. monochloro- acetate	27%, wt.	S	10 ^c
DH11-DH19	Sod. monochloro- acetate	20%, wt.	F	9
DH20-DH29	Sod. monochloro- acetate	20%, wt.	P	10
DH30-DH39	2,4-D ester	8# a.h.g.	S	10
DH40-DH49	2,4-D ester	16# a.h.g.	S.	10
DH50-DH59	2,4,5-T ester	8# a.h.g.	S	10
DH60-DH69	2,4,5-T ester	16# a.h.g.	S	10
DH70-DH79	2,4,5-T ester	8# a.h.g.	F	10
<u>July 15 treatments</u>				
DH120-DH129	2,4-D ester	8# a.h.g.	S	10
DH130-DH139	2,4-D ester	8# a.h.g.	F	10
DH140-DH149	2,4-D ester	16# a.h.g.	S	10
DH150-DH159	2,4,5-T ester	8# a.h.g.	S	10
DH160-DH169	2,4,5-T ester	8# a.h.g.	F	10
DH170-DH179	2,4,5-T ester	16# a.h.g.	S	10
DH180-DH189	2,4,5-T ester	24# a.h.g.	S	10
DH190-DH196	2,4,5-T ester	8# a.h.g.	P	7 ^d
<u>August 11 treatments</u>				
DJ5-DJ9	Sod. arsenite		F	5 ^e
DJ10-DJ14	Sod. monochloro- acetate	24%, wt.	F	5 ^f
DJ15-DJ24	Sod. monochloro- acetate	24%, wt.	F	10
DJ25-DJ34	2,4,5-T ester	8# a.h.g.	S	10
DJ35-DJ44	2,4-D ester	8# a.h.g.	S	10

^a Acid equivalent per 100 gallons of treating solution.

^b One gallon of solution per tree group unless otherwise specified.

^c Carrier was half water and half diesel oil.

^d Carrier was water.

^e About one-eighth pint per tree; poured into frill.

^f One-half gallon used; trees frilled half way around stem.

TABLE II
SCHEDULE OF TREATMENTS MADE AT JENNISON DURING 1953
(S means basal spray and F means frilled girdle.)

Tree numbers within group	Chemical used	Strength	Method	Number trees per group
<u>April 24 treatments</u>				
J95-J96	Sod. monochloro- acetate	20%, wt.	F	2 ^a
J97-J98	2,4-D ester	8# a.h.g. ^b	S	2
J99-J100	2,4,5-T ester	8# a.h.g.	S	2
<u>May 8 treatments</u>				
J80	2,4-D ester	8# a.h.g.	S	1
J81	2,4,5-T ester	8# a.h.g.	S	1
J82	Sod. monochloro- acetate	20%, wt.	F	1
<u>May 15 treatments</u>				
J70 & J72	Sod. monochloro- acetate	20%, wt.	F	2 ^c
J73 & J74	Sod. monochloro- acetate	20%, wt.	F	2 ^d
J71 & J75	Sod. monochloro- acetate	20%, wt.	F	2

^a Solution applied at the rate of 10 trees/gallon, unless specified otherwise.

^b Acid equivalent per 100 gallons of treating solution.

^c Solution applied at the rate of 40 trees/gallon.

^d Solution applied at the rate of 20 trees/gallon.

completed; on April 24 a heavy rain fell at night, starting about 6 hours after treating.

The treatments at Dunbar were made during a rather heavy infestation of the Forest Tent Caterpillar, Malacosoma disstria Hubn. Some of the trees were partially defoliated at the time of treatment; others were getting a second set of leaves which was not fully developed.

The April 24 treatments at Jennison were made before bud break; the May 8 treatments were made when the trees were beginning to leaf out, and the May 15 treatments made when the leaves were about three-quarters developed.

Collection of samples for microscopic study. Before any samples were collected, the objectives of this phase of the problem were listed, and individual collection schedules drawn up to obtain the necessary material. It was apparent that some samples could be used for two or more purposes, thus reducing the total number of samples required. After individual schedules had been completed, a master schedule was drawn up, from which all samples were obtained.

Because of the importance of the tree stem in the manufacture of pulp and paper, and because of the significance of the cambium in bark peeling, the major emphasis of this problem was placed on the cambial region of the treated tree stem. Accordingly, the first collection schedule was designed to secure samples which would provide information

relative to this portion of the treated tree. The information desired was as follows: (1) to determine whether or not the application of 2,4-D ester, 2,4,5-T ester or sodium monochloroacetate would cause any structural changes in the cambium region of the tree stems; (2) if changes did occur, to determine the progressive effects of each chemical; (3) to determine, if possible, the relationship between these progressive effects and the loosening of the bark; (4) to determine which chemical was most effective in loosening the bark; and (5) to determine what the effect would be of doubling the strength of one of the chemical solutions.

Four trees, selected from the June 24 treatments, were used to obtain these samples. The following four treatments were used on these trees: sodium monochloroacetate in a frill; 2,4-D ester in a basal spray; 2,4,5-T ester in a basal spray; and a double strength solution of 2,4,5-T ester in a basal spray. The first samples were collected on July 7 and at approximately weekly intervals thereafter until September 3.

Most samples were taken from the tree stem at approximately 5 feet above the ground; a few were also obtained from the sprayed area of the stem (18 inches from the ground), for comparison purposes. The collection schedule for these samples is presented in Table III.

TABLE III

SCHEDULE OF STEM SAMPLES TAKEN FROM TREES TREATED WITH THREE DIFFERENT CHEMICALS AND COLLECTED AT APPROXIMATELY WEEKLY INTERVALS DURING THE SUMMER OF 1952

(Trees were treated on June 24, 1952.)

Sample Number	Chemical used	Collection date	Days since treatment
DK-17-1S ¹	Sod. monochloro-acetate ²	July 9	15
DK-27-1S	2,4,5-T ester	July 9	15
DK-49-1S	2,4,5-T ester ³	July 9	15
DK-61-1S	2,4-D ester	July 9	15
DK-17-2S	Sod. monochloro-acetate	July 17	23
DK-27-2S	2,4,5-T ester	July 17	23
DK-49-2S	2,4,5-T ester ³	July 17	23
DK-61-2S	2,4-D ester	July 17	23
DK-17-3S	Sod. monochloro-acetate	July 24	30
DK-27-3S	2,4,5-T ester	July 24	30
DK-49-3S	2,4,5-T ester ³	July 24	30
DK-17-4S	Sod. monochloro-acetate	July 30	36
DK-27-4S	2,4,5-T ester	July 30	36
DK-49-4S	2,4,5-T ester ³	July 30	36
DK-61-3S	2,4-D ester	July 30	36
DK-17-5S	Sod. monochloro-acetate	Aug. 8	45
DK-27-5S	2,4,5-T ester	Aug. 8	45
DK-61-4S	2,4-D ester	Aug. 8	45
DK-17-6S	Sod. monochloro-acetate	Aug. 14	51
DK-27-6S	2,4,5-T ester	Aug. 14	51
DK-49-5S	2,4,5-T ester ³	Aug. 14	51
DK-61-5S	2,4-D ester	Aug. 14	51

TABLE III (continued)

Sample Number	Chemical used	Collection date	Days since treatment
DK-17-7S	Sod. monochloro- acetate	Aug. 20	57
DK-27-7S	2,4,5-T ester	Aug. 20	57
DK-61-6S	2,4-D ester	Aug. 20	57
DK-17-8S	Sod. monochloro- acetate	Aug. 27	64
DK-27-8S	2,4,5-T ester	Aug. 27	64
DK-49-6S	2,4,5-T ester ³	Aug. 27	64
DK-61-7S	2,4-D ester	Aug. 27	64
DK-17-9S	Sod. monochloro- acetate	Sept. 3	71
DK-27-9S	2,4,5-T ester	Sept. 3	71
DK-49-7S	2,4,5-T ester ³	Sept. 3	71
DK-61-8S	2,4-D ester	Sept. 3	71

¹Explanation of sample numbers: Each sample was given a number at the time of collection, corresponding to the tree from which it had been taken. In addition, a set of letters and numbers, following the tree number, was used to describe how many times the tree had been sampled and the portion of the tree used. For example, DK-17-1S was used to describe the sample taken from tree DK-17 (See Table I). The "1" following the second dash means that it was the first time that tree DK-17 had been sampled; the S means that it was a stem sample. The letters L, B, and R were used to designate leaf, branch, and root samples respectively. These letters occur in Table VI.

²Sodium monochloroacetate was used in conjunction with a frilled girdle; 2,4,5-T ester and 2,4-D ester were used as a basal spray.

³Applied double strength (16# a. h. g.).

A second set of stem samples, also taken approximately 5 feet above the ground, was obtained to determine the influence of time of treatment on the effectiveness of each of the three chemicals. These samples were removed from trees treated on different dates, and in each case they were collected 36 days after treating. This information is presented in Table IV.

Eighty days after treating, one tree which had been treated with 2,4,5-T ester on June 4, was felled and stem samples taken at 5-foot intervals from the sprayed area to the top of the tree; the purpose of this sampling was to determine if the action of the chemical was the same throughout the length of the tree. Branch and root samples were also taken from this tree at the same time, to determine if the chemical had caused any anatomical changes in these tree parts.

Seventy-one days after treating, root samples were collected from tree DK-17, which had been treated with sodium monochloroacetate on June 24. This was the tree from which stem samples had been taken all summer, as presented in Table III.

Branch and leaf samples were collected throughout the summer when definite signs of injury could be detected in the trees. No particular schedule was followed in the col-

TABLE IV

COLLECTION SCHEDULE OF STEM SAMPLES TAKEN 36 DAYS
AFTER TREATING, FROM TREES WHICH HAD BEEN TREATED
ON VARIOUS DATES DURING 1952

Sample number	Chemical used	Treatment date	Collection date	Days since treatment
DK-17-4S	Sod. monochloro-acetate*	June 24	July 30	36
DK-27-4S	2,4,5-T ester	June 24	July 30	36
DK-61-3S	2,4-D ester	June 24	July 30	36
DK-111-2S	Sod. monochloro-acetate	July 3	Aug. 8	36
DK-130-1S	2,4,5-T ester	July 3	Aug. 8	36
DK-147-1S	2,4-D ester	July 3	Aug. 8	36
DH-16-1S	Sod. monochloro-acetate	July 9	Aug. 14	36
DH-58-1S	2,4,5-T ester	July 9	Aug. 14	36
DH-38-1S	2,4-D ester	July 9	Aug. 14	36
DH-159-1S	2,4,5-T ester	July 15	Aug. 20	36
DH-127-1S	2,4-D ester	July 15	Aug. 20	36

*Sodium monochloroacetate was used in conjunction with a frilled girdle; 2,4,5-T ester and 2,4-D ester were used as a basal spray.

lection of these samples, because it was not desired to study progressive effects of chemicals on leaves.

Other miscellaneous stem samples were taken during the summer to obtain additional information on the effect of chemicals on the structure of the tree stem. These samples were not obtained according to a fixed schedule, but were collected at any convenient time.

Table V shows all the samples collected at the Dunbar Station during the summer of 1952. Sample numbers, treatment dates, chemicals and collection dates are given. It should be noted that every time a sample was taken from a treated tree, a corresponding sample was obtained from an untreated tree. In addition to these samples collected at Dunbar, additional untreated samples were taken from the Jennison area during 1953 and 1954 to provide complete information on the condition of the cambial region at all seasons of the year; these samples are also included in Table V.

Field observations. Field observations were made at Dunbar throughout the summer of 1952. They were usually made at the same time that samples were collected and were, for the most part, confined to the trees being sampled. These observations were made primarily on the appearance of leaves, twigs and stems of the trees. It was hoped that some correlation might be found between the outward appearance of the tree and the internal, microscopic changes oc-

TABLE V

COLLECTION SCHEDULE OF ALL SAMPLES TAKEN AT DUNBAR DURING
1952 AND THE JENNISON AREA DURING 1953 AND 1954

(S means basal spray, F means frilled girdle,
and P means peeled girdle.)

Sample number	Chemical used	Treat- ment date	Method	Collec- tion date	Days since treat- ment
DUN-1-1S ¹	Untreated	--	--	7/1/52	--
DK-1-1S	Sod. monochloro- acetate	6/24/52	P ³	7/9/52	15
DK-21-1S	Sod. monochloro- acetate	6/24/52	S	7/9/52	15
DK-27-1S	2,4,5-T ester	6/24/52	S	7/9/52	15
DK-49-1S	2,4,5-T ester ²	6/24/52	S	7/9/52	15
DK-61-1S	2,4-D ester	6/24/52	S	7/9/52	15
DK-17-1S	Sod. monochloro- acetate	6/24/52	F	7/9/52	15
DUN-2-1S	Untreated	--	--	7/9/52	--
DK-15-1LB	Sod. monochloro- acetate	6/24/52	F	7/9/52	15
DUN-3-1S	Untreated	--	--	7/17/52	--
DK-17-2S	Sod. monochloro- acetate	6/24/52	F	7/17/52	23
DK-27-2S	2,4,5-T ester	6/24/52	S	7/17/52	23
DK-49-2S	2,4,5-T ester ²	6/24/52	S	7/17/52	23
DK-61-2S	2,4-D ester	6/24/52	S	7/17/52	23
D-1-1S	2,4,5-T ester	6/4/52	S	7/19/52	45
DUN-4-1S	Untreated	--	--	7/19/52	--
DK-17-3S	Sod. monochloro- acetate	6/24/52	F	7/24/52	30
DK-27-3S	2,4,5-T ester	6/24/52	S	7/24/52	30
DK-49-3S	2,4,5-T ester ²	6/24/52	S	7/24/52	30
DUN-5-1S	Untreated	--	--	7/24/52	--
DUN-6-1SEL	Untreated	--	--	7/30/52	--
DUN-7-1S	Untreated	--	--	7/30/52	--
DK-17-4S	Sod. monochloro- acetate	6/24/52	F	7/30/52	36
DK-27-4S	2,4,5-T ester	6/24/52	S	7/30/52	36
DK-49-4S	2,4,5-T ester ²	6/24/52	S	7/30/52	36
DK-61-3S	2,4-D ester	6/24/52	S	7/30/52	36

TABLE V (continued)

Sample number	Chemical used	Treatment date	Method	Collection date	Days since treatment
DK-111-1LB	Sod. monochloroacetate	7/3/52	F	7/30/52	27
D-1-2SB	2,4,5-T ester	6/4/52	S	8/5/52	62
DUN-4-2SLB	Untreated	--	--	8/5/52	--
DK-17-5S	Sod. monochloroacetate	6/24/52	F	8/8/52	45
DK-27-5S	2,4,5-T ester	6/24/52	S	8/8/52	45
DK-61-4S	2,4-D ester	6/24/52	S	8/8/52	45
DK-111-2S	Sod. monochloroacetate	7/3/52	F	8/8/52	36
DK-130-1S	2,4,5-T ester	7/3/52	S	8/8/52	36
DK-147-1S	2,4-D ester	7/3/52	S	8/8/52	36
DUN-6-2S	Untreated	--	--	8/8/52	--
DK-17-6S	Sod. monochloroacetate	6/24/52	F	8/14/52	51
DK-27-52	2,4,5-T ester	6/24/52	S	8/14/52	51
DK-49-5S	2,4,5-T ester ²	6/24/52	S	8/14/52	51
DK-61-5S	2,4-D ester	6/24/52	S	8/14/52	51
DH-16-1S	Sod. monochloroacetate	7/9/52	F	8/14/52	36
DH-38-1S	2,4-D ester	7/9/52	S	8/14/52	36
DH-44-1S	2,4-D ester ²	7/9/52	S	8/14/52	36
DH-58-1S	2,4,5-T ester	7/9/52	S	8/14/52	36
DUN-6-3S	Untreated	--	--	8/14/52	--
DK-6-1LB	Sod. monochloroacetate	6/24/52	P	8/18/52	55
DK-28-1LB	2,4,5-T ester	6/24/52	S	8/18/52	55
DH-22-1LB	Sod. monochloroacetate	7/9/52	P	8/18/52	40
DH-30-1LB	2,4-D ester	7/9/52	S	8/18/52	40
DH-52-1LB	2,4,5-T ester	7/9/52	S	8/18/52	40
DH-122-1LB	2,4-D ester	7/15/52	S	8/18/52	34
DJ-8-1LB	Sodium arsenite	8/11/52	F	8/18/52	7
DJ-23-1LB	Sod. monochloroacetate	8/11/52	F	8/18/52	7
DUN-8-1LB	Untreated	--	--	8/18/52	--
DK-17-7S	Sod. monochloroacetate	6/24/52	F	8/20/52	57
DK-27-7S	2,4,5-T ester	6/24/52	S	8/20/52	57

TABLE V (continued)

Sample number	Chemical used	Treatment date	Method	Collection date	Days since treatment
DK-61-6S	2,4-D ester	6/24/52	S	8/20/52	57
DH-159-1S	2,4,5-T ester	7/15/52	S	8/20/52	36
DH-127-1S	2,4-D ester	7/15/52	S	8/20/52	36
DUN-6-4S	Untreated	--	--	8/20/52	--
DJ-6-1S	Sodium arsenite	8/11/52	F	8/20/52	9
DJ-22-1S	Sod. monochloro-acetate	8/11/52	F	8/20/52	9
D-1-3SBR	2,4,5-T ester	6/4/52	S	8/23/52	80
DUN-4-3SBR	Untreated	--	--	8/23/52	--
DH-58-2S	2,4,5-T ester	7/9/52	S	8/23/52	45
DK-17-8S	Sod. monochloro-acetate	6/24/52	F	8/27/52	64
DK-27-8S	2,4,5-T ester	6/24/52	S	8/27/52	64
DK-49-6S	2,4,5-T ester ²	6/24/52	S	8/27/52	64
DK-61-7S	2,4-D ester	6/24/52	S	8/27/52	64
DUN-6-5S	Untreated	--	--	8/27/52	--
DJ-6-2S	Sodium arsenite	8/11/52	F	8/27/52	16
DJ-22-2S	Sod. monochloro-acetate	8/11/52	F	8/27/52	16
DH-159-2S	2,4,5-T ester	7/15/52	S	8/29/52	45
DK-17-9SR	Sod. monochloro-acetate	6/24/52	F	9/3/52	71
DK-27-9S	2,4,5-T ester	6/24/52	S	9/3/52	71
DK-49-7S	2,4,5-T ester ²	6/24/52	S	9/3/52	71
DK-61-8S	2,4-D ester	6/24/52	S	9/3/52	71
DUN-6-6SR	Untreated	--	--	9/3/52	--
J-89-1S	Untreated	--	--	4/24/53	--
J-89-2S	Untreated	--	--	5/8/53	--
J-89-3S	Untreated	--	--	5/15/53	--
J-89-4S	Untreated	--	--	5/22/53	--
J-89-5S	Untreated	--	--	5/29/53	--
J-89-6S	Untreated	--	--	6/5/53	--
J-89-7S	Untreated	--	--	2/18/54	--

¹See first footnote under Table III for explanation of sample numbers.

²Double strength (16# a. h. g.).

curing in the treated trees. In addition to these general observations, a detailed inspection of every treated tree was made during the summer of 1952; another was made during the summer of 1953. These inspections noted the peelability of the bark as well as the general appearance of the trees.

Field observations were also made during 1953 and 1954 on the trees which had been treated at the Jennison area. The primary purpose of these observations was to compare the effects of very early treatments with those applied later in the season.

In order to make further observations, two inspection trips, during the summers of 1953 and 1954, were made to the properties of the Patten Logging Company at Anasa, Michigan, and the William Bonifas Lumber Company at Iron Mountain, Michigan. These companies were both conducting chemical debarking experiments on aspen, using the same chemicals as were used at Dunbar.

Methods of sample removal. Samples were removed from the stems and roots of trees with a set of wood-carving chisels. The chisels were of an ordinary square-edge type, and a hollow, half-round type. Leaf and twig samples were extracted with the aid of a razor-sharp scalpel.

Since it was desired to study the cambium and adjacent tissues only, and because it facilitated sample removal, the outer portion of the bark was carefully removed with the

square-edge chisel before stem or root samples were extracted (see Fig. 1). After the outer portion of the bark had been removed, a rectangle, measuring approximately one-half inch wide and three-quarters of an inch long, was formed by cutting through the phloem and cambium, into the outer layers of the xylem. This was accomplished by removing a number of very thin layers of material with the half-round chisel, as shown in Fig. 2. A clean cut was made through the outermost growth rings by placing the square-edged chisel along the perimeter of the rectangle and pushing the blade into the wood. The chisel was then placed diagonally across each corner of the rectangle and pushed into the xylem forming an octagon (see Fig. 3). The square-edge chisel was then inserted in the groove which formed the base of the octagon and pressed into the wood, care being taken that it was not pressed in too far. The handle of the chisel was then pushed down, prying the sample out of the tree, as illustrated in Fig. 4. Each sample when removed contained one or two growth rings as well as the cambial region, functioning phloem, and at least a portion of the non-functioning phloem.

Small twigs, measuring between one-sixteenth and three-sixteenths of an inch in diameter, were sampled by making clean, cross-sectional cuts through the twigs with a sharp

Fig. 1. Showing method of removing outer bark
of a tree with a chisel.

Photograph through the courtesy of John L. Hill.



Fig. 2. Formation of rectangle by cutting through
the phloem and into the outer layers
of the xylem.

Photograph through the courtesy of John L. Hill.



Fig. 3. Formation of octagon by cutting across
the corners of previously formed rectangle.

Photograph through the courtesy of John L. Hill.



Fig. 4. Method of prying out sample composed of phloem, cambium, and outermost rings of xylem.

Photograph through the courtesy of John L. Hill.



scalpel. The samples were all cut to approximately three-eighths of an inch in length.

The mid-rib portion of leaves was sampled by removing a rectangular portion of the leaf blade which included the mid-rib. The rectangles were approximately three-sixteenths of an inch wide (at right angles to the mid-rib), and one-quarter of an inch long (parallel with the mid-rib).

When the samples were removed from the tree, they were immediately placed in numbered bottles containing FAA killing and fixing fluid made in accordance with the formula given by Johansen (42) for woody tissues. Upon returning from the field, the samples were transferred to test tubes and aspirated by means of a water aspirator until they sank to the bottom of the tubes. A small strip of white cardboard, with the sample number written on it with India ink, was placed in each test tube.

A 4 by 6 inch file card was made out for each sample collected and all pertinent information relative to treating, collecting and subsequent handling was recorded thereon.

Sectioning and staining of material. All material was embedded in paraffin and mounted on wooden blocks prior to sectioning; the identifying number of each sample was written on the wooden block in india ink.

A 3 by 5 inch file card was then made out for each sample when it was sectioned; information relative to thickness of sections, staining schedule followed, and number of slides made was recorded.

Leaf sections were cut on a rotary microtome and affixed to glass slides by means of Haupt's adhesive. Only cross sections were made of leaves and they were all cut 10 microns thick.

All other sections were made on a sliding microtome and were cut between 8 and 14 microns in thickness. Cross sections and radial sections were cut for all stem and root samples, and for some twig samples. The sections were placed between wire screens, as described by Hyland (40) to prevent curling and to expedite staining. All wood sections were stained with safranin and fast green, and were made into permanent slides.

V. FIELD OBSERVATIONS

The effects of chemical treatment on tree appearance.

The first treatments, involving all the standard chemicals and treating methods, were made on June 24 and June 25. Therefore, in order to observe the effects of the chemicals for the longest period of time, most field observations were made on trees treated on these dates.

The first trees to show signs of injury from chemical treatment were those which had been frilled and treated with sodium monochloroacetate. These trees were treated on June 24 and an inspection made 15 days later. All the leaves were turning black and most of them had a dried, distorted appearance. The bark of the stems was turning a dark brown immediately above the frilled areas. None of the other trees, treated at the same time, showed signs of injury.

Twenty-three days after treating it was observed that all trees treated with sodium monochloroacetate in frills were completely defoliated. Stem bark appeared to be completely blackened from the frills to a point about 3 feet above the frills. A few trees which had received a basal spray treatment of 2,4,5-T ester showed some effects of the treatment. The leaves were starting to display brilliant red and yellow colors; small brown and orange-colored spots

began to appear on the lower 5 or 6 feet of the stem bark. No other treated trees appeared to be affected at this time.

Observations made 30 days after treating revealed that the phloem and cambial regions of trees, treated with sodium monochloroacetate in frills, appeared to be completely killed beneath the large areas of blackened bark. These areas extended to about 5 feet above the frills at this time. In contrast to this condition, the only phloem and cambial tissues that appeared dead on trees treated with 2,4,5-T ester, were confined to small areas found beneath the discolored spots described previously. These spots were more numerous and larger than reported at 23 days. However, extensive areas of green bark existed between discolored spots, and the phloem and cambial regions beneath these green areas appeared to be healthy. The first evidence of damage to trees which had received a basal spray of 2,4-D ester was observed on this date; the effects were very similar to those produced by 2,4,5-T ester.

Defoliation of trees treated with 2,4,5-T ester did not occur until 45 days after treatment and was then only taking place on some of the trees. This defoliated condition can be seen in Figure 5. It was noted at this time that sodium monochloroacetate killed leaves much more quickly than either 2,4,5-T or 2,4-D; leaves of trees treated with sodium monochloroacetate had turned from green to black within a

few days' time and had fallen within 23 days. The leaves of trees treated with 2,4,5-T ester had first turned a brilliant red or yellow color and had remained on the trees for a much longer time. At the time of this examination some leaves on trees treated with 2,4-D were still green. It was also noted that the stem-wood beneath the discolored patches of 2,4,5-T treated trees, contained black rings of tissue; the rings were smaller and were contained within the perimeters of the discolored areas. These rings are plainly visible in Figure 6. It was also noted at this time that the sprayed area of stems, and the tips of branches of 2,4,5-T treated trees, were evidently in an advanced state of injury. The branches, between the tree trunks and the branch tips, appeared to be healthy; the trunks themselves appeared to be healthy from just below the tips of the leaders to a point about 10 feet above the ground. Between 5 and 10 feet above the ground, the outer surface of the bark seemed to be green. However, the phloem and cambial regions were observed to be turning a light brown color. Below 5 feet the bark contained numerous discolored patches, and the sprayed area of the trunks was turning a solid orange-brown color.

Trees treated with 2,4-D ester began to develop many discolored spots on the lower portion of the stems at 51 days after treating. These spots were very similar to those caused by 2,4,5-T ester. As seen in Figure 7, these

blotches were exuding a resinous liquid, especially those of the 2,4-D treated trees. The leaves of the 2,4-D treated trees were now in full autumn color, while those of most of the 2,4,5-T treated trees were blackened and shrivelled; a few were completely defoliated. However, some of the 2,4,5-T treated trees still retained numerous colored leaves. It was also noted at this time that almost all trees which had been treated with a double strength solution of 2,4,5-T ester were completely defoliated, although the extent of damage to the stem bark seemed to be about the same as that on trees treated with single strength.

Trees treated with sodium monochloroacetate applied to a peeled girdle first displayed signs of injury 55 days after treatment; the leaves of some trees were turning a brownish color. At the same time, it was observed that the bark could easily be stripped, for about a foot, above the frilled areas of trees treated with this type girdle and sodium monochloroacetate.

At 62 days after treating, the discolored spots on 2,4,5-T treated trees had spread all over the stem, from the ground to the crown; in many places they were coalesced, forming large areas of orange-colored bark. However, areas of green bark could still be seen on most trunks. The tips of most branches were completely killed, and the portions

between tips and trunks displayed an orange color similar to that of the stem.

The appearance of trees treated with 2,4-D was quite similar to those treated with 2,4,5-T, although the degree of damage was much less severe. On the contrary, trees which had been frilled and treated with sodium monochloroacetate were quite different in appearance from either the 2,4-D or 2,4,5-T treated trees. The stem bark of trees treated with sodium monochloroacetate was now a solid black color from the frills to the crowns. On some trees, green bark was still visible, but it was confined to very narrow vertical streaks, sometimes extending from the frills to the tops of the trees. It was seen that most of the bark between frills and the ground was still green.

An examination made 71 days after treating showed that the bark of trees which had been frilled and treated with sodium monochloroacetate could easily be stripped off the trunk, from the frills to a height of about 7 feet above the ground. On many trees treated with 2,4,5-T, very little green bark could be found above the sprayed area; the sprayed area itself had turned a solid brown color. In most cases, the bark of these trees still resisted peeling. Trees treated with 2,4-D were similar in appearance to 2,4,5-T treated trees but were not damaged as much.

It was interesting to note that although trees treated with double strength 2,4,5-T had been defoliated more quickly than those with single strength solutions, there did not seem to be any difference in appearance or peelability of the stem bark at this time. The roots of all treated trees apparently had not been damaged by the chemical treatments, even though many of the trees appeared to be killed down to the root collar.

Eighty days after treating, the bark of trees which had been treated with 2,4,5-T on June 4, 1952, displayed an orange and brown, variegated color above the sprayed areas. Branches were blackened for a distance of about 8 inches back of the tips; the portions between tips and trunks exhibited a coloration similar to the stem. This coloration can be seen in Figure 8. The bark of the sprayed areas was a solid brown color and could be peeled off fairly easily; bark above the sprayed areas was still tight and could not be peeled. The roots still seemed to be healthy, even immediately below the root collars.

Effectiveness of chemicals and treatments used to facilitate peeling. A summary of a 100 percent inspection made on September 5 can be found in Table VI. The condition of all trees, treated during the summer of 1952, is presented in this table. Condition of trees was determined by a careful examination of leaves and stem bark.

TABLE VI

SUMMARY OF INSPECTION MADE ON SEPTEMBER 5, 1952,
OF ASPEN TREATED DURING 1952

(S means basal spray, P means peeled girdle,
and F means frill girdle.)

Treat- ment date	Group	Chemical used	Meth- od	Number of trees				
				T ¹	D	ND	I	H
6/4	DL-D13	2,4,5-T ester	S	12	12			
6/24	DK1-DK10	Sod. monochloro- acetate	P	10	2	2	1	5
6/24	DK11-DK20	Sod. monochloro- acetate	F	9	9			
6/24	DK21-DK25	Sod. monochloro- acetate	S	5	1			4
6/24	DK26-DK35	2,4,5-T ester	S	9	8		1	
6/24	DK36-DK45	2,4,5-T ester	F	10	6		4	
6/24	DK46-DK55	2,4,5-T ester ²	S	10	10			
6/24	DK56-DK64	2,4-D ester	S	9	9			
6/24	DK65-DK74	Pentachlorophenol	S	10				10
6/25	DK75-DK85	$\frac{1}{2}$ Pentachlorophenol: $\frac{1}{2}$ 2,4,5-T ester	S	11	2		7	2
6/25	DK86-DK90	None	F	5				5
6/25	DK91-DK95	None	P	5			5	
7/3	DK95-DK104	Sod. monochloro- acetate	P	9	4	1	4	
7/3	DK105-DK114	Sod. monochloro- acetate	F	10	6	2	2	
7/3	DK115-DK124	2,4,5-T ester	F	10	4		3	3
7/3	DK125-DK134	2,4,5-T ester	S	10	9	1		
7/3	DK135-DK144	2,4,5-T ester ²	S	10	10			
7/3	DK145-DK153	2,4-D ester	S	9	9			
7/9	DH1-DH10	Sod. monochloro- acetate: $\frac{1}{2}$ water, $\frac{1}{2}$ diesel oil	S	10	2	1	3	4
7/9	DH11-DH19	Sod. monochloro- acetate	F	9	9			
7/9	DH20-DH29	Sod. monochloro- acetate	P	10	8	1	1	
7/9	DH30-DH39	2,4-D ester	S	10	3	1	3	3
7/9	DH40-DH49	2,4-D ester ²	S	10	5	1	3	1

TABLE VI (continued)

Treat- ment date	Group	Chemical used	Meth- od	Number of trees				
				T ¹	D	ND	I	H
7/9	DH50-DH59	2,4,5-T ester	S	10	7	2	1	
7/9	DH60-DH69	2,4,5-T ester ²	S	10	10			
7/9	DH70-DH79	2,4,5-T ester	F	10	7		3	
7/15	DH120-DH129	2,4-D ester	S	10		2	6	2
7/15	DH130-DH139	2,4-D ester	F	10		1	4	5
7/15	DH140-DH149	2,4-D ester ²	S	10	2	1	6	1
7/15	DH150-DH159	2,4,5-T ester	S	10	7		2	1
7/15	DH160-DH169	2,4,5-T ester	F	10	4	4	2	
7/15	DH170-DH179	2,4,5-T ester ²	S	10	5	2	3	
7/15	DH180-DH189	2,4,5-T ester ³	S	10	10			
7/15	DH190-DH196	2,4,5-T ester, water solution	P	7				7
8/11	DJ5-DJ9	Sodium arsenite	F	5	5			
8/11	DJ10-DJ14	Sod. monochloro- acetate ⁴	F	5	2		3	
8/11	DJ15-DJ24	Sod. monochloro- acetate	F	10	10			
8/11	DJ25-DJ34	2,4,5-T ester	S	10		3	6	1
8/11	DJ35-DJ44	2,4-D ester	S	10	1	2	4	3

¹T means total, D means dead, ND means nearly dead, I means showing injury, and H means healthy.

²Double strength (16# a. h. g.).

³Triple strength (24# a. h. g.).

⁴Frilled and treated on one side of tree only.

In general it can be said that the only trees which could be peeled easily, at the time of examination, were those which had been frilled and treated with sodium monochloroacetate, and were listed as dead. Even those trees which had been treated as late as August 11 were in a peelable condition.

Trees treated with 2,4,5-T and listed as dead were showing signs of bark loosening, especially in the vicinity of the sprayed areas. The trees killed with 2,4-D were fairly resistant to peeling, as were those which had been treated by means of sodium monochloroacetate applied to a peeled girdle.

A check made in October, 1952, indicated that in addition to the frilled trees, treated with sodium monochloroacetate, most of those treated with 2,4,5-T in June, and now dead, appeared to be in a satisfactory peeling condition.

Table VII is a summary of another inspection, made on the same trees, on June 13, 1953. Practically every tree listed as dead in this summary was in good peeling condition. The bark of many trees was splitting from the ground to the top of the trunk. This was especially true of those trees treated with sodium monochloroacetate, although not confined exclusively to them (see Figures 9 and 10). Trees listed as showing injury or nearly dead peeled about the same as untreated trees in some cases; in others they were definitely harder to peel.

TABLE VII

SUMMARY OF INSPECTION MADE ON JUNE 13, 1953,
OF ASPEN TREATED DURING 1952(S means basal spray, P means peeled girdle,
and F means frill girdle.)

Treat- ment date	Group	Chemical used	Meth- od	Number of trees				
				Tl	D	ND	I	H
6/4	DL-D13	2,4,5-T ester	S	12 ²	12			
6/24	DK1-DK10	Sod. monochloro- acetate	P	9	3	1	5	
6/24	DK11-DK20	Sod. monochloro- acetate	F	9	9			
6/24	DK21-DK25	Sod. monochloro- acetate	S	4	1		1	2
6/24	DK26-DK35	2,4,5-T ester	S	9	9			
6/24	DK36-DK45	2,4,5-T ester	F	9	9			
6/24	DK46-DK55	2,4,5-T ester ³	S	10	10			
6/24	DK56-DK64	2,4-D ester	S	9	9			
6/25	DK65-DK74	Pentachlorophenol	S	10			3	7
6/25	DK75-DK85	1 Pentachlorophenol, 2 $\frac{1}{2}$ 2,4,5-T ester	S	11	4	5	2	
6/25	DK86-DK90	None	F	4			2	2
6/25	DK91-DK95	None	P	3	1	1	1	
7/3	DK95-DK104	Sod. monochloro- acetate	P	9	7	2		
7/3	DK105-DK114	Sod. monochloro- acetate	F	10	8	2		
7/3	DK115-DK124	2,4,5-T ester	F	10	9	1		
7/3	DK125-DK134	2,4,5-T ester	S	10	10			
7/3	DK135-DK144	2,4,5-T ester ³	S	9	9			
7/3	DK145-DK153	2,4-D ester	S	6	6			
7/9	DH1-DH10	Sod. monochloro- acetate: $\frac{1}{2}$ water, $\frac{1}{2}$ diesel oil	S	10	2	3	1	4
7/9	DH11-DH19	Sod. monochloro- acetate	F	9	9			
7/9	DH20-DH29	Sod. monochloro- acetate	P	10	9	1		
7/9	DH30-DH39	2,4-D ester	S	10	6	1	2	1
7/9	DH40-DH49	2,4-D ester ³	S	10	9	1		

TABLE VII (continued)

Treat- ment date	Group	Chemical used	Meth- od	Number of trees				
				T ¹	D	ND	I	H
7/9	DH50-DH59	2,4,5-T ester	S	10	9	1		
7/9	DH60-DH69	2,4,5-T ester ³	S	10	10			
7/9	DH70-DH79	2,4,5-T ester	F	10	9	1		
7/15	DH120-DH129	2,4-D ester	S	9	2	2	3	2
7/15	DH130-DH139	2,4-D ester	F	10		4	5	1
7/15	DH140-DH149	2,4-D ester ³	S	10	4	1	3	2
7/15	DH150-DH159	2,4,5-T ester	S	10	8	2		
7/15	DH160-DH169	2,4,5-T ester	F	10	10			
7/15	DH170-DH179	2,4,5-T ester ³	S	10	8	2		
7/15	DH180-DH189	2,4,5-T ester ⁴	S	10	10			
7/15	DH190-DH196	2,4,5-T ester, water solution	P	7		7		
8/11	DJ5-DJ9	Sodium arsenite	F	5	5			
8/11	DJ10-DJ14	Sod. monochloro- acetate ⁵	F	5	2	1	2	
8/11	DJ15-DJ24	Sod. monochloro- acetate	F	10	10			
8/11	DJ25-DJ34	2,4,5-T ester	S	10	5	3	2	
8/11	DJ35-DJ44	2,4-D ester	S	10	5	3	1	1

¹T means total, D means dead, ND means nearly dead, I means showing injury, and H means healthy.

²Total number of trees may not agree with those given in Table VI, because some trees were felled for examination and others were down due to natural causes.

³Double strength (16# a. h. g.).

⁴Triple strength (24# a. h. g.).

⁵Frill on one side of tree only.

From Tables VI and VII it can be seen that sodium monochloroacetate applied in frills, and 2,4,5-T ester applied as a basal spray appeared to be the most effective treatments tried in this study.

The influence of time of treatment on the effectiveness of chemicals and methods. Tables VI and VII show that sodium monochloroacetate apparently was as effective when applied late in the summer as it was when applied earlier. On the other hand, 2,4-D and 2,4,5-T appeared to be much less effective when applied in August than when applied in June.

Additional information concerning time of treatment was obtained from observations made on trees treated at the Jennison forest during the spring of 1953. An examination of these trees on February 18, 1954, showed that, with one exception, all of them were dead, and that the bark could easily be stripped from their trunks. These trees had all been treated in late April and early May and appeared to have responded to treatment as well as those which had been treated during June and July. Information pertaining to chemicals and type of treatment used on these trees can be obtained from Table II.

Miscellaneous observations. It was observed that trees which had been frilled and treated with sodium monochloroacetate on one side only did not develop the charac-

teristic black bark as quickly as those which had been frilled and treated around the entire circumference. Twenty-two days after treating, the bark immediately above the frilled area was turning a dark orange color, while the bark on the untreated side of the tree remained green. By cutting away the outer portion of the bark it was observed that the phloem and cambial regions immediately above the frilled portion of the bark were turning a brownish color, while the tissue above the untreated portion of the stem appeared to be normal (see Figure 11). However, the crowns appeared to be dying uniformly on all sides of these trees. Upon re-examining these trees in June of 1953, it was found that two were dead and three were still producing a few green leaves; the bark on the treated side of the stems was splitting and was blackened but appeared to be healthy on the untreated sides of all but one tree.

During the summers of 1952 and 1953, it was noted that in the majority of cases damage to the stem bark was first seen on the southeast side of the tree. In the case of trees treated with sodium monochloroacetate, the bark appeared to turn black more quickly. The discolored spots and exudations of resinous fluid, characteristic of trees treated with 2,4,5-T and 2,4-D, were also noted first on the southeast side of the trees. The death of leaves, however, was observed to occur simultaneously throughout the crowns of the trees.

Of the 361 trees treated during 1952, evidence of attraction to wildlife was only found in one group. This group had been treated with a basal spray consisting of a mixture of sodium monochloroacetate in water and diesel oil. The group consisted of 10 trees and every tree showed signs of wildlife activity. The moss from around the base of each tree had been eaten, exposing the root collar. In addition, numerous holes had been dug between, and under, the roots of the trees. (See Figure 12.)

The depth of frills appeared to have a pronounced effect on the success of this type of treatment. It was noted that deep frills, cutting into the sapwood for at least one-half inch, were much more effective than shallow frills which barely scored the sapwood. Trees with deep frills were not only killed more quickly but could be peeled cleaner and at an earlier date. It was also established that trees with deep frills responded to treatment more readily than trees which had received a peeled girdle prior to spraying.

Regardless of the type of treatment used, it was noted that the size of the trees appeared to influence the effectiveness of treatments. Even when additional solution was applied in an effort to compensate for size, the best results were obtained on trees which ranged from 5 to 9 inches in diameter at breast height. Many trees above 10 inches in

diameter were found to be healthy and vigorous one year after treating; others, although dead, did not peel as satisfactorily as the smaller trees.

In most cases, it was found that treated trees peeled better the spring or summer following treatment than they did the autumn following treatment. One objection to this procedure was the discoloration that developed, in the outer inch of sapwood, as the trees stood over winter. This discoloration necessitated additional bleaching for certain types of paper products. Discolored sapwood seemed to be more prevalent in the trees treated with sodium monochloroacetate than with trees treated with the other chemicals. It was present to some degree, however, in most chemically treated trees which had stood over the winter.

It was also found that the bark of pulpwood sticks which came from the upper portions of the tree usually were more difficult to peel than those which came from the lower portions. This was more noticeable in trees treated with 2,4,5-F and 2,4-D than with those treated with sodium monochloroacetate. This condition held true one year after treating as well as during the same year.

It was noted that the bark of some trees, treated with sodium monochloroacetate, could not be peeled off in large sheets but broke up into very small pieces. This bark appeared to be in an advanced stage of disintegration and in

many cases long fibrous strands of bark adhered to the tree trunk.

Fig. 5. Aspen tree, treated with 2,4,5-T ester, showing complete defoliation 45 days after treatment.

Fig..6. Stem of aspen treated with 2,4,5-T ester showing rings of blackened xylem tissue resulting from treatment.

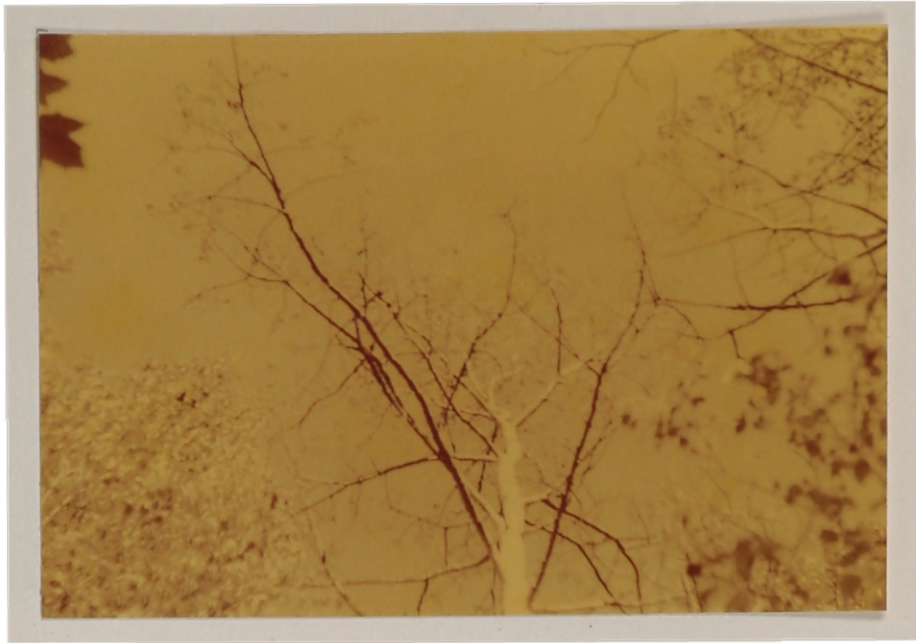


Fig. 7. Stem of aspen treated with 2,4-D ester, showing exudation of resinous fluid from discolored spots.

Fig. 8. Branch of aspen tree treated with 2,4,5-T ester, showing typical variegated color found 80 days after treatment.



Fig. 9. Base of tree treated with 2,4,5-T ester, showing splitting and discoloration of bark one year after treatment.

Fig. 10. Upper portion of aspen tree treated with 2,4,5-T ester, showing splitting of stem bark one year after treating.

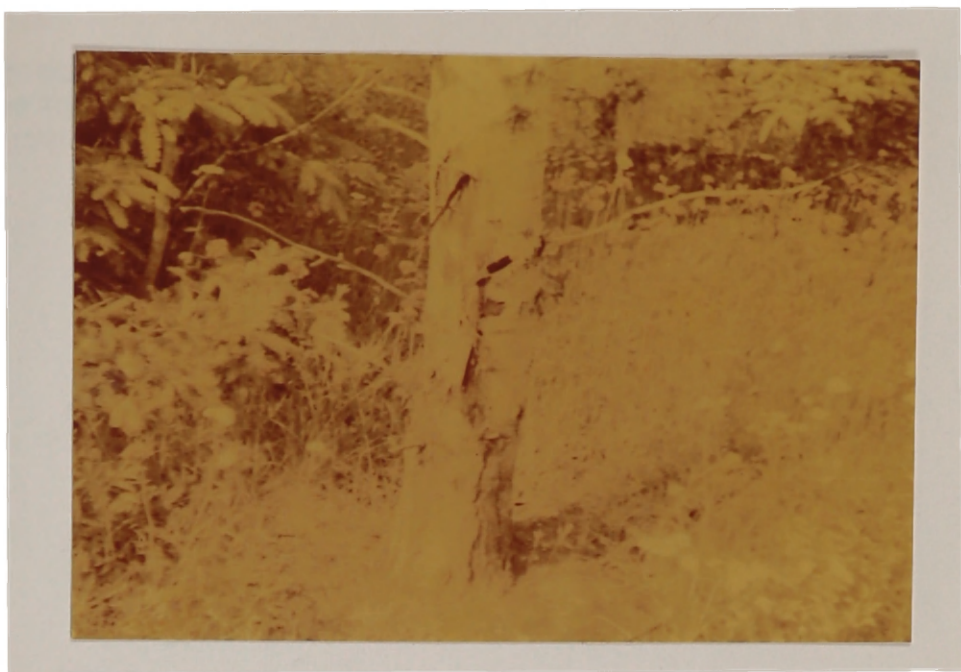


Fig. 11. Aspen tree treated with sodium monochloroacetate, showing discoloration of phloem above treated half of stem, and showing healthy tissue above untreated half of stem.

Fig. 12. Base of tree treated with sodium monochloroacetate, showing evidence of wildlife activity. Moss has been eaten from root collar and numerous holes were dug under roots.



VI. LABORATORY DATA ON THE ANATOMY OF STEMS, TWIGS,
LEAVES, AND ROOTS--WITH APPLICABLE
FIELD OBSERVATIONS

A. Stem Anatomy of Untreated Trees

1. Description of the Cambial Region and Adjacent Tissues of
the Stem

This description is based on samples taken from stems at approximately 5 feet above the ground, and collected at various times during the growing season.

Cambial region. The cambial region, including undifferentiated xylem and phloem cells, varies from 5 to 15 cells in width, depending on the time of season. The amount and nature of the cell contents also varies during the growing season, ranging from abundant to scarce, and from a granular to a fluid state.

Xylem. The xylem of aspen stems is composed of vessels, fibers, longitudinal parenchyma, and ray parenchyma. The wood is typically diffuse porous, with vessels of approximately the same diameter throughout the growth rings. Vessels are in multiples of two or more, equipped with simple perforation plates. Longitudinal parenchyma is mostly terminal, forming a broken line at the extremity of each growth

ring. Rays are uniseriate and follow a rather straight course through the xylem. Fibers are relatively numerous, especially in the outer portion of the rings; walls are relatively thin.

Phloem. Phloem was found to consist of longitudinal parenchyma, ray parenchyma, sieve tubes, companion cells, fibers, and some sclereids. As seen in Figure 13, sieve tubes are much larger and have thicker walls than the surrounding cells; sieve plates, principally on the radial walls, are plainly visible because of their thick appearance, which is due to depositions of callose. Extruded nucleoli are a constant feature of the sieve tubes during the growing season; one can be seen at the extreme right side of Figure 13. It was noted that the sieve tube elements possess long, sloping end walls, containing compound sieve plates. Each sieve plate contains from 8 to 12 sieve areas. Figure 14 shows the compound sieve plates and extruded nucleoli of the sieve tubes. Longitudinal parenchyma cells are smaller in diameter and have thinner walls than the sieve tubes; companion cells are the smallest in diameter, occupying the corners of the sieve tubes. Sieve tubes, companion cells, and longitudinal parenchyma cells form broad tangential bands of tissue which alternate with narrower, tangential bands of fibers. Crystalliferous cells are frequently found in the vicinity of the fibers (see Fig. 15). Scler-

eids are occasionally found adjacent to the cambial region; farther out they are quite numerous and interspersed among the fibers.

2. Condition of the Cambial Region and Adjacent Tissues of the Stem at Different Times of the Year

a. Condition on February 18 (see Fig. 16)

Cambial region. Cell walls were firm and relatively thick; cells were rectangular in shape, aligned in orderly radial rows, and were in a contracted state. The entire region was approximately 5 cells wide. Cell contents were very abundant, rather granular, and stained quite dark with safranin.

Xylem. A distinct dividing line could be seen between the cambial region and mature xylem cells. Vessels and fibers, formed the previous year, were all fully developed and possessed relatively thick walls. Ray parenchyma, adjacent to the cambial region, had cell contents similar to those of the cambial region.

Phloem. The last formed cells of the previous year were still undifferentiated. They were approximately the same size in diameter and possessed contents similar to those already described. External to these cells, the differentiated elements could be seen. Sieve tubes appeared to be lacking in cytoplasm; sieve plates were devoid of callose, and no extruded nucleoli could be detected. Companion

cells and longitudinal parenchyma cells were rich in cell contents.

b. Condition on April 24 (see Fig. 15)

Field observations. Buds were green and expanded but not open.

Cambial region. Cells were no longer in orderly rows; walls were not as firm or thick as reported for February. Cell contents were changing from a granular to a more or less fluid state; they were not as dense and did not take up stain as readily as those reported for February.

Xylem. No change noted.

Phloem. Cells reported as being undifferentiated in February now were found to be differentiating into the various types of specialized tissues. Cell contents throughout the phloem were dispersing and approaching a fluid state.

c. Condition on May 29 (see Fig. 17)

Field observations. Leaves of the sample trees were fully developed.

Cambial region. Cambium was actively dividing, forming new xylem and phloem cells. Cambial cells were wider in a radial direction than previously noted. The cambial region was much wider than reported before. It was not possible to distinguish with certainty between cambial region and adjacent tissues because of many undifferentiated cells. Cell

contents were now in a fluid state and evidently had lost much of their chromaticity.

Xylem. First formed vessels and fibers already had secondary walls; those which had just differentiated possessed thin primary walls. Xylem cells were forming at a rapid rate. Cell contents were abundant in newly formed ray cells but scarce in others.

Phloem. Sieve tubes had enlarged and now were three to four times as large in diameter as the surrounding cells of longitudinal parenchyma; the walls also were thicker than those of the parenchyma cells. Sieve plates were acquiring a deposit of callose and as a result were very prominent. Extruded nucleoli were appearing in the mature sieve tubes, and cytoplasm was found lining the walls.

d. Condition on July 30 (see Fig. 13)

Cambial region. The cambial region was much narrower than on May 29; cells were more nearly in rows and were approaching a rectangular shape. Cell contents were approaching a granular state again, and the entire region had an appearance of decreased activity.

Xylem. Most xylem cells were fully differentiated. Very few thin-walled vessels or fibers could be detected, and evidently very few new cells were being formed.

Phloem. Phloem cells were still forming very slowly; sieve tubes adjacent to the cambial region had not expanded

to their full size. Callose and extruded nucleoli could still be detected in the mature sieve tubes.

e. Condition on August 20 (see Fig. 18)

Cambial region. Appearance of cells was about the same as reported on July 30, except that cell contents seemed to be more granular.

Xylem. No change noted.

Phloem. Some sieve plates were thicker than reported on July 30, evidently as a result of increased callose deposits. A distinct absence of extruded nucleoli was also noted in most sieve tubes. The contents of many phloem cells were quite granular, and became darkly stained with safranin.

B. Progressive Effects of Chemicals on the Stem Anatomy of Trees Treated During 1952

1. Effects of Sodium Monochloroacetate on the Cambial Region and Adjacent Tissues

Unless otherwise stated, all samples were taken at approximately 5 feet above the ground from the stem of tree DK-17. This tree was frilled and sprayed on June 24, and samples removed periodically during the summer (see Table III).

a. Fifteen days after treating (see Fig. 19)

Field observations. All leaves were turning brown and

withered in appearance; stem bark in the vicinity of the frill was also turning brown.

Cambial region. The walls of most cells were distorted and in a state of partial collapse; in some places it was almost impossible to distinguish individual cells. Contents and walls of all cells were stained a dark brown due to chemical treatment, and in spots they were fused together.

Xylem. Some cells immediately adjacent to the cambial region were becoming distorted. Ray cells adjacent to the cambial region contained dark contents, similar to those of the cambium.

Phloem. It was noted that most sieve tubes were partially collapsed; walls were much thicker than those of normal tubes and were stained a dark brown color. In some cases it was very difficult to locate sieve plates because they resembled the walls of other cells in thickness and coloration. When visible they had very heavy depositions of callose. Figure 20, at high magnification, shows sieve tubes with plates. It was also noted that most sieve tubes were devoid of extruded nucleoli. The walls of many longitudinal parenchyma cells were becoming thick-walled; they were distorted and in an early stage of collapse. Ray tissue was very much distorted. In some areas it was extremely difficult to trace the path of a ray through the cambium into the phloem. Cell contents of the rays were stained dark

brown and were fused with the walls near the cambial region. The only elements in the phloem that did not seem to be affected by the treatment were the bands of fibers and sclereids. The appearance of the phloem can be compared with that of untreated phloem collected at the same time by comparing Figures 19 and 21.

b. Twenty-three days after treating

Field observations. Tree was completely defoliated. Bark from frill to about 3 feet above the frill was completely blackened; from 3 feet above frill to top of tree, and from frill to about 18 inches below, the bark had an orange-brown color.

Cambial region. The amount of collapse, and the degree of disintegration of the walls was more advanced than at 15 days. The dark brown color of cell contents was more pronounced and the contents were disintegrating in some cells.

Xylem. Many newly formed cells were beginning to show signs of collapse and general distortion. Ray cells were becoming distorted and in an early state of collapse adjacent to the cambial region.

Phloem. Very few sieve tubes could be distinguished. Sieve plates were hard to detect in those sieve tubes which could be seen. Ray cells adjacent to the cambium were starting to disintegrate and had blackened walls and contents. No damage to fibers or sclereids could be detected.

c. Thirty days after treating

Field observations. Tree had approximately the same appearance as described at 23 days; blackened bark areas extended about 2 feet further up the trunk.

Cambial region. It was impossible to distinguish individual cells in the cambium. Collapse had become severe and general throughout the entire region. Cell contents could not be distinguished from the walls; both appeared as blackish masses of material, apparently joined together. In many places, rays lost all continuity through the cambium.

Xylem. Not much change in appearance of the xylem could be detected. The degree of disintegration of ray cells was greater than at 23 days.

Phloem. Almost all cell walls, except those of fibers and sclereids were in an advanced state of collapse and disintegration. Cells close to the cambial region were more distorted than those farther out. The lumens of many cells were smaller in diameter than those of untreated material because of the thick walls. Blackened cell contents were general throughout the phloem. Even longitudinal parenchyma in the outer regions contained dark contents. In many areas the continuity between groups of fibrous cells and adjacent parenchymatous tissue was broken, leaving isolated groups of fibers and sclereids.

d. Thirty-six days after treating (see Fig. 22)

Field observations. Stem bark was blackened from the frill to the top of the tree with only a few narrow, green streaks remaining.

Cambial region. No change noted.

Xylem. No change noted.

Phloem. Cell walls were more distorted than at 30 days. In many places, adjacent to the cambial region, the phloem was in the form of tangential bands of black material. Cell collapse was complete and no individual cell shapes could be determined. Rays appeared as solid black lines twisting through the phloem. Fibers and sclereids still seemed to be intact, but the spaces between groups of fibers and sclereids, and adjacent parenchymatous material were much more pronounced than at 30 days.

e. Forty-five days after treating

Field observations. Appearance of tree was the same as at 30 days. It was noted, however, that the bark immediately above the frilled area could be stripped off by hand.

Cambial region. The cambium had the appearance of a disorganized tangential band of blackened tissue. Radial walls were severely collapsed and it was impossible to distinguish individual cells.

Xylem. Most cells immediately adjacent to the cambial zone were in an intermediate stage of disintegration. In

some areas distinct separations could be seen between cambial zone and xylem.

Phloem. The only intact cells in the phloem were bands of fibers and sclereids. All other cells were undergoing a process of disintegration.

f. Fifty-one, 57, and 64 days after treating

Field observations. Not much change was noted in appearance of the tree. The bark was almost completely black above the frill; only a few green patches remained near the top of the trunk. Bark could be stripped off more easily as time went on; at 64 days it was difficult to remove stem samples without separating bark from wood.

Cambial region, xylem, and phloem. There was very little change in appearance during this period. All cells, except fibers and sclereids, were disintegrating very slowly.

g. Seventy-one days after treating (see Figures 23 and 24)

Field observations. Tree was completely dead above frill; no green bark was visible. Bark was also completely blackened from frill to a point about one foot below frill. Below this point there still were numerous green bark patches. The bark above the frill was very loose and extreme care had to be used in order to remove samples in an undamaged condition.

Cambial region. Cambial region had completely collapsed. It was impossible to determine where the cambial region ended and phloem or xylem began. All traces of cell cavities had been obliterated, and the entire region appeared as a coal-black mass of distorted tissue.

Xylem. The last-formed cells of the xylem were in an advanced state of disintegration, leaving many small lacunae between xylem and cambial region. Some ray cells adjacent to the cambial zone had disintegrated; others had blackened walls and contents.

Phloem. The sieve tubes, companion cells, and parenchyma cells adjacent to the cambial region had completely collapsed, forming a wide tangential band of twisted, blackened wall material. Farther out collapse was not so pronounced, but the walls were very thick and had the same blackened appearance as those adjacent to the cambial region. Rays could not be distinguished close to the cambial region and farther out they were completely dead, containing blackened cell contents. This condition can be seen in Fig. 24. In many places there were numerous void areas between bands of intact fibrous cells and adjacent parenchymatous material. This condition was general throughout the phloem, even in the outer portions of this region (see Fig. 23).

2. Effects of 2,4,5-T Ester on the Cambial Region and Adjacent Tissues

Unless otherwise stated, all samples were taken at approximately 5 feet above the ground from the stem of tree DK-27. This tree was treated by the basal spray method on June 24 and samples removed periodically during the summer (see Table III).

a. Fifteen days after treating (see Fig. 25)

Field observations. Tree appeared to be healthy and vigorous at this time. All foliage and bark areas were green.

Cambial region. No damage could be detected.

Xylem. No damage could be detected.

Phloem. Many cells possessed very thick walls. Although no evidence of collapse could be detected in most cells of longitudinal parenchyma, it did appear in some sieve tubes. Other sieve tubes had very small cell cavities because of their extremely thick walls. No extruded nucleoli were seen and it was very difficult to detect sieve plates. Cell contents were granular and stained quite dark as a result of treating. No damage could be seen in the fibers or sclereids.

b. Twenty-three days after treating

Field observations. Leaves were beginning to turn from green to various shades of red and yellow. Stem bark was green except for some small discolored spots on the lower 5 or 6 feet of the trunk. Samples taken at this time were from the center of a small discolored spot, located about 2 feet above the ground.

Cambial region. Cambial cells were slightly distorted and a few seemed to be in an early state of collapse.

Xylem. No damage could be detected.

Phloem. The phloem was about the same as at 15 days. Cell contents were a little darker.

c. Thirty days after treating

Field observations. Approximately one-half of the leaves had turned brown; the others were colored red and yellow. The lower portion of the trunk appeared to be dying in localized spots; the upper portion did not seem to be damaged. Sample was taken from a height of 2 feet, from an area of green bark.

Cambial region. Cambial region was about the same as reported for 23 days.

Xylem. No damage could be detected.

Phloem. The phloem was in about the same condition as reported at 23 days.

d. Thirty-six days after treating (see Figures 26 and 27)

Field observations. Tree appearance was about the same as at 30 days. More leaves had turned brown but the condition of the trunk had not changed appreciably.

Cambial region. Cell walls, especially radial walls, were starting to collapse and presented a distorted appearance. Cell contents could not be seen.

Xylem. In some places, newly formed cells were collapsing and walls were disintegrating, causing numerous small lacunae between the cambial region and xylem.

Phloem. Extruded nucleoli and sieve plates were not visible in the sieve tubes. Thick tangential bands of collapsed wall material could be seen at various places in the phloem. In other regions, cell walls were thick and very dark but had not collapsed. Uncollapsed cells were rounded or oval, resembling expanded longitudinal parenchyma cells. This condition can be seen in Figure 27.

e. Forty-five days after treating

Field observations. All leaves were completely dead; tree was partially defoliated. Discolored areas in bark were larger, and black rings of tissue were discovered in the wood beneath these areas.

Cambial region. Cell walls were blackened and started to disintegrate. Collapse was more general than reported at 36 days.

Xylem. Collapse of newly formed cells was more advanced than at 36 days. Lacunae were becoming larger and more numerous between cambial region and xylem. Many rays had blackened cell contents, in most cases extending out to the end of the growth ring.

Phloem. Not much change from 36 days except that extruded nucleoli were very numerous in the sieve tubes, immediately adjacent to the cambium. Farther out, the phloem had the same appearance as described at 36 days.

f. Fifty-one days after treating

Field observations. Discolored spots in the bark of the trunk were much larger than reported previously. Many spots had coalesced, forming large areas of orange-colored bark. Many of the discolored areas were exuding a resinous liquid. The sample was taken at 5 feet from an area of green bark immediately adjacent to a discolored spot.

Cambial region. The cambial region was in approximately the same condition as reported at 30 days. The cells were just starting to show collapse, and a few walls were displaying the first signs of disintegration. Cell contents were breaking into fragments.

Xylem. No damage could be detected.

Phloem. Sieve tubes were in an early state of collapse; those adjacent to the cambial region were noted to have thick walls. Walls were lined with dark-stained cyto-

plasm. It was observed that the outer portion of the phloem was much less affected than that closer to the cambial region. Some sieve plates could still be detected, as well as extruded nucleoli.

g. Fifty-seven days after treating (see Figures 28 and 29)

Field observations. Discolored bark areas now extended from the sprayed area to the top of the tree. Sample taken at 5 feet from discolored area.

Cambial region. Cells were in an advanced state of collapse. Walls were distorted and disintegrating, making identification of individual cells impossible. Cell contents were granular and blackened in appearance. Many were disintegrating into fragments.

Xylem. Walls and contents of longitudinal and ray parenchyma cells were stained coal black. Rays were in advanced state of disintegration. Walls of many cells adjacent to the cambial region were collapsed and torn. The intervessel pits in many spots were very black and were observed to be bulging into the vessel cavities. There also was a preponderance of vessels and very few fibers adjacent to the cambial region.

Phloem. All cells adjacent to the cambial region were found in an advanced state of collapse and disintegration. Farther out, cells with thick walls were noted and very little collapse was observed. Rays were normal except im-

mediately adjacent to the cambial region where they had disintegrated. A few extruded nucleoli were in evidence and occasionally a sieve plate could be detected.

h. Sixty-four days after treating

Field observations. Large areas of the trunk were now discolored. The sprayed area was turning an orange-brown. The sample was taken from the sprayed area, about 2 feet from the ground.

Cambial region. Cells were completely collapsed. It was impossible to distinguish individual cells because the entire region appeared as a solid, tangential band of blackened, distorted material.

Xylem. Some disintegration of newly-formed cells was noted. However, the degree of distortion and blackening was not as great as reported at 57 days. Ray cells were damaged most. Many of them displayed blackened walls and contents.

Phloem. Numerous tangential bands of blackened material were noted throughout the phloem. Cell contents appeared as solid black masses of material. Walls of the majority of cells, even those that had not collapsed, were very dark. Ray cells appeared as solid masses of black tissue; no distinction could be made between contents and walls. Many large void spaces were noted between intact fibers and sclereids, and adjacent material.

i. Seventy-one days after treating (see Fig. 30)

Field observations. Trunk above sprayed area was almost solid orange, with little green bark visible. The sprayed area was almost a solid brown. The bark of the sprayed area was in a peelable condition, but above this area it was tight. Sample was taken from 5 feet.

Cambial region. The cambial region had the same appearance as reported at 64 days.

Xylem. Many newly-formed cells had collapsed, forming numerous small lacunae between cambial region and xylem. It was noted that some ray and longitudinal parenchyma cells had dark walls and dark cell contents. However, the degree of damage to the xylem was much less than at either 64 or 57 days. This can be observed by comparing Figures 28 and 30.

Phloem. General appearance of the phloem was about the same as at 57 days; many sieve tubes were partially collapsed and most cells had very thick walls. Tangential bands of blackened material were in evidence throughout the phloem. Fibers and sclereids were intact.

3. Effects of 2,4-D Ester on the Cambial Region and Adjacent Tissues

Unless otherwise stated, all samples were taken at approximately 5 feet above the ground from the stem of tree DK-61. This tree was treated by the basal spray method on

June 24 and samples removed periodically during the summer (see Table III).

a. Fifteen days after treating

Field observations. No evidence of damage could be detected.

Cambial region. No damage could be detected.

Xylem. No damage could be detected.

Phloem. No damage could be detected.

b. Twenty-three days after treating

Field observations. No evidence of damage could be detected.

Cambial region. No damage could be detected.

Xylem. No damage could be detected.

Phloem. No damage noted except that the cell contents of many longitudinal parenchyma cells were stained dark brown, and were disintegrating and moving toward the cell walls, giving the walls a thick appearance.

c. Thirty-six days after treating

Field observations. The leaves were starting to turn various shades of red and yellow. A few discolored spots were found on the lower 10 feet of the trunk; a resinous fluid was observed on some of the spots. Sample was taken from a discolored spot.

Cambial region. No damage could be detected.

Xylem. No damage could be detected.

Phloem. Cell contents had a darker color than noted before. The walls of most parenchymatous cells, especially sieve tubes and longitudinal parenchyma, were becoming very thick; no evidence of collapse was detected.

d. Forty-five days after treating

Field observations. Not much change in appearance; some leaves still green; discolored spots on trunk more numerous.

Cambial region. No damage could be detected.

Xylem. No damage could be detected.

Phloem. Cell contents were very dark and fragmentary; most of them were lining the walls.

e. Fifty-one days after treating (see Fig. 31)

Field observations. All leaves had changed color. Many discolored spots had developed on the lower half of the trunk; upper portion was still entirely green. Much fluid was being exuded by discolored spots.

Cambial region. Very little collapse or distortion was found at this time; cell walls were darker than those of untreated material and they were more rigid. Cambial region was devoid of cell contents as compared with untreated material (compare Figures 31 and 32).

Xylem. No damage could be detected.

Phloem. Cell contents and walls of most parenchymatous cells were stained black; walls of most were very thick. Ray cells were very much distorted. Longitudinal parenchyma and sieve tubes did not show much collapse, although sieve plates and extruded nucleoli were very difficult to detect.

f. Fifty-seven days after treating

Field observations. Most leaves had turned brown; discolored spots were spreading to upper portions of the trunk.

Cambial region. Cell walls were very thick, distorted, and were starting to collapse.

Xylem. Contents of ray cells closest to the cambium started to darken.

Phloem. Condition about the same as at 51 days.

g. Sixty-four days after treating

Field observations. Appearance of tree about the same as at 57 days.

Cambial region. Collapse of cells was becoming very general throughout the entire cambial region.

Xylem. No change from 57 days.

Phloem. No change from 57 days.

h. Seventy-one days after treating (see Figures 33 and 34)

Field observations. All leaves were brown but very little defoliation had taken place. Sprayed area of trunk

had turned brown; above sprayed portion large areas of orange bark were forming.

Cambium. Entire region was in a state of severe collapse; cell walls were black and distorted, forming a tangential band of disintegrated material.

Xylem. A few cells immediately adjacent to the cambial region were observed to be collapsing. Except for these cells, the xylem was intact.

Phloem. General collapse and distortion was noted adjacent to the cambial region. Farther out, most cells, except fibers and sclereids, had abnormally thick walls and many were in a state of partial collapse. This condition gave the appearance of very small cell cavities (compare Figures 34 and 35). Sieve plates and extruded nucleoli could not be detected. Fibers and sclereids were not damaged. The phloem was very similar in appearance to the phloem of 2,4,5-T treated material. This condition can be seen by comparing Figures 30 and 33.

C. Appearance of the Cambial Region and Adjacent Stem
Tissues Resulting from Chemical Treatments
Applied on Different Dates

Trees used for this study were treated on different dates during the growing season. All samples were obtained

36 days after treating, and were removed from the stem at approximately 5 feet above the ground (see Table IV).

1. The Appearance of Stem Tissues Taken from Trees Treated on Different Dates with Sodium Monochloroacetate in Frills

a..Tree treated on June 24 (see Fig. 22)

Field observations. Tree was completely defoliated. Stem bark was black from the frill to the top of the tree with only a few narrow green streaks remaining.

Cambial region. It was impossible to distinguish individual cells in the cambium. The entire region was severely collapsed. Cell contents and walls were stained black and seemed to be fused together. In many places the path of rays could not be followed.

Xylem. Many newly-formed cells were beginning to show signs of collapse. Ray cells adjacent to the cambial region were distorted and in an early state of collapse.

Phloem. Collapse and disintegration was general throughout the entire region. Fibers and sclereids often appeared as isolated groups because of the disintegration and collapse of parenchymatous cells around them. It was extremely difficult to identify different types of parenchymatous cells in some places. Rays appeared as solid black lines twisting through the phloem. Where sieve tubes

could be distinguished, it was difficult to locate sieve plates. Extruded nucleoli were not observed in the phloem.

b. Tree treated on July 3(see Fig. 36)

Field observations. Tree was partially defoliated; all remaining leaves were brown. Stem bark was blackened for about 4 feet above frill.

Cambial region. Cells were observed to be in an early stage of collapse; walls were disintegrating in some places, in others they were thickening.

Xylem. A few of the last-formed cells were in a state of collapse, leaving small, isolated lacunae between cambial region and xylem. Ray cells were disintegrating in some places adjacent to the cambium. Other ray cells were observed to have blackened cell contents.

Phloem. Much of the phloem adjacent to the cambium was in an advanced stage of disintegration. Farther out many cells had thick black walls; some collapse was evident and in some areas fibers and sclereids appeared as isolated groups. Ray cells were becoming blackened and very much distorted.

c. Tree treated on July 9.

Field observations. Tree was partially defoliated; all remaining leaves had turned black. Trunk was green except

for one black streak about 6 inches wide which extended from the frill to the top of the tree.

Cambial region. Very little damage was noted; some cells were in an early state of collapse, while others apparently were not damaged.

Xylem. No damage could be detected.

Phloem. Very little damage was noted in the longitudinal and ray parenchyma cells. Some sieve tubes adjacent to the cambial region were in an early state of collapse. Many appeared to be normal, having normal-appearing sieve plates but no extruded nucleoli. Very little wall thickening was observed. Blackening of walls and cell contents was found in isolated cells; most of them were near the cambial region.

2. The Appearance of Stem Tissues Taken from Trees Treated on Different Dates with a Basal Spray of 2,4,5-T Ester

a. Tree treated on June 24 (see Fig. 26)

Field observations. Approximately one half of the leaves had turned brown; the others were colored red and yellow. The lower portion of the trunk was dying in localized spots; upper region of the stem did not seem to be damaged.

Cambial region. Cell walls were starting to collapse and become distorted. Cells seemed to be devoid of contents.

Xylem. In some places, newly-formed cells were collapsed and walls were disintegrating, causing numerous small lacunae between cambial region and xylem.

Phloem. The phloem was characterized by collapsed cells, thick dark walls, and absence of extruded nucleoli and sieve plates. Thick tangential bands of wall material could be seen at various places in the phloem. In others, cell walls were thick and very dark but the cells had not collapsed. Those which had not collapsed were rounded or oval.

b. Tree treated on July 3

Field observations. All leaves turned brown, but apparently all were still attached.

Cambial region. Some evidence of walls becoming rigid; otherwise no damage was observed.

Xylem. No damage noted except a few ray cells had dark-brown contents.

Phloem. Some collapse and some wall thickening was noted, but not as pronounced as in the tree treated on June 24. Many longitudinal and ray parenchyma cells had dark contents.

c. Tree treated on July 9

Field observations. Most leaves were colored red and yellow; only a few were brown. No evidence of stem injury could be detected.

Cambial region. Cells were in approximately the same condition as those described for tree treated on July 9.

Xylem. No damage could be detected.

Phloem. Some collapse and wall thickening was noted. Parenchyma cells had dark-colored contents.

d. Tree treated on July 15 (see Fig. 37)

Field observations. Appearance was about the same as that of tree treated on July 9.

Cambial region. No damage could be detected.

Xylem. Damage could be detected in cell walls. The contents of some ray and longitudinal parenchyma cells were stained a very dark brown.

Phloem. Very little wall distortion was noted, but wall thickening was becoming quite general. Sieve plates were extremely difficult to detect. Cell contents in many cells were quite granular and in many cells were stained a deep purple.

3, The Appearance of Stem Tissues Taken from Trees Treated on Different Dates with 2,4-D Ester Applied as a Basal Spray

a. Tree treated on June 24.

Field observations. Leaves were starting to turn various shades of red and yellow. A few discolored spots were

found on the lower 10 feet of the trunk. Most of the discolored spots were exuding a resinous fluid.

Cambial region. No damage could be detected.

Xylem. No damage could be detected.

Phloem. Cell contents were very dark. Thw walls of most parenchymatous cells, especially sieve tubes, were becoming very thick; no evidence of collapse was detected.

b. Tree treated on July 3

Field observations. A few leaves were turning color. Trunk appeared to be undamaged.

Cambial region. No damage could be detected.

Xylem. No damage could be detected.

Phloem. About the same as reported for tree treated on June 24.

c. Tree treated on July 9

Field observations. A few colored leaves were observed. No evidence of stem damage was found.

Cambial region. No damage could be detected.

Xylem. No damage could be detected.

Phloem. Very little damage was observed. Some evidence of wall thickening was seen, but no collapse or disintegration could be found.

d. Tree treated on July 15 (see Fig. 38)

Field observations. No damage of any kind was observed.

Cambial region, xylem, and phloem. No damage of any type was noted. All cells appeared to be normal. This can be seen by comparing Figures 18 and 38.

D. The Effects of 2,4,5-T Ester at Different Heights
in the Tree Stem

Samples for these observations were obtained from tree D1. This tree was treated with a basal spray on June 4 and felled 80 days after treating. Samples were removed from the stem at various intervals, from the sprayed area to the tip of the leader.

At the time of felling, the tree was completely defoliated, and the bark of the stem was completely discolored. The bark of the sprayed area was a solid brown; above the sprayed area it was a variegated orange and brown. The bark could be peeled from around the sprayed area but was tight on the remainder of the stem. Branches were blackened and bark was peeling for a distance of about 18 inches back to the tips. The portion from the trunk out to the tip was colored the same as the trunk. The tip of the leader was blackened the same as the branch tips.

a. Observations on a sample taken from the sprayed area (see Fig. 39)

Cambial region. Cells of the cambial area were in a general state of collapse. The walls appeared to be in an

advanced stage of disintegration; it was very difficult to determine individual cells. Walls and contents were observed to be very dark. Ray cells could not be traced through the cambial region.

Xylem. The walls of some cells adjacent to the cambial region had collapsed, causing small lacunae between cambial zone and xylem.

Phloem. Many cells, mostly sieve tubes, adjacent to the cambial region were in a partial state of collapse. Rays were very distorted near the cambial region, and wall thickening of all cells was general.

b. Observations on a sample from 5 feet above ground (see Fig. 40)

All tissues appeared to be the same as those of the sprayed area. This can be illustrated by comparing Figures 39 and 40.

c. Observations on a sample from 10 feet above the ground.

Cambial region. Very little damage was observed in the cambial region. Some collapse was noted in a few cells.

Xylem. Most cells appeared undamaged; a small number of last-formed cells had collapsed walls.

Phloem. Very little collapse was noted; cell wall thickening was general throughout the phloem.

d. Observations on a sample from 15 feet above the ground.

Cambial region. Very early stages of collapse were noted in a few cells.

Xylem. No damage could be detected.

Phloem. Some wall thickening was noted. Some sieve plates were visible, but walls containing them were becoming thick and were in an early stage of collapse.

e. Observations on a sample from 20 feet above the ground.

Cambial region. No damage was detected.

Xylem. No damage was detected.

Phloem. Some wall thickening noted; very little collapse was seen. Some extruded nucleoli were visible and also a few sieve plates.

f. Observations on a sample from 35 feet above the ground

(see Fig. 41)

All tissues were in about the same condition as reported for 20 feet.

g. Observations on a sample from the blackened portion in the tip of the leader (see Fig. 42)

Cambial region. Cambial region was completely killed and appeared as an almost solid, black, tangential band of material. It was impossible to distinguish between walls and cell contents; both were black and fused together.

Xylem. Last-formed cells were badly distorted in spots; many appeared to be in an intermediate stage of collapse.

Phloem. Cell contents and walls were blackened and fused together. It was impossible to distinguish different types of parenchymatous cells. The fibers and sclereids were the only cells that appeared to be undamaged.

E. Miscellaneous Observations

1. The Effects of Doubling the Concentration of 2,4,5-T Ester

These observations were made on samples removed from the stem of tree DK-49, treated by the basal spray method on June 24. The samples were obtained on the same days as those for the tree treated with single strength 2,4,5-T (see Table III). Information regarding observations on single strength samples can be found in section VI, B-2.

Even though leaves of the tree treated with double strength solution had been damaged more quickly than those of the tree treated with a single strength solution, the appearance of the tree stems in the field was quite similar.

Observations made on the anatomy of the cambial region and adjacent tissues failed to disclose any significant differences between the two concentrations used. This was true for slides examined at each time interval after treating. A

comparison between Figures 30 and 43 will show that the degree of injury, 71 days after treating, was about the same for both concentrations.

2. The Effects of Chemicals on Twigs

Samples were removed from twigs when the outermost 6 to 8 inches were blackened. In each instance one sample was taken from the blackened portion; twigs were approximately one-eighth inch in diameter in this region. A second sample was also removed approximately 2 feet below the point of leaf attachment. Twigs averaged about three-eighths of an inch in diameter at this point. Twig bark was not blackened in this area, although it frequently was discolored.

The effects of the various chemical treatments were very similar on twigs. Therefore, only observations made on samples taken from a twig treated with 2,4,5-T have been described. The only noticeable effect in samples taken 2 feet below leaf attachment was a slight thickening of some of the parenchymatous cell walls. In samples removed from the blackened portion of the twigs, the cambium was distorted and collapsed; walls and cell contents were completely blackened. All parenchyma cells, including those of the phloem, xylem, and pith had dark-colored walls and blackened cell contents. Cells in the phloem were affected more than those of the xylem or pith. A comparison between untreated and treated twigs can be seen in Figures 44 and 45.

3. The Effects of Chemical Treatment on Leaves

Leaves were collected when they had become blackened and were partially dried out. Samples were removed from that portion of the blade containing the midrib of the leaf. The effects of the chemical treatments were very similar, and only the observations made on a leaf of a tree treated with 2,4-D ester have been described.

a. Description of portions of an untreated leaf (see Figures 46 and 47)

Midrib portion. The midrib portion of the leaf blade contains one large vascular bundle. The bundle is encircled by a bundle sheath of fibers. This ring appears to be thicker in the dorsal and ventral portions than in the side areas, as described by Eames and McDaniels (96). The dorsal portion of the vascular bundle contains the elements of the phloem, principally sieve tubes, companion cells and longitudinal parenchyma. The ventral portion of the bundle is composed of xylem elements: namely, vessels, fibers, and longitudinal parenchyma. Between the bundle and the leaf epidermis, undifferentiated parenchyma cells, crystalliferous cells, and a layer of thick-walled collenchyma cells could be seen.

Blade portion adjacent to midrib. The blade of the leaf, adjacent to the midrib, is made up of upper and lower epidermis, palisade cells, and spongy mesophyll. The pali-

sade layer is located in the ventral portion of the blade and is arranged in two horizontal layers, immediately below the upper epidermis. These layers occupy well over the upper half of the leaf blade. The spongy mesophyll is evident in the dorsal portion of the blade, and occupies all the space between the palisade layer and lower epidermis. The spongy mesophyll appears as a loose network with numerous void spaces between cells. At the time of collection, chloroplasts were very numerous and could be detected in the cells of the palisade layer and spongy mesophyll.

b. Description of portions of a treated leaf (see Figures 48 and 49)

Midrib portion. All phloem cells in the vascular bundle were completely collapsed. Walls appeared distorted and dried up. Contents were blackened, and in many cases could not be separated from wall material. Identity of the individual cell types was impossible. All parenchymatous tissue in the xylem appeared to be dead. Cell contents and walls were black and could not always be separated. No damage was detected in vessel walls. Parenchymatous cells outside the vascular bundle had blackened cell contents and thickened walls which were partially collapsed. Epidermal cells and underlying collenchyma also had dark cell contents.

Blade portion adjacent to midrib. It was practically impossible to identify individual cells except in the epi-

dermal layers. Walls and contents were blackened and fused together. No chloroplasts could be detected because of this condition. The entire blade portion seemed to have contracted, resulting in a much thinner cross section than that of untreated material.

4. The Effects of Chemical Treatments on the Main Roots

Samples for this study were obtained from tree D1. This was the same tree that was used for the stem study described under section VI-D. It was treated with a basal spray of 2,4,5-T on June 4 and samples were collected 80 days after treating. At the time of sample collection the tree was completely defoliated, the stem bark above the sprayed area was entirely discolored, and the bark of the sprayed area, down to the root collar, was blackened and could be peeled off.

Samples were also collected from tree DK17. This tree was treated with sodium monochloroacetate on June 24. The condition of the main roots of this tree was so similar to those of tree D1, that only the root of tree D1 has been described.

The anatomy of the main root of an untreated tree was found to be essentially the same as that of the stem, with a few modifications (see Fig. 50). The cambial region of the root appears to be narrower than that of the stem, and the phloem is much wider. Bands of fibers and sclereids are

much less frequent in the root, and where they occur, the bands are narrower. At the time of examination, cambial and phloem cells were rich in cell contents. Sieve tubes were well developed showing sieve plates, callose, and extruded nucleoli. Xylem and phloem cells immediately adjacent to the cambial region were still differentiating.

The cells of the cambial region of the treated root seemed to be much more rigid than those of the untreated root. The xylem cells all appeared to be mature and no cells still in the process of differentiation could be found (see Fig. 51). A distinct absence of extruded nucleoli was observed in the phloem. Sieve plates were very difficult to locate and those that were present were devoid of callose. The sieve tubes themselves had much smaller diameters when compared with untreated material. Cell contents in the phloem were quite scarce in contrast to the untreated root. A few of the walls were a little darker than those of the untreated cells, but there was no indication of collapse or distortion at this time. The root had the appearance of being in a dormant condition.

Fig. 13. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing prominent sieve tubes and sieve plates with callose. One extruded nucleolus can be seen at extreme right of section. (205 X.) Sample was taken 5 feet above the ground from the stem of an untreated tree on July 30.

Fig. 14. Radial section of the cambial region and adjacent tissues of an aspen stem. Sieve plates showing connecting strands enclosed in heavy callose can be seen at right of the section; two extruded nucleoli are also visible. (312 X.) Sample was taken 5 feet above the ground from the stem of an untreated tree on August 23.

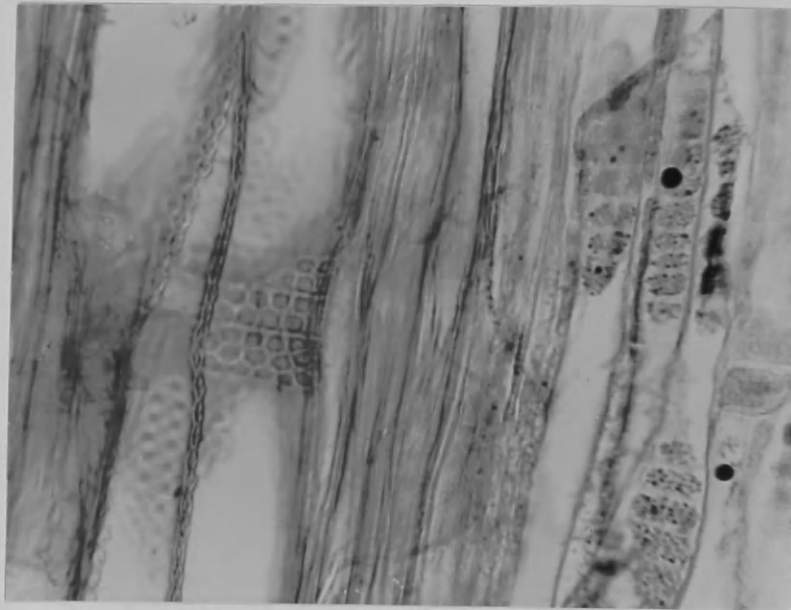
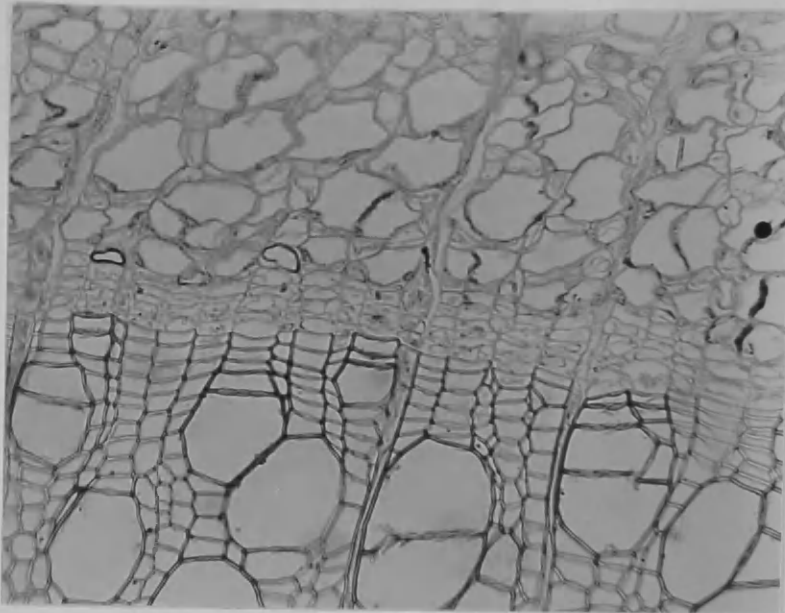


Fig. 15. Transverse section of the cambial region and adjacent tissues of an aspen stem showing start of phloem and cambial activity. Cells are expanding and contents changing from a granular to a semi-fluid state. Xylem is still inactive. (205 X.) Sample was taken 5 feet above the ground from the stem of an untreated tree on April 24.

Fig. 16. Transverse section of the cambial region and adjacent tissues of an aspen stem. Cambium is in typical dormant condition, characterized by firm walls and rectangular cell shape. All cell contents are granular and dark in the cambium. Note absence of extruded nucleoli. (205 X.) Sample was taken 5 feet above the ground from the stem of an untreated tree.

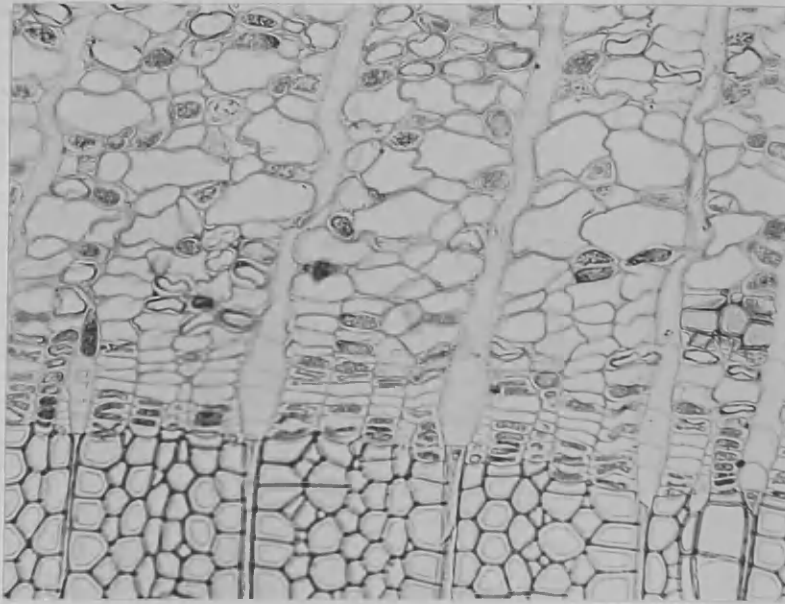
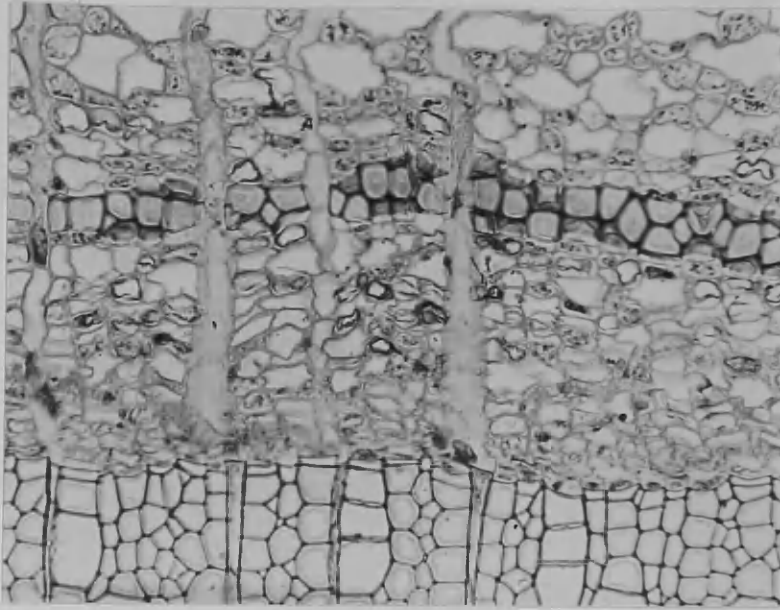


Fig. 17. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing actively dividing cambium, expanding sieve tubes, and newly-formed xylem cells with thin walls. (205 X.) Sample was taken from 5 feet above the ground from the stem of an untreated tree on May 29.

Fig. 18. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing dark, granular cell contents, narrow cambial region, and heavy callose formations. (205 X.) Sample was taken 5 feet above the ground from the stem of an untreated tree on August 20.

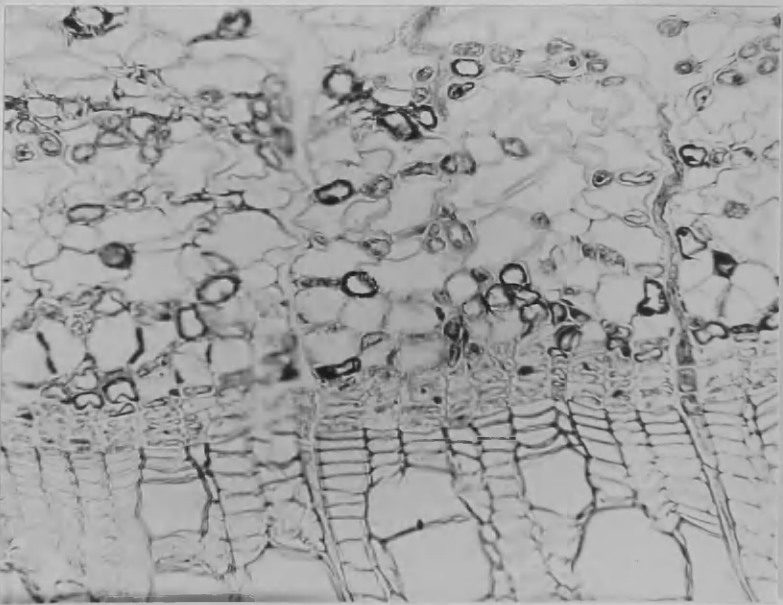
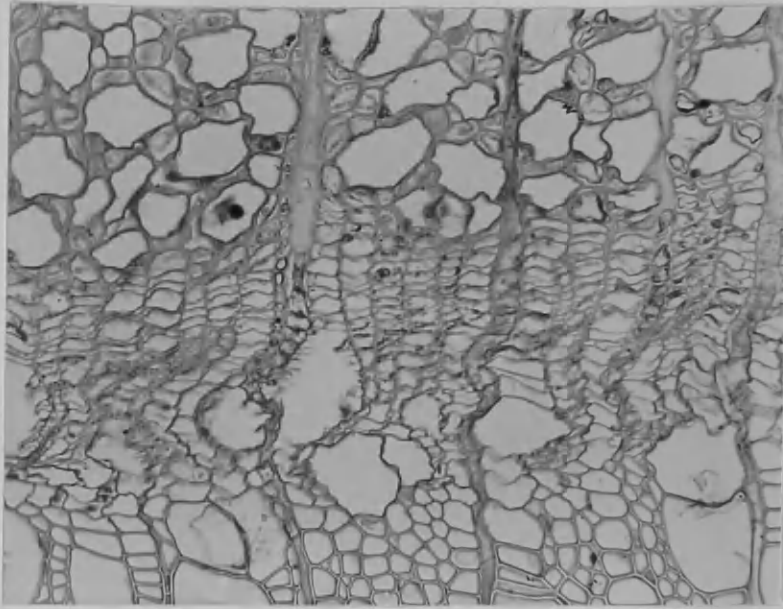


Fig. 19. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing distorted cells in the cambium, absence of extruded nucleoli, and very thick, dark cell walls in the phloem. (205 X.) Sample was taken 5 feet above the ground from the stem of a tree treated with sodium monochloroacetate on June 24; sample was collected 15 days after treating.

Fig. 20. Transverse section of the phloem, adjacent to the cambial region, showing thick cell walls. Sieve tube at right of the section contains sieve plate with heavy accumulation of callose. (700 X.) Section was made from same sample as was used for Figure 19.

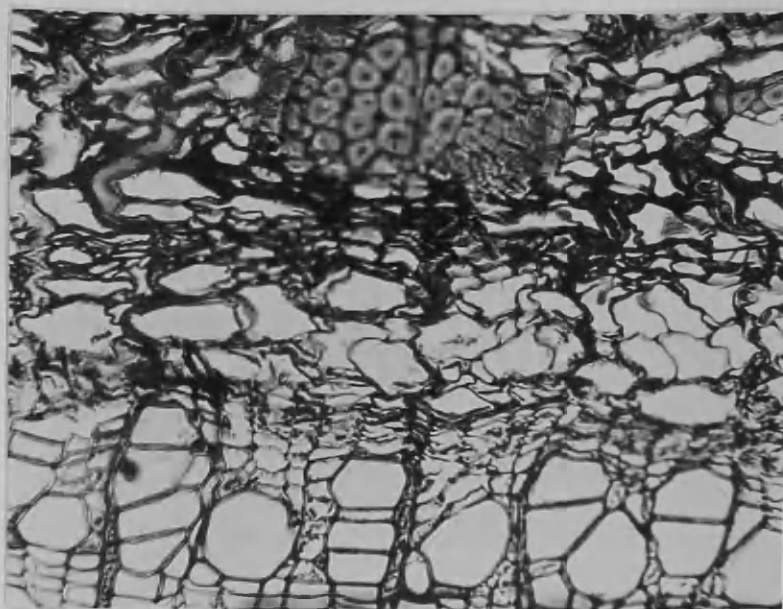


Fig. 21. Transverse section of the cambial region and adjacent tissues of an aspen stem. Note large, expanded sieve tubes, well defined sieve plates with connecting strands, and extruded nucleoli. (205 X.) Sample was taken 5 feet above the ground from the stem of an untreated tree on July 9.

Fig. 22. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing severe collapse in the cambium and adjacent tissues, blackened ray cells, and void areas in the phloem. (205 X.) Sample was taken 5 feet above the ground from the stem of a tree treated with sodium monochloroacetate on June 24; sample was collected 36 days after treatment.

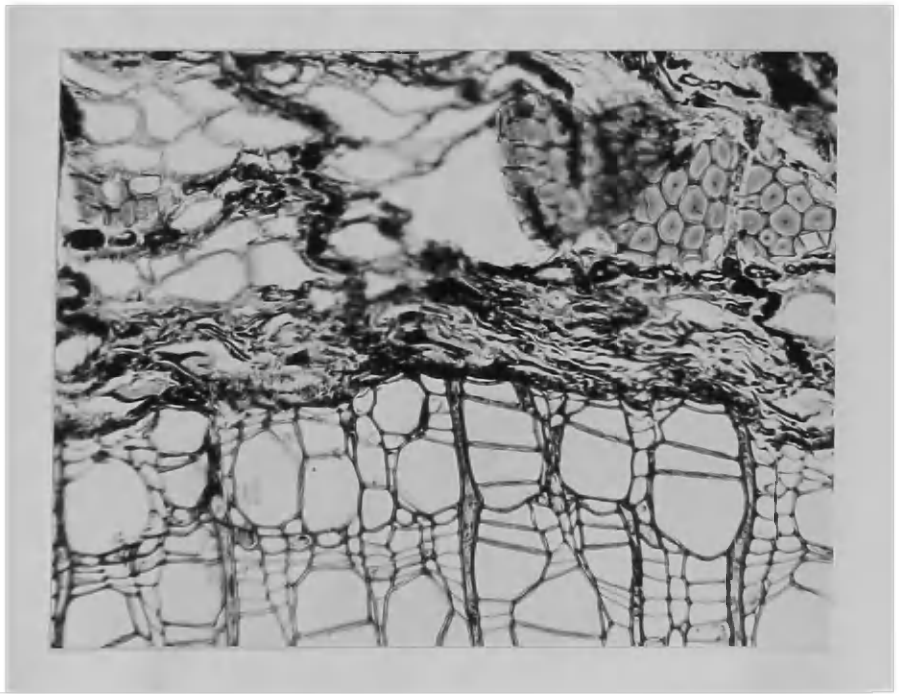
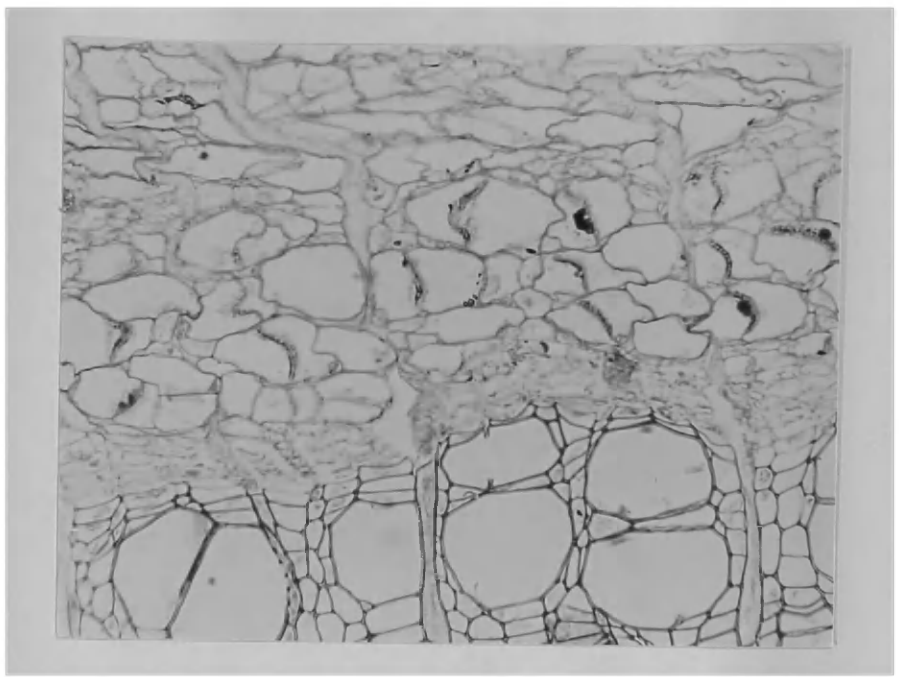


Fig. 23. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing blackened, distorted tissue of the cambial region. Note numerous lacunae between xylem and cambium, and large voids in phloem. (205 X.) Sample was taken 5 feet above the ground from the stem of a tree treated with sodium monochloroacetate on June 24; sample was collected 71 days after treatment.

Fig..24. Radial section of cambium and phloem of an aspen stem. Blackened cambium can be seen at extreme left; blackened ray cells and disintegration of phloem parenchyma cells can be seen at the right of the cambium. (205 X.) Section made from the same sample as used for Fig. 23.

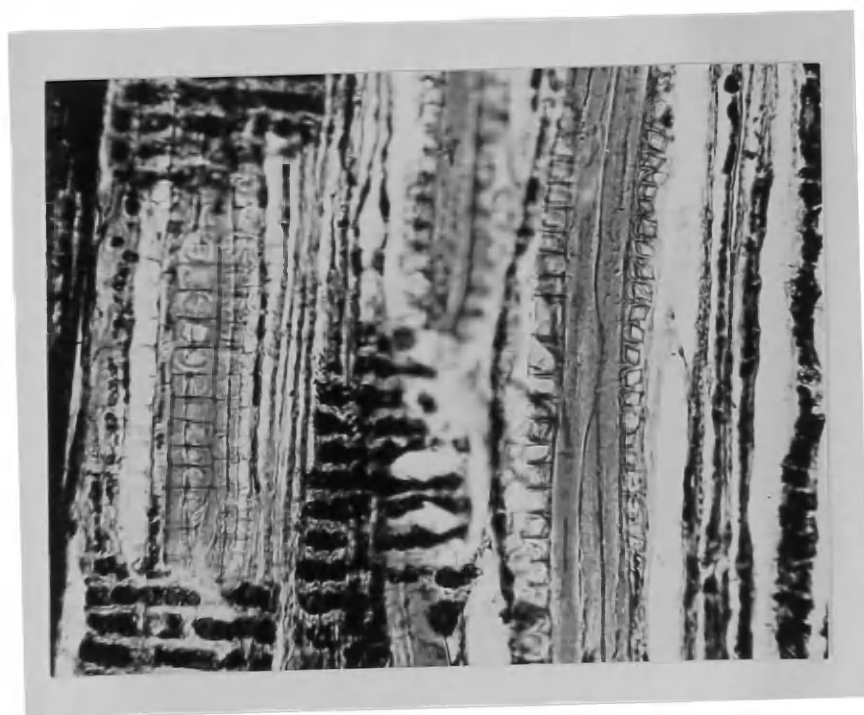
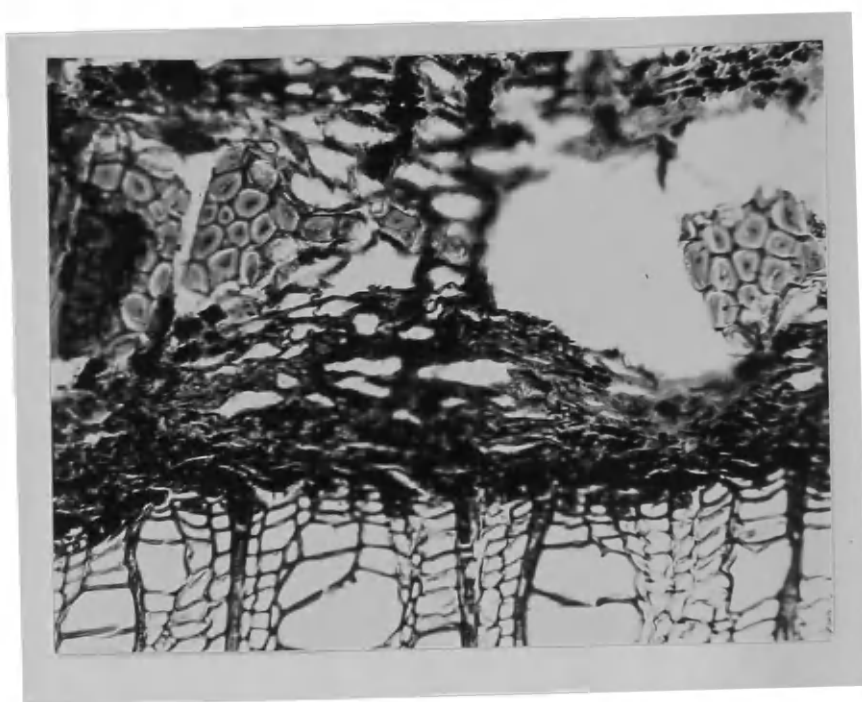


Fig. 25. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing intact cambial region and xylem; some parenchymatous cells of the phloem have thickened walls. (205 X.) Sample was taken at 5 feet above the ground from the stem of a tree treated with 2,4,5-T ester on June 24; sample was collected 15 days after treatment.

Fig. 26. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing early stages of collapse in the cambium, and thick-walled parenchymatous cells in the phloem. (205 X.) Sample was taken 5 feet above the ground from the stem of a tree treated with 2,4,5-T ester on June 24; sample was collected 36 days after treatment.

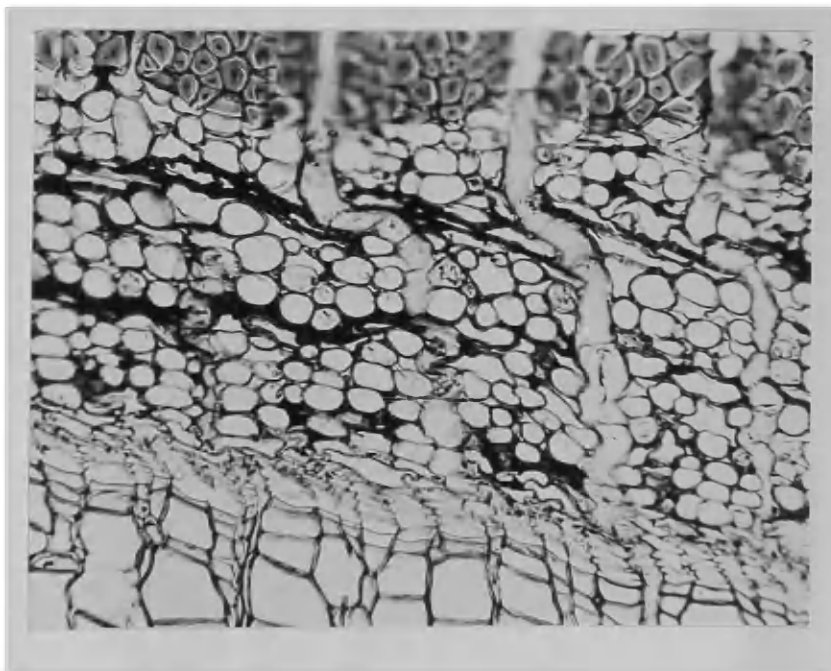
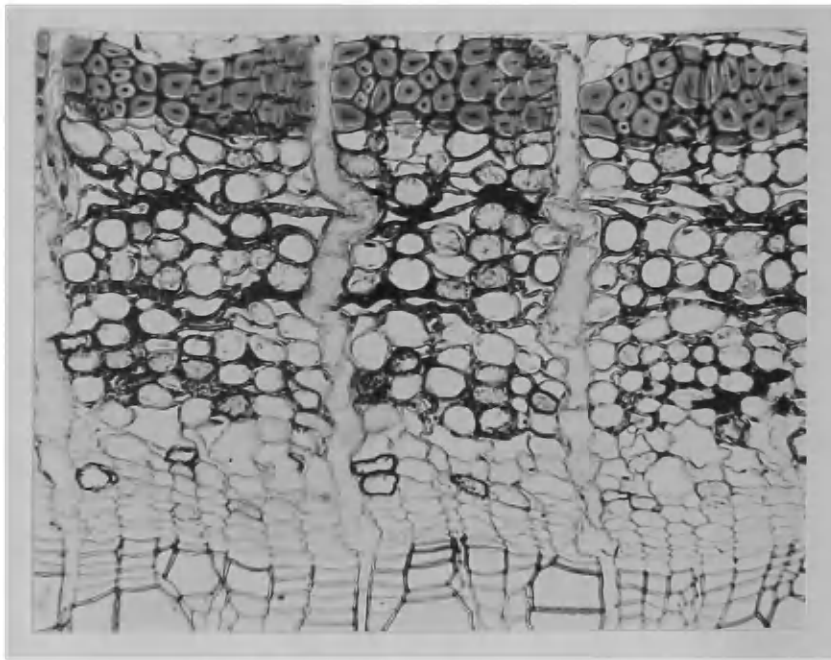


Fig. 27. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing details of thickened walls and collapsed cells in the phloem. (700 X.) Section was made from sample used for Figure 26.

Fig. 28. Transverse section of the cambial region and adjacent tissues of the stem of an aspen, showing advanced state of collapse in the cambium and adjacent phloem tissues; parenchymatous cells in xylem have blackened cell contents. (205 X.) Sample was taken 5 feet above the ground from the stem of a tree treated with 2,4,5-T ester on June 24; sample was collected 57 days after treatment.

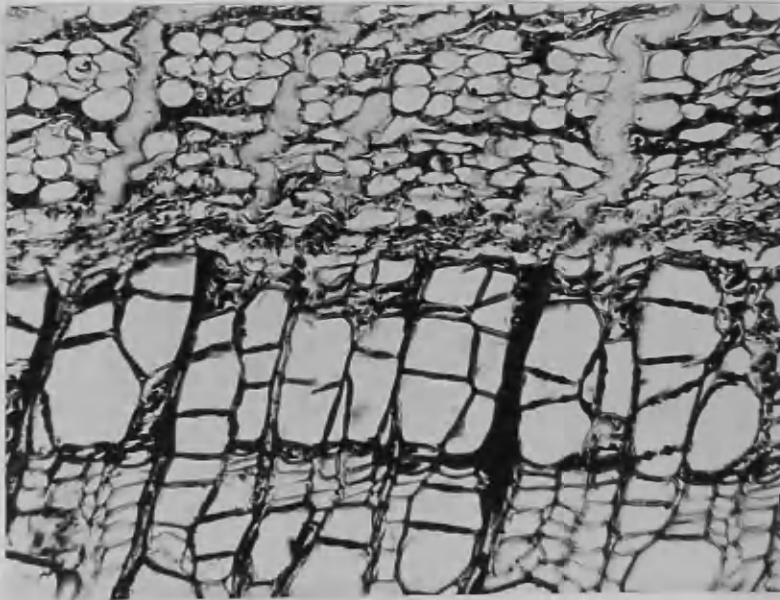
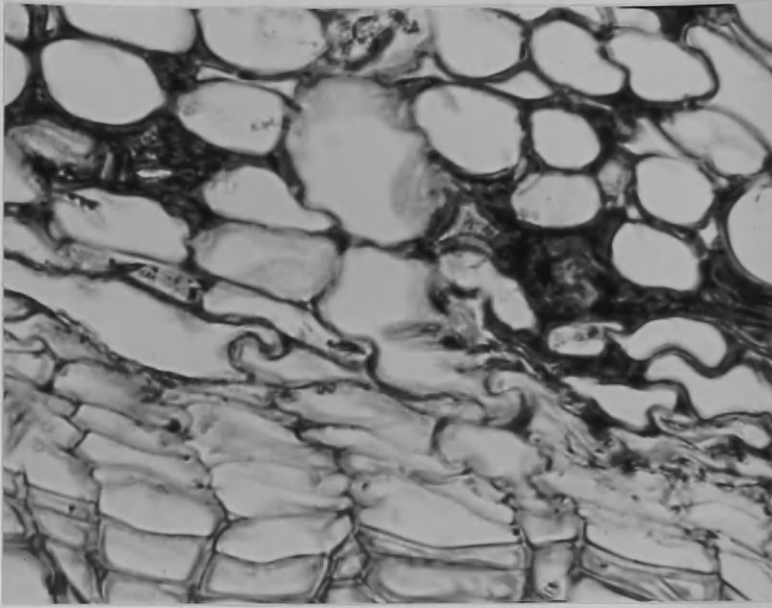


Fig. 29. Radial section of the cambial region and adjacent tissues of the stem of aspen, showing blackened cell walls and contents in the vicinity of the cambium. (205 X.) Section was made from same sample used in Figure 28.

Fig. 30. Transverse section of the cambial region and adjacent tissue of an aspen stem, showing advanced disintegration of cambium, and thickened walls of parenchymatous cells in the phloem. Note lacunae between xylem and cambium. (205 X.) Sample was taken from 5 feet above the ground from the stem of a tree treated with 2,4,5-T ester on June 24; sample was collected 71 days after treatment.

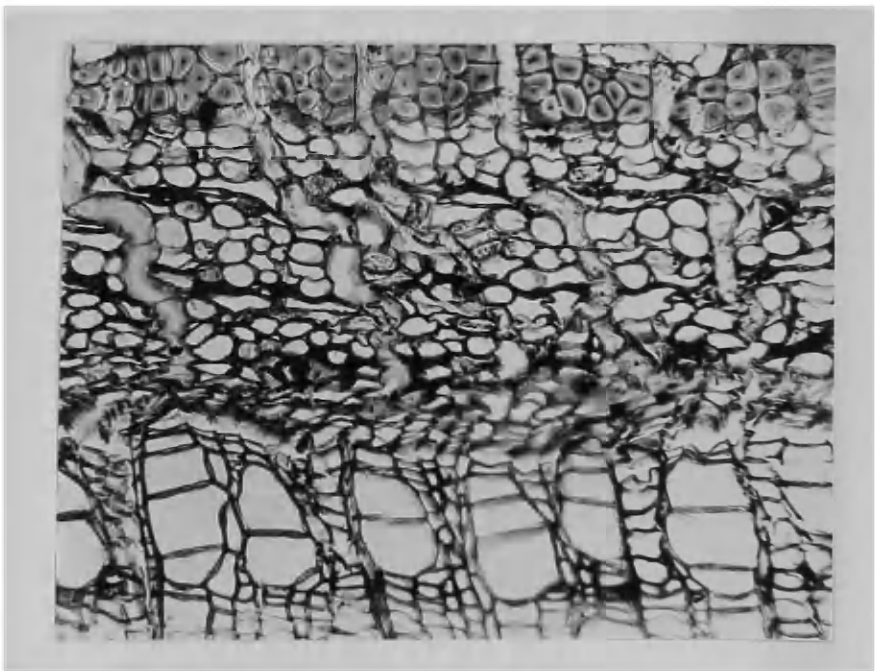


Fig. 31. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing blackened cell walls of the cambial region and thick, blackened walls in the phloem. (205 X.) Sample was taken 5 feet above the ground from the stem of a tree treated with 2,4-D ester on June 24; sample was collected 51 days after treatment.

Fig. 32. Transverse section of the cambial region and adjacent tissue of an aspen stem, showing numerous extruded nucleoli, prominent sieve plates, and cells rich in contents. (205 X.) Sample was taken 5 feet above the ground from the stem of an untreated tree on August 14.

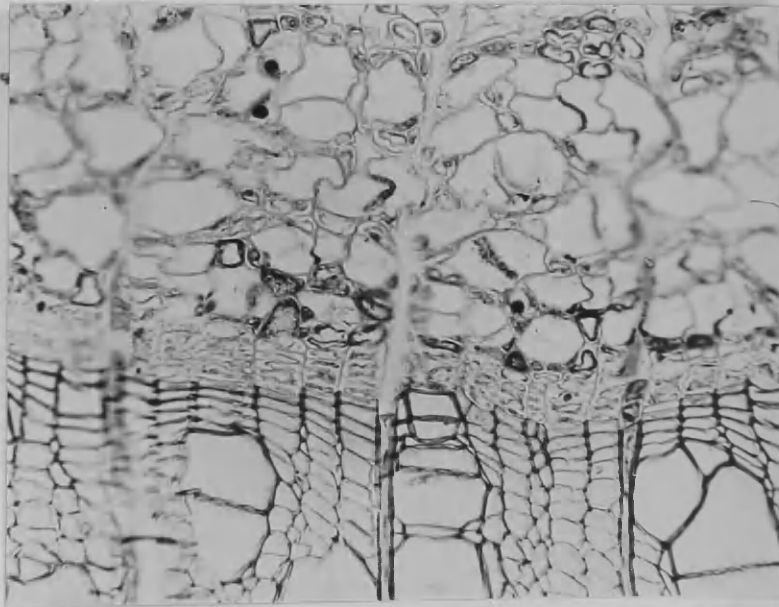
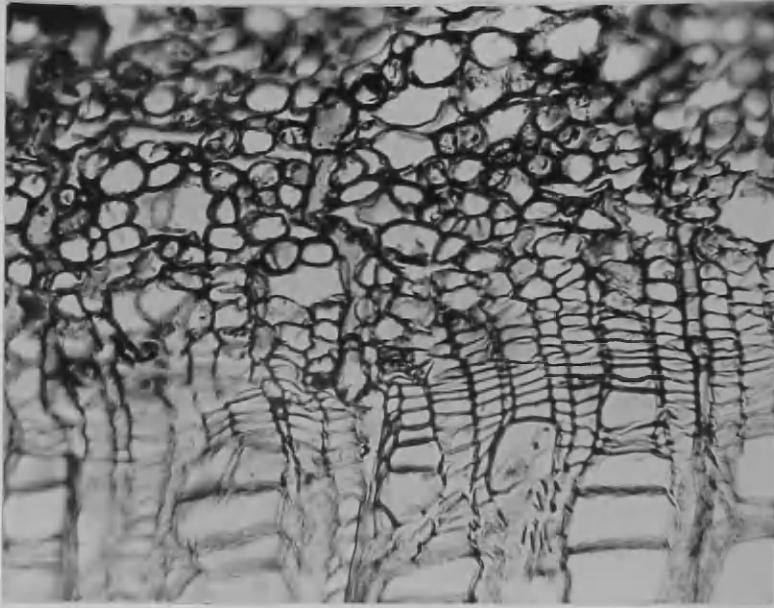


Fig. 33. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing blackened cell contents of cambium and phloem, thick walls in the phloem, and collapse of cambial cells. (205 X.) Sample was taken 5 feet above the ground from the stem of a tree treated with 2,4-D ester on June 24; sample was collected 71 days after treatment.

Fig. 34. Phloem of Figure 33 at higher magnification, showing thickened walls, and absence of sieve plates and extruded nucleoli in sieve tubes. (700 X.)

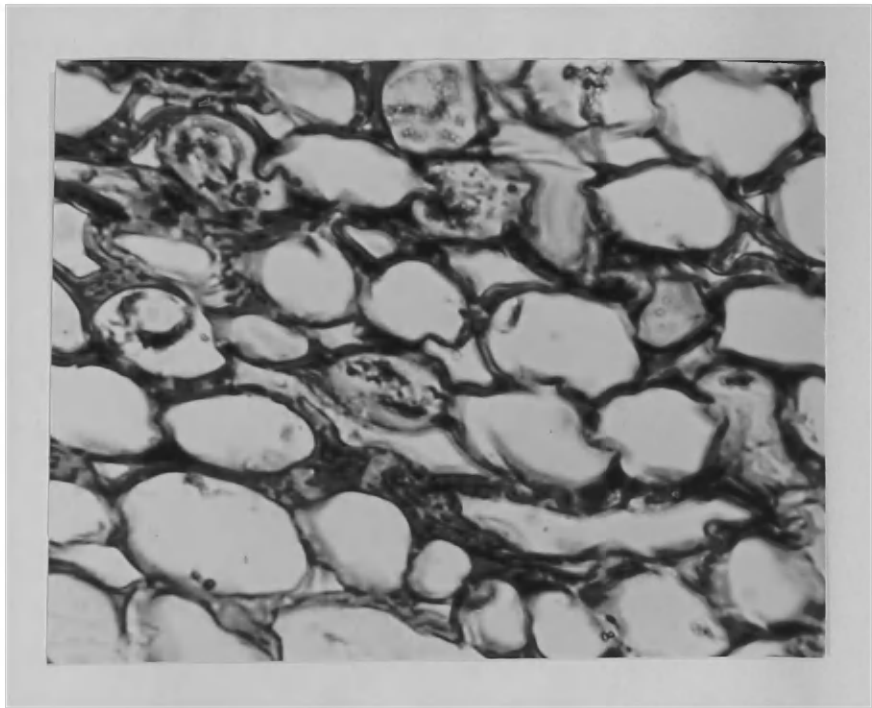
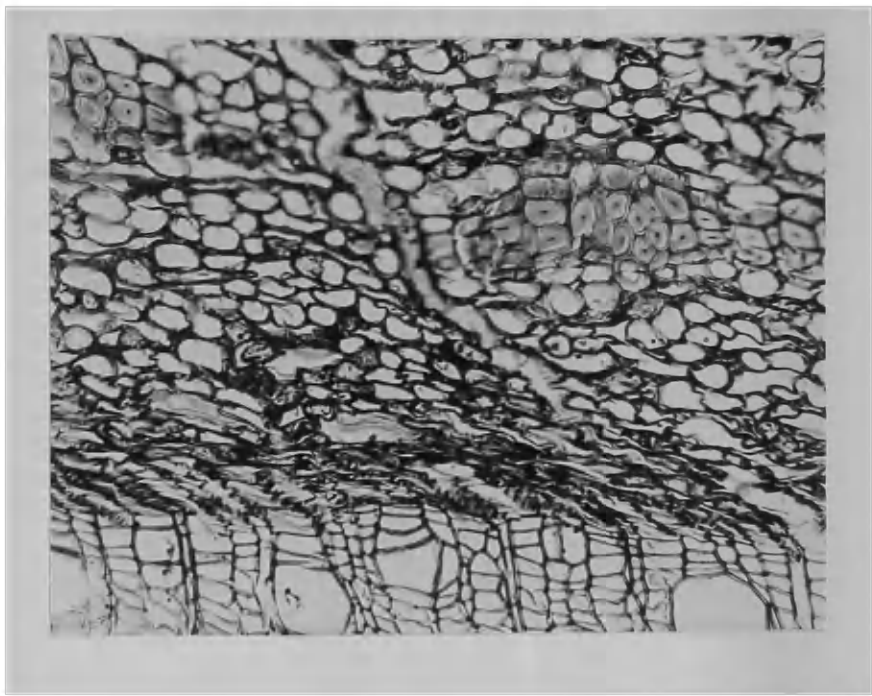


Fig. 35. Transverse section of the phloem, immediately adjacent to the cambial region, showing expanded sieve tubes with prominent sieve plates. (700 X.) Sample was taken from 5 feet above the ground from the stem of an untreated tree on September 3.

Fig. 36. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing early stages of collapse in the cambial region, blackened phloem and xylem ray tissue, and disintegration of phloem cells adjacent to the cambium. (205 X.) Sample was taken 5 feet above the ground from the stem of a tree treated with sodium monochloroacetate on July 3; sample was collected 36 days after treatment.

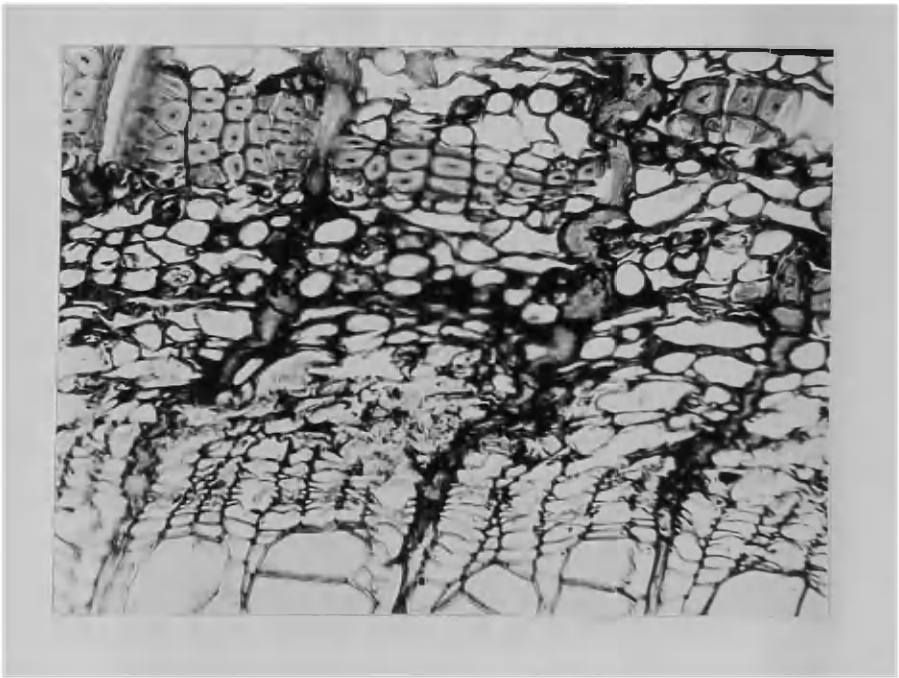
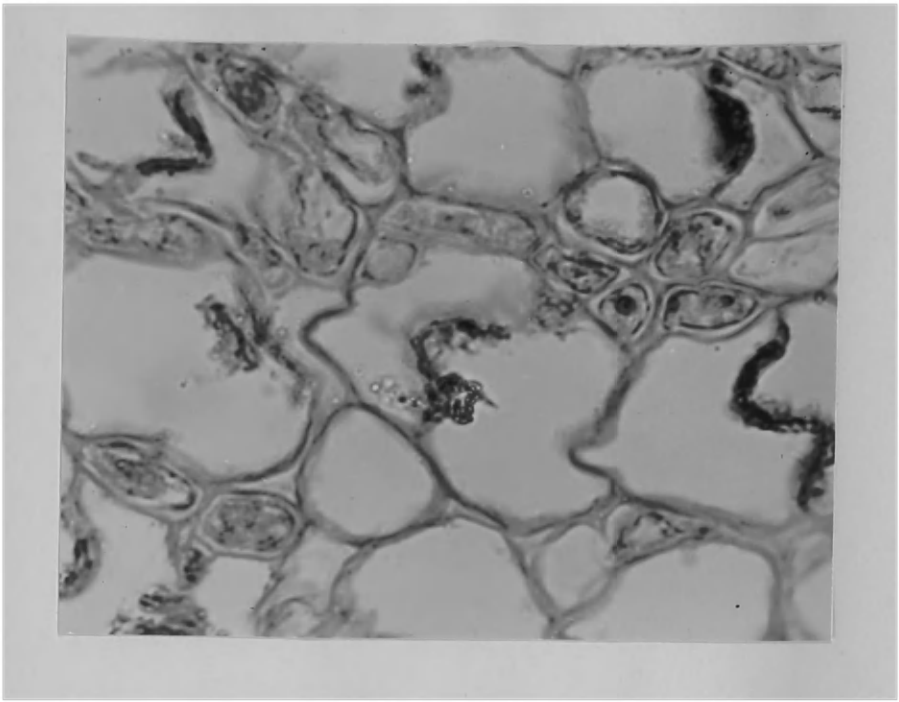


Fig. 37. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing intact cell walls in the cambium and xylem, and early stages of collapse in the phloem. Note the darkened cell contents in the xylem and phloem. (205 X.) Sample was taken 5 feet above the ground from the stem of a tree treated with 2,4,5-T ester on July 15; sample was collected 36 days after treatment.

Fig. 38. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing no evidence of damage from chemical treatment. (205 X.) Sample was taken 5 feet above the ground from the stem of a tree treated with 2,4-D on July 15; sample was collected 36 days after treatment.

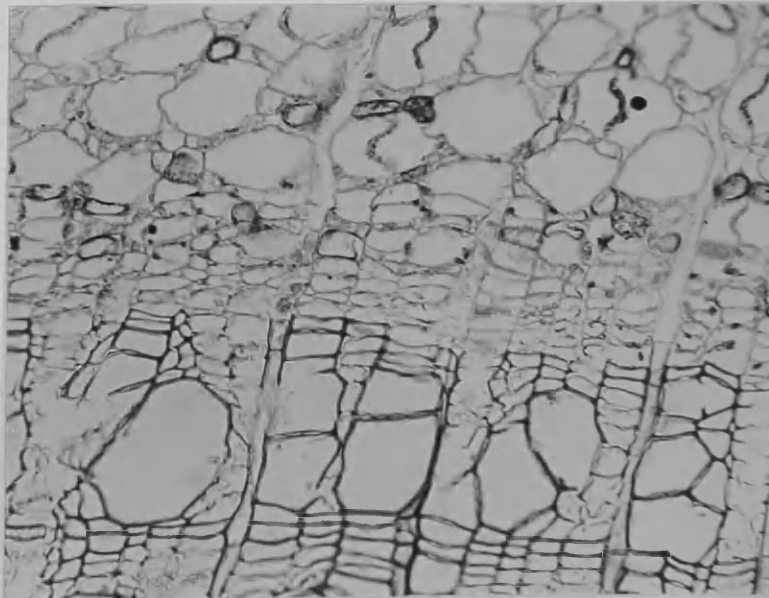
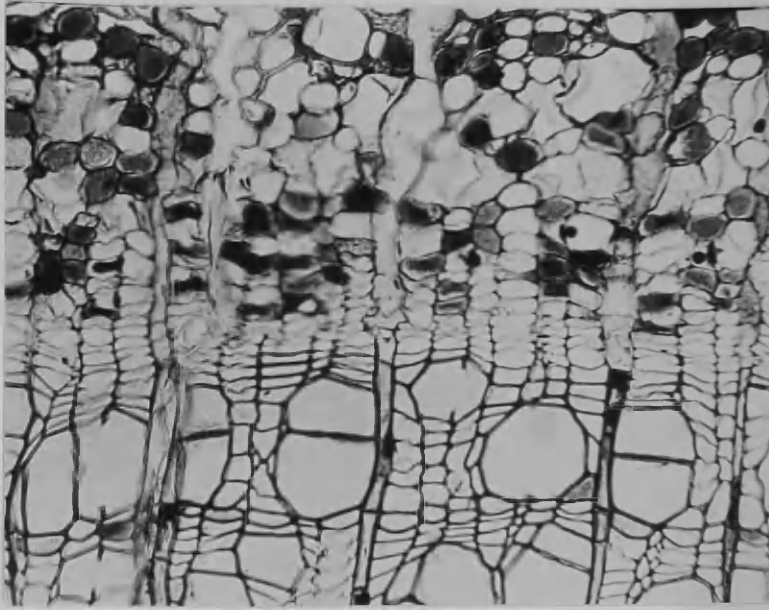


Fig. 39. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing collapse of cambium and phloem parenchyma cells adjacent to cambium. (205 X.) Sample was taken 18 inches above the ground from the stem of a tree treated with 2,4,5-T ester on June 4; sample was collected 80 days after treatment.

Fig. 40. Transverse section of the cambial region and adjacent tissues of the stem of an aspen. Condition of this section is comparable to that of Figure 39. (205 X.) Sample was taken at 5 feet from same tree as used for Figure 39.

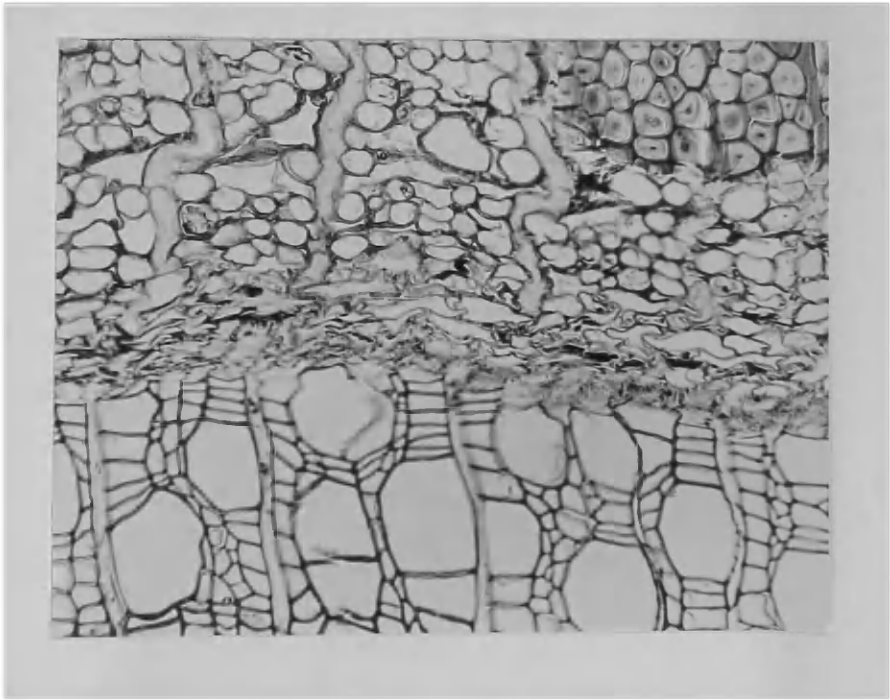
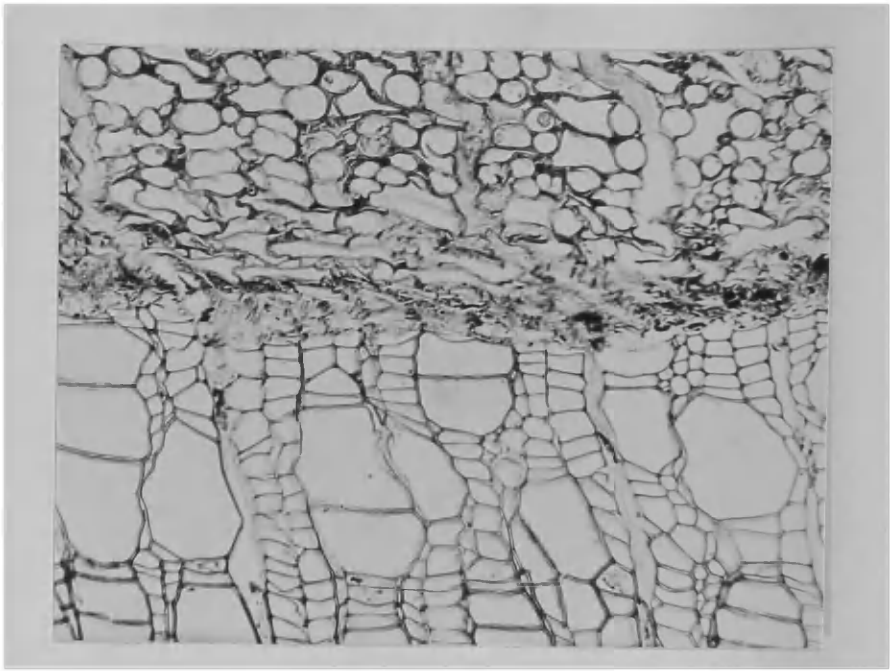


Fig. 41. Transverse section of the cambial region and adjacent tissues of the stem of an aspen. Cambium and xylem are intact; early stages of collapse and some wall thickening visible in phloem. (205 X.) Sample was taken 35 feet above the ground from the same tree as used in Figures 39 and 40.

Fig. 42. Transverse section of the tip of the leader of an aspen stem, showing completely blackened and collapsed cambium. Xylem is distorted and phloem is blackened and distorted. Note intact fibers and sclereids in phloem. (205 X.) Sample was taken from the leader of the same tree as used for Figures 39, 40, and 41.

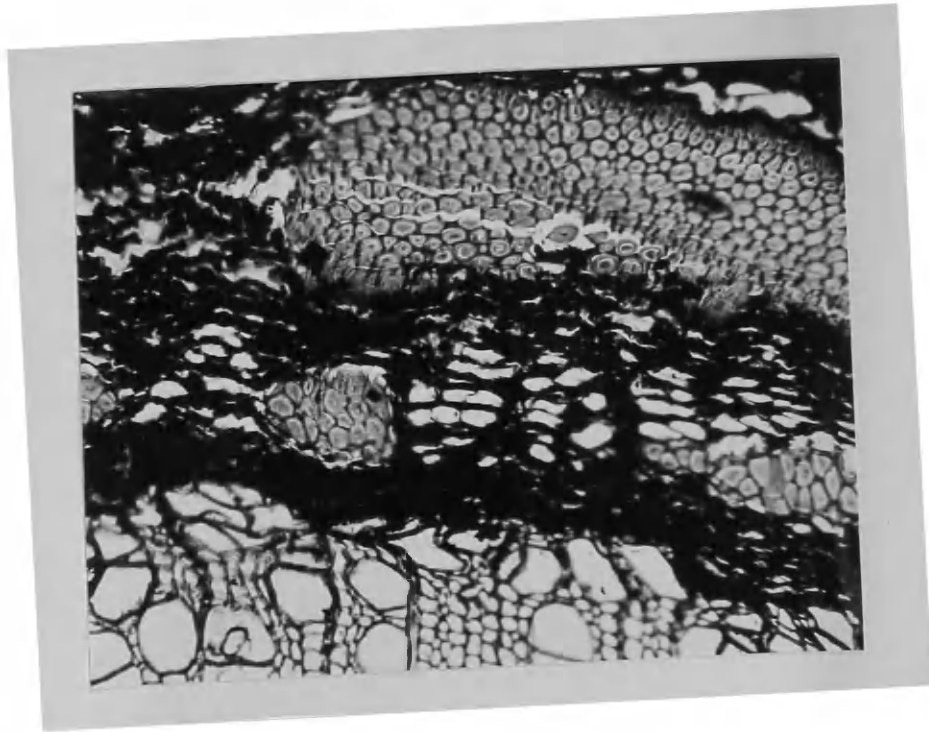
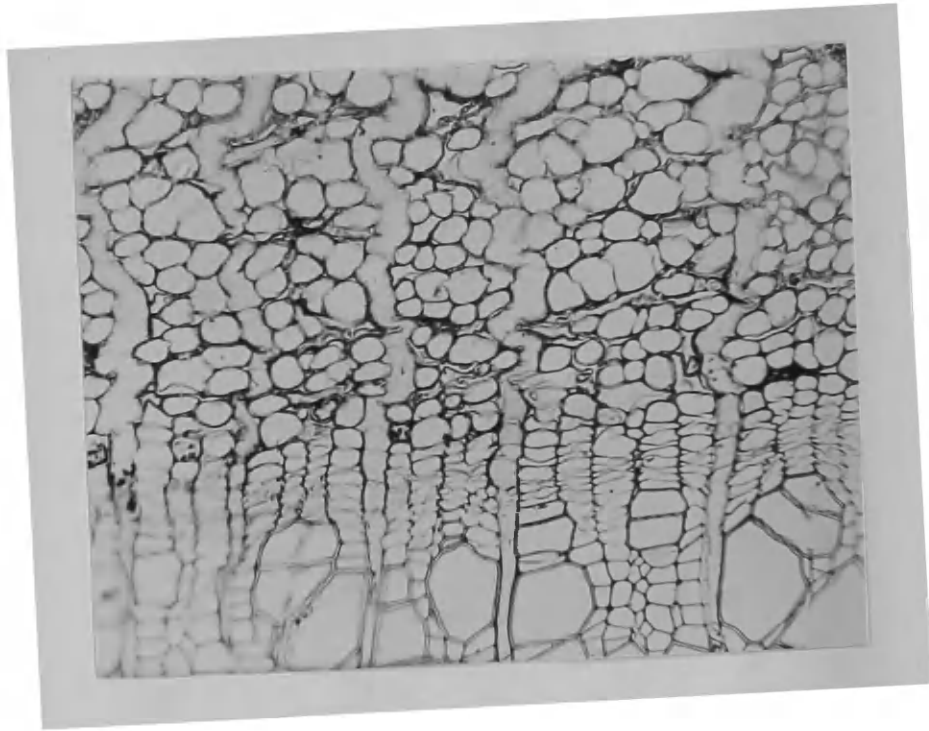


Fig. 43. Transverse section of the cambial region and adjacent tissues of an aspen stem, showing advanced stage of disintegration of cambial cells, lacunae between xylem and cambium, and thick parenchyma walls in the phloem. (205 X.) Sample was taken from 5 feet above the ground from the stem of a tree treated with a double strength solution of 2,4,5-T ester on June 24; sample was collected 71 days after treatment.

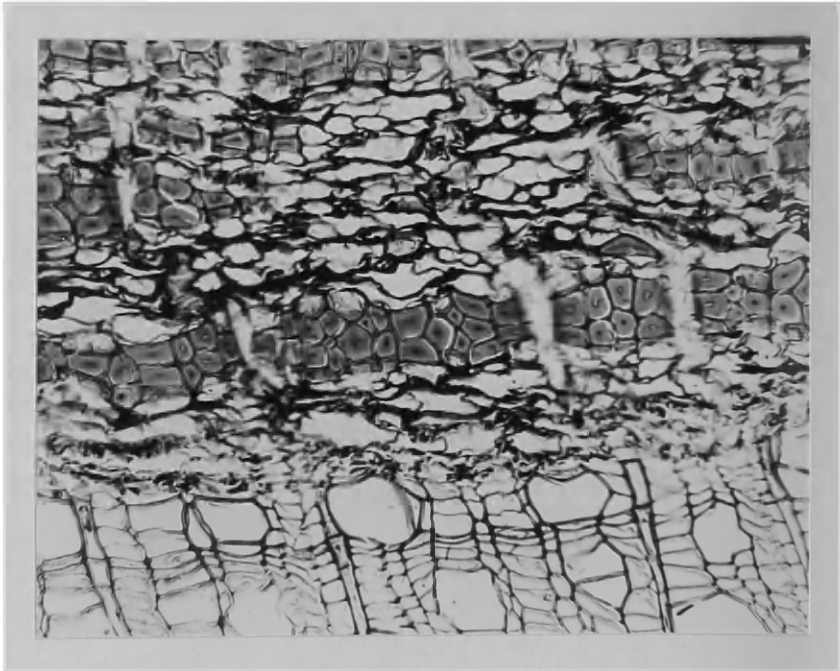


Fig. 44. Transverse section of a twig from an untreated tree; collected on August 18 and showing xylem, cambium, phloem, and bark cells. (205 X.)

Fig. 45. Transverse section of a twig from a tree treated with 2,4,5-T ester on July 9. Sample was collected 40 days after treatment. Section shows completely collapsed cambium, blackened and disintegrated phloem parenchyma cells, and some blackened pith cells. (205 X.)

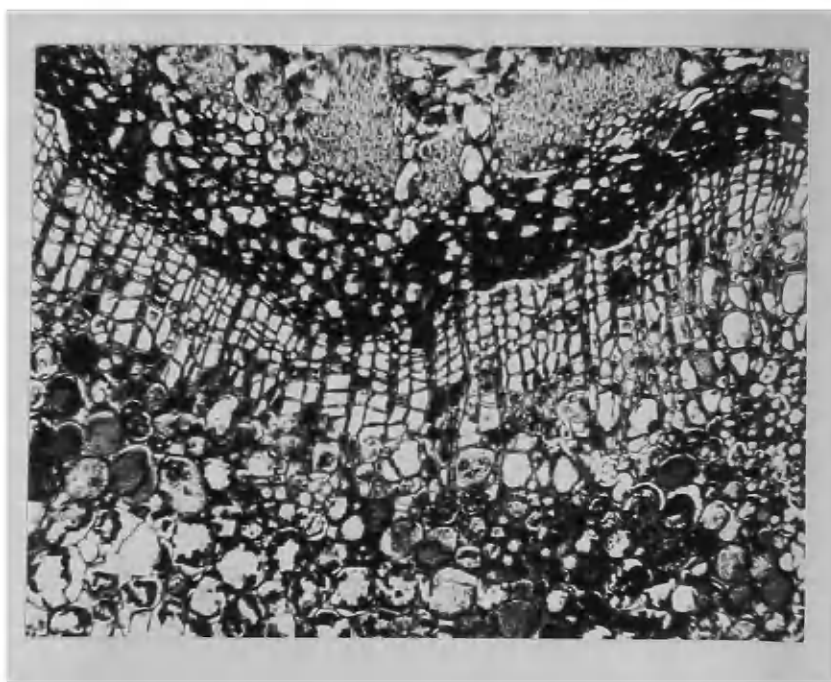
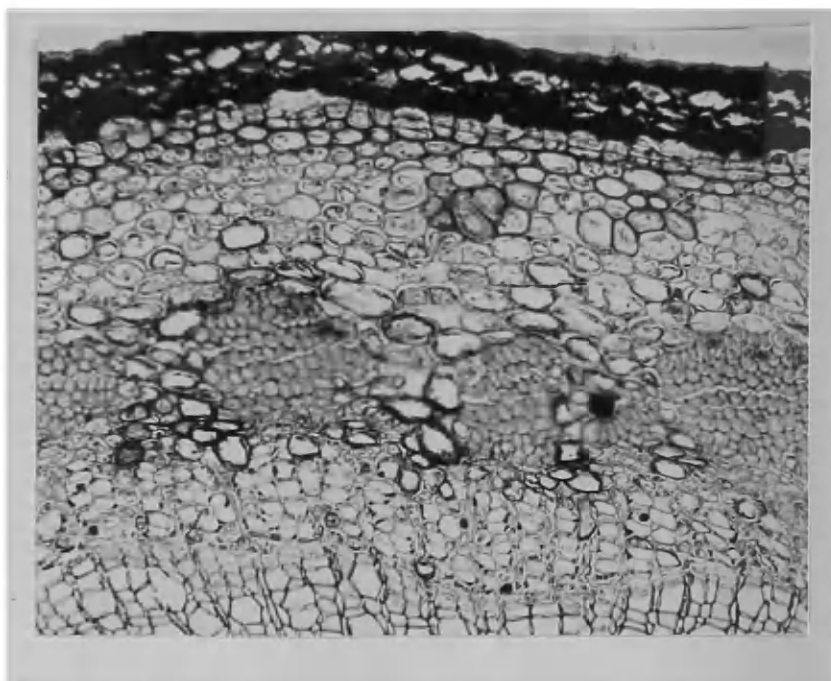


Fig. 46. Transverse section of the midrib portion of an aspen leaf, showing vascular bundle, bundle sheath, and surrounding cells. (205 X.)

Fig. 47. Transverse section of the leaf blade immediately adjacent to the midrib section of Figure 46. Epidermal layers, palisade layers, and spongy mesophyll are visible. Note prominent chloroplasts in palisade layers and spongy mesophyll. (205 X.)

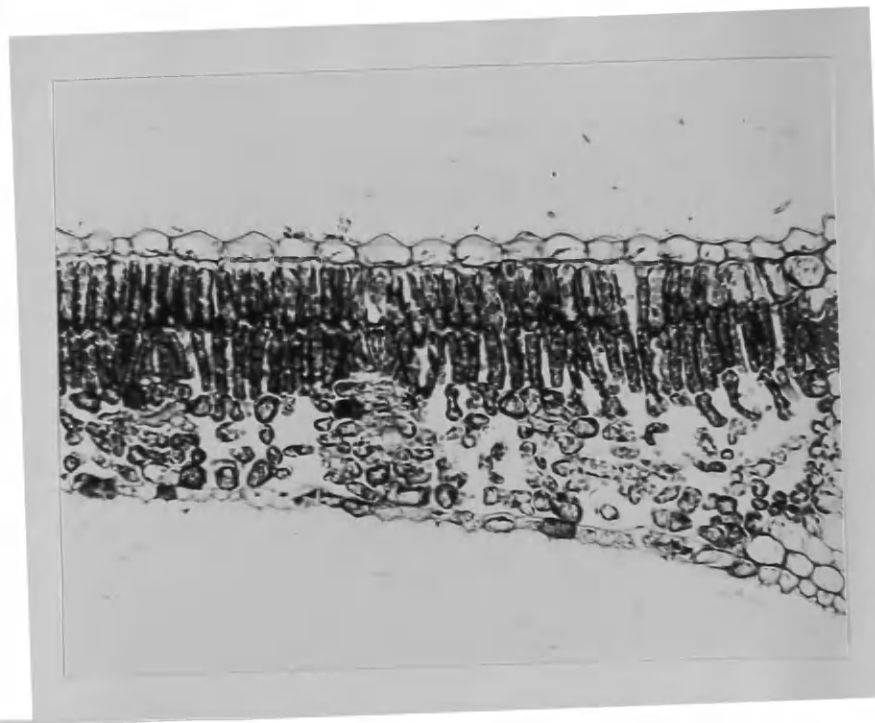
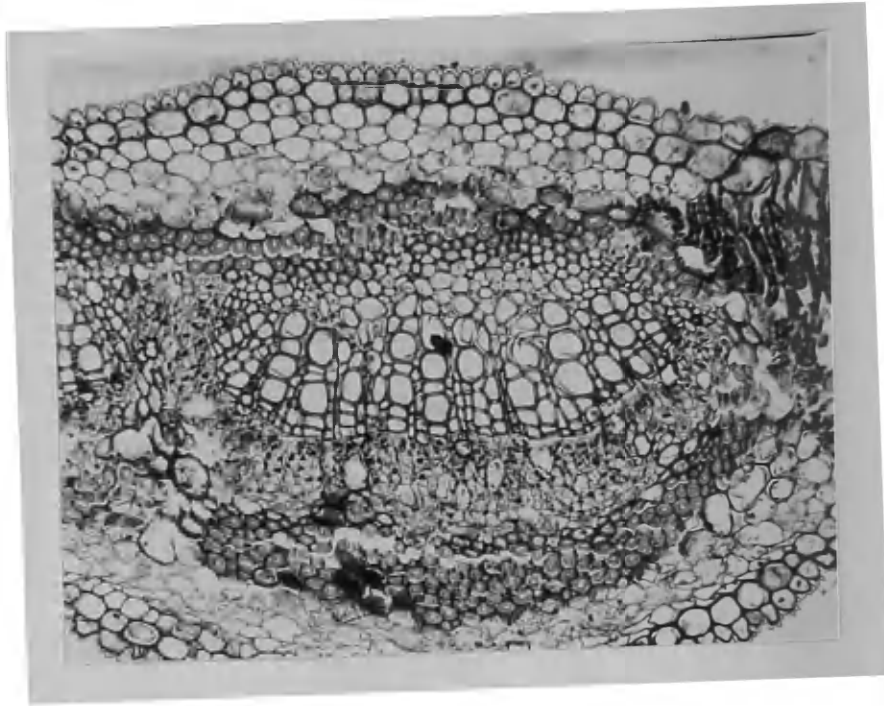


Fig. 48. Transverse section of the midrib of a leaf. From an aspen tree treated with 2,4-D ester on July 9; sample was collected 40 days after treatment. Completely blackened and disintegrated phloem is visible within vascular bundle. Blackened ray cells in the xylem, and blackened parenchymatous cells outside the bundle sheath can also be seen. (205 X.)

Fig. 49. Portion of leaf blade adjacent to the midrib described in Figure 48. All cells of palisade layer and spongy mesophyll are blackened and distorted except for a few intact fibers. (205 X.)

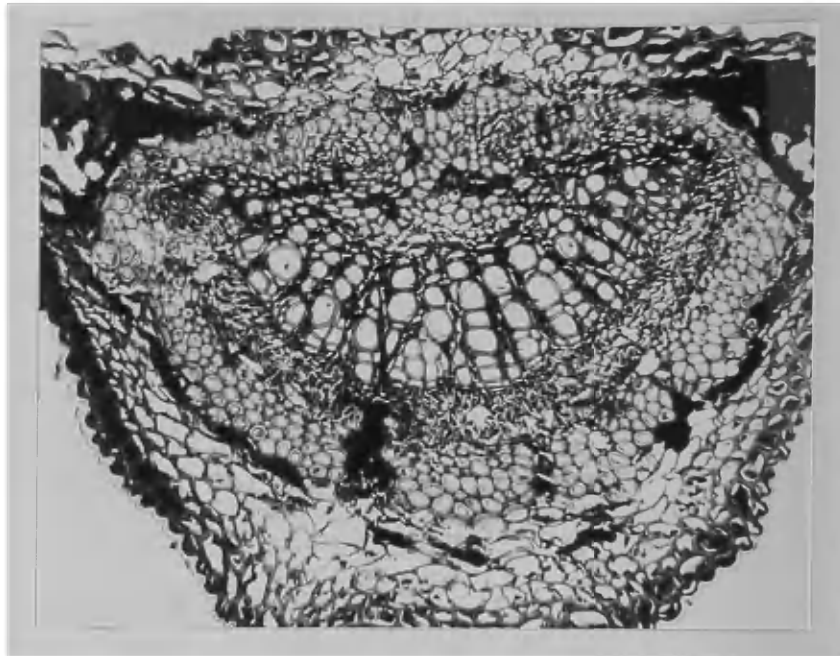
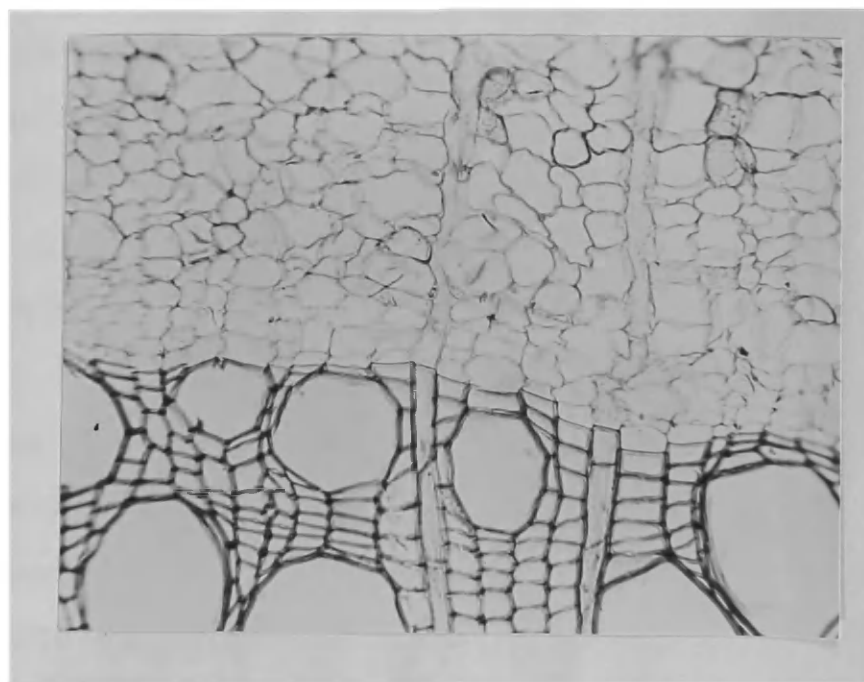
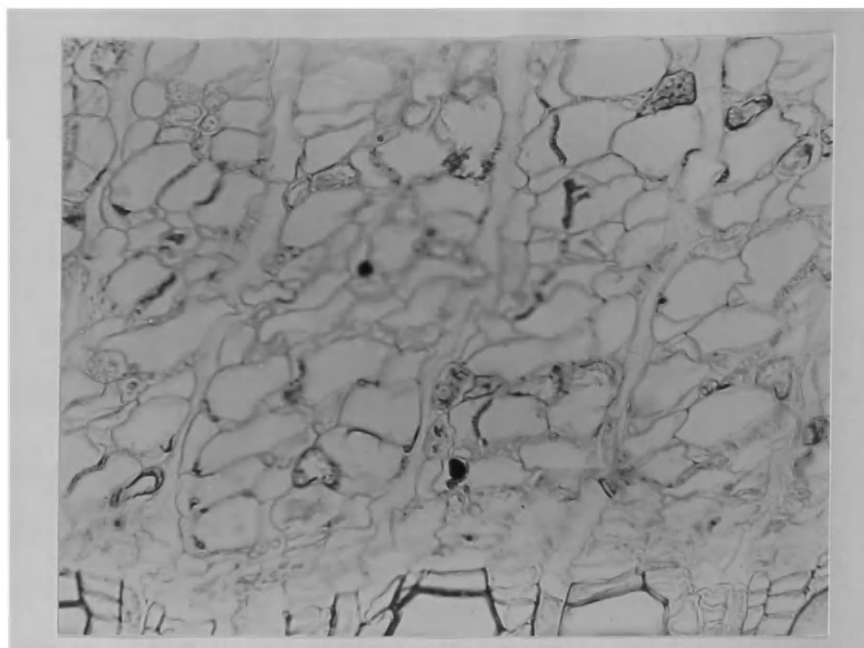


Fig. 50. Transverse section of the cambial region and adjacent tissues of an aspen root, showing extruded nucleoli, expanded sieve tubes, and prominent sieve plates. Sample was taken from the main root of an untreated tree, about 18 inches from the root collar. Sample was collected on September 3.

Fig. 51. Transverse section of the cambial region and adjacent tissues of an aspen root, showing contracted appearance of sieve tubes, absence of extruded nucleoli, and lack of visible sieve plates. (205 X.) Sample was taken from the main root of an aspen tree treated with 2,4,5-T ester on June 4. Sample was collected 80 days after treatment.



VII. DISCUSSION AND CONCLUSIONS

A. Discussion

1. Field Observations

Effects of chemical treatment on the appearance of trees and condition of the bark. All chemical treatments resulted in death of the trees. However, the speed of killing and the extent of bark loosening obtained were quite different.

Sodium monochloroacetate applied in frills was the fastest-acting chemical. The leaves of trees treated by this method turned from green to brown within a few days and had fallen from most trees by the end of 23 days. Bark of the stem turned a solid black color within a relatively short time and could be stripped from the stem to a height of about 7 feet above the ground when examined 71 days after treating.

Basal sprays of 2,4,5-T ester and 2,4-D ester were the only other treatments that showed extensive injury during summer and autumn immediately following treatment. Sodium monochloroacetate applied to a peeled girdle did injure some trees quite severely but most of the damage occurred the

year after treating. Other treatments were only partially effective, even one year after treatment.

The leaves of trees treated with 2,4,5-T and 2,4-D turned a brilliant red and yellow for about 3 weeks before changing to brown and remained attached to the tree much longer than those of trees treated with sodium monochloroacetate in frills. The bark of the stems became discolored in small areas at first. These areas later coalesced and the outer bark became a solid orange color.

Although the appearance of trees treated with 2,4,5-T and 2,4-D was quite similar, the killing effect of the 2,4,5-T treatment was much more rapid, and bark loosening more pronounced. The bark of the sprayed area of trees treated with 2,4,5-T was fairly loose when inspected 71 days after treating, but the remainder of the stem bark was tight. On 2,4-D treated trees, the bark was still tight 71 days after treatment, even on the sprayed areas.

An examination made one year after treating showed that most trees treated with sodium monochloroacetate in frills and trees treated with 2,4,5-T ester could be peeled. On the other hand, many trees treated with 2,4-D still resisted peeling.

The effectiveness of treatments when applied on different dates. The date of treating did have some effect on the speed and effectiveness of chemical treatments. It was found

that early treatments, even before bud-break, were just as quick and effective in their action as those which were applied when trees were in full leaf. Treatments made later than July 3 took longer to kill trees and were less effective in loosening the bark than treatments applied earlier. This was especially true of trees treated with 2,4,5-T or 2,4-D. Trees treated with sodium monochloroacetate after July 3 did not die as fast as those treated earlier in the season, but the bark peeled in September just as easily as it did from the trees with the earlier treatments.

Miscellaneous observations. Examinations made on the roots of treated trees failed to disclose any damage. Roots of trees treated with sodium monochloroacetate were inspected 71 days after the chemical was applied, and the roots of trees treated with 2,4,5-T ester were examined 80 days after treatment. In both instances the roots appeared to be healthy, although the trees apparently were dead down to the root collar.

All chemical treatments were more effective in the lower half of the trunk than they were in the upper half. Pulpwood sticks obtained from the lower region of the trunks usually peeled much easier than those obtained from the upper portion. This was true for all chemical treatments but was especially pronounced in sticks obtained from trees treated with 2,4,5-T and 2,4-D.

It was noted that aspen treated with sodium monochloroacetate in frills died faster and eventually peeled better than that treated with the same chemical applied to a peeled girdle. It was also found that frills which had been cut into the sapwood for at least one-half inch were more effective than those which barely scored the wood.

Very little translocation in the lateral direction was found in the case of sodium monochloroacetate. Trees frilled and treated on one side of the stem showed stem injury on the same side only. These findings are in agreement with those of other workers (20,65).

Allowing treated trees to stand over winter before felling and peeling was found to increase greatly the effectiveness of treatments. In many cases the bark contained long vertical splits and was sloughing off naturally when examined one year after treatment. However, it was also discovered that standing over winter caused a discoloration in the outer one inch of sapwood. This discoloration is undesirable for certain types of paper products, since additional amounts of bleach must be used in order to produce a pure white product.

2. Anatomical Changes in the Tissues of Stems, Twigs, Leaves, and Roots.

a. Progressive effects of chemical treatments on the cambial region and adjacent tissues of the stem.

Effects of sodium monochloroacetate. Cells of the cambial region were distorted and partially collapsed 15 days after treatment. Cell contents became dark and, as more time elapsed, cell collapse became general and very severe throughout the region. This was followed by disintegration of cell walls and contents. Eventually the entire cambial region was transformed into tangential bands of blackened, structureless cell walls and cell contents. This condition can be observed in Figure 23.

The mature vessels and fibers of the xylem were not affected by the treatment. On the other hand, the walls of newly-formed xylem elements showed signs of collapse 15 days after treatment. This condition became more pronounced until collapse was general in this part of the xylem, followed by cell wall disintegration. The result was the appearance of numerous lacunae between xylem and the cambial region. Ray and longitudinal parenchyma cells of the xylem frequently exhibited blackened walls and contents, although these cells collapsed only immediately adjacent to the cambial region.

The phloem was severely attacked, even as early as 15 days after treatment. The walls of most parenchymatous cells became blackened and increased in thickness; cells were twisted and distorted, and in partial collapse. Later on, collapse became severe, especially in the sieve tubes, and then a gradual disintegration took place. This disintegration became progressively greater, and after 71 days large voids were formed between bands consisting of intact fibers and sclereids and remaining parenchymatous cells. Sieve plates were very difficult to detect in the treated sections. This apparently was due to the fact that the walls containing the plates were thickened and lined with dark cytoplasm which masked the plates. The plates were also devoid of callose in many instances, which made their detection more difficult. Extruded nucleoli were very rare. In many cases none could be found in the entire phloem. The absence of sieve plates and extruded nucleoli can be seen in Figure 23. Ray and longitudinal parenchyma cells of the phloem became blackened and distorted as a result of treatment. In many cases rays disintegrated in the vicinity of the cambial region and assumed a twisted appearance throughout the rest of the phloem (see Fig. 22).

The ultimate effects of this chemical, as seen 71 days after treating, were complete collapse of the cambial region, collapse of newly-formed xylem elements, severe col-

lapse of some parenchymatous cells in the phloem, and disintegration of others. Numerous large voids were present in the phloem. The only cells that remained intact were fibers and sclereids in the phloem, and vessels and fibers in the xylem.

Effects of 2,4,5-T ester. The effects of 2,4,5-T ester were quite similar to those of sodium monochloroacetate but much less severe. The chemical did not injure the cells as fast as the sodium monochloroacetate did, and when samples of the two treatments were collected on the same day, and a comparison made, the 2,4,5-T sample was always affected less.

Fifteen days after treating, only a few parenchymatous cells of the phloem were damaged. These had thickened walls and dark cell contents but no collapse could be detected. No damage to the cambial region or xylem could be seen. Eventually the cambial region did collapse, but 71 days after treatment very little collapse had occurred in either the phloem or xylem as compared with the sample from the tree treated with sodium monochloroacetate. Probably the greatest single difference between the two treatments was the appearance of the phloem. The amount of collapse and destruction never became as great in the 2,4,5-T treated samples. Parenchymatous cells developed very thick walls and the cavities were very small in comparison with untreat-

ed cells. However, most cell walls retained a rounded or oval appearance, even 71 days after treating. Very few voids developed in the phloem of 2,4,5-T treated trees.

Effects of 2,4-D ester. The effects of 2,4-D were almost identical to those of 2,4,5-T. The degree of injury was less in 2,4-D samples than in those of 2,4,5-T collected at the same time. Injury to most cells started at a later date and never was as severe as 2,4,5-T injury.

Comments. The blackening of cell contents and walls, as described for these three treatments, is thought by some investigators to be caused by a release of tannin and phlobaphene compounds by dying ray and longitudinal parenchyma cells (31). Dark-stained sieve tube contents have been interpreted as the results of disorganization of the cytoplasm (57).

The thickened walls of the phloem probably were caused by a combination of actual wall thickening and a lining of cytoplasm stained the same as the wall, due to action of the chemicals. This condition can be observed in Figures 27 and 33.

The absence of callose on sieve plates and the disappearance of extruded nucleoli in sieve tubes have been associated with decreased activity of the phloem (28,29). This was probably the case with the treated trees. The phloem was apparently in a state of decreased activity as a result

of approaching death, causing an absence of extruded nucleoli and callose material. This condition was a constant feature of practically every sample which displayed other signs of injury due to chemical treatment.

b. The effectiveness of chemicals when applied on different dates.

Sodium monochloroacetate. Based on an examination of samples taken 36 days after treatment, it was found that the June 24 application produced the greatest amount of damage. The July 3 treatment was next in effectiveness and the July 9 treatment was least effective. However, even though the degree of injury was not as great for the last two dates, it was still quite severe and the treatments were judged satisfactory.

2,4,5-T ester. Only the June 24 treatments showed extensive damage. The July 3 treatment was next in effectiveness, and treatments made on July 9 and July 15 showed very little damage.

2,4-D ester. The 2,4-D treatments followed the same pattern as the 2,4,5-T treatments. The degree of injury was less in these samples and no damage of any kind could be found in the July treatments.

Comments. The results of this study are related somewhat to the observations made by other workers.

Wilcox (72) reported that natural peeling of trees in New York State was easiest when the mean temperature was above 40 degrees Fahrenheit. He also observed that the penetration of an arsenic solution into the sapwood was most rapid during the height of the sap-peeling season.

Czabator (21), working on the same experiment with Wilcox, observed that the best chemical treatment of hardwoods resulted when the chemical was applied between May 30 and July 1.

It is evident that although the average temperature must be above a certain point for successful chemical treatment, it is not the deciding factor. Observations made on the stems of untreated aspen show that the cambial region is most active between May 29 and July 1. On the basis of these findings and those summarized above for treated trees, it would appear that success of treatment coincides more closely with cambial activity rather than changes in temperature alone.

c. The effects of 2,4,5-T ester at different heights in the trunk.

By taking samples from different heights in the trunk, it was found that only the samples from the lower 10 feet and from the tip of the leader showed damage to any degree. The samples from the portion between 10 feet above the

ground and the top showed very little damage. This can be seen by comparing Figures 39, 41, and 42.

d. Miscellaneous data

Double strength 2,4,5-T solution. No difference could be detected in the degree of injury caused by single strength and double strength 2,4,5-T ester. Samples taken the same number of days after treating, showed approximately the same amount of damage. The appearance of the stem samples 71 days after treatment can be seen by comparing Figures 30 and 43.

Effects of chemicals on twigs and leaves. The leaves and twigs of treated trees showed very severe injury due to chemical treatment. In all cases extensive collapse and disintegration occurred, sometimes before damage to the trunk could be detected.

It is possible that the greatest amount of the chemical is transported up to the leaves but is not carried back down because of the death of the leaves and phloem tissue of the twigs immediately below the leaves. This assumption, coupled with the fact that the lower portion of the trunk is usually saturated with treating solution, may explain why the base of the trees and the tops are damaged more severely than the intervening portions.

Effect of chemical treatments on roots. An examination of root samples showed a distinct absence of extruded nucle-

oli and sieve plates in the phloem. It was also noted that the diameters of the sieve tubes were not as large as those of untreated samples. The phloem showed no other injury. There was not much activity in the cambial region, but it did not show any damage. Xylem did not appear to be damaged.

B. Conclusions

Effectiveness of chemicals as debarking agents. Sodium monochloroacetate applied in frills was the most effective chemical used in this study for the purpose of loosening the bark of aspen. However, good results can only be achieved by allowing treated trees to stand over winter; the bark of some of the upper sticks was tight even after standing for one year.

2,4,5-T ester, applied as a basal spray, was fairly effective as a debarking agent. Sticks from the lower portion of the trunks could be peeled after the trees had remained standing over winter, but not as easily as those of trees treated with sodium monochloroacetate. The occurrence of tight bark on the upper sticks was found to be more common on 2,4,5-T treated trees than on those treated with sodium monochloroacetate. Further studies on methods of application are needed to obtain the best results with this chemical.

2,4-D ester was not a satisfactory chemical for the chemical debarking of aspen. The results were too inconsistent to warrant further study.

Bark loosening due to chemical action. The results of this study suggest that bark loosening is caused by a combination of chemical and physical changes occurring in the cambial region and adjacent tissues of the tree stem. The collapse of newly-formed xylem cells and the accompanying lacunae, in conjunction with collapse and disintegration of cambial and phloem cells, evidently results in the formation of a belt of weakened material, consisting of remnants of cell walls and cell contents, which can be ruptured very easily. Collapse of cells seems to be partially caused by a contraction of the entire phloem region. This contraction is evidently restrained by the xylem, causing the long vertical splits to occur in the bark after treatment.

Effect of chemicals on roots. The chemicals appear to have little effect on the roots and apparently are not translocated from a treated tree to a healthy one. In spite of the changes reported in root samples, no evidence of damage to adjacent trees could be detected two years after treatment. In view of the fact that most aspen trees originate as suckers and are connected by an extensive lateral root system (22), it would seem that any damage to adjacent trees should have been apparent within this period of time.

Limitation of tree size. There apparently is a maximum tree stem diameter, beyond which the chemicals used in this study lose their effectiveness. In almost every group of treated aspen where one or two individuals remained healthy, they were trees of 10 inches or more in diameter at breast height. This was true even where additional solution had been applied to compensate for tree size.

Peeling characteristics of trees treated with chemicals. The bark of many trees treated with sodium monochloroacetate breaks up into small fragments when it is peeled from the tree. This apparently is caused by severe disintegration of the phloem. This region was found so completely disintegrated 71 days after treatment that in many places only isolated groups of fibers and sclereids were intact. This results in bark crumbling when it is pulled off the tree, allowing the long strands of intact fibers and sclereids to remain attached to the trunk.

The bark of trees treated with 2,4-D ester and 2,4,5-T ester was found to peel off the trees in large sheets. This condition is probably due to the fact that relatively little disintegration occurs in the phloem, while collapse is rather severe in the cambial region. This forms a plane of weakness between cambial region and phloem, and allows the bark to be peeled off intact.

Treatment dates. Treatments made before July 1 are faster acting and more effective than those made later in the season. Applications made just before bud-break produce results comparable to those made when leaves are fully developed. Date of treatment apparently has more influence on the results of 2,4,5-T and 2,4-D treatments than on those made with sodium monochloroacetate.

Applicability of treatments to commercial operations. Although trees treated with sodium monochloroacetate and 2,4,5-T ester could be peeled one year after treatment, it is felt that the amount of tight bark remaining on the upper sticks is too great to warrant application of these methods to commercial operations. The amount of discolored wood resulting from trees standing over winter is also a distinct drawback to the extensive use of these methods.

Further investigations, however, of application methods may eliminate these objections and result in treatments applicable to commercial use.

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