TREE INJECTION EXPERIMENTS IN THE STUDY OF BIRCH DIEBACK¹

By R. F. MORRIS²

INTRODUCTION

The injection of chemicals into trees has attracted interest in many countries, and Roach (11) has found that some of the earliest experiments date back as far as the twelfth century. The possibilities offered by injection methods have been tested in connection with various problems in tree physiology, silviculture, entomology and pathology. The injection of non-toxic dyes has been used frequently in physiological research to study the movement of water in the tree. Stains have also been used in living trees in attempts to make the wood resemble that of more valuable species, particularly for the manufacture of furniture; however, the elaborate injection methods required to secure even distribution of the stain in large trees have discouraged any extensive commercial application. The injection of poisons to kill the tree and, at the same time, to prevent attack by bark beetles, borers and decay organisms has been attended by varying degrees of success. Injection for this purpose has received a great deal of study in connection with barkbeetle control, as illustrated by the work of Craighead and his associates (6, 7). In silviculture the injection of poisons to kill undesirable tree species and to prevent sprout growth from the stumps has been shown to be both effective and inexpensive (8). The possibility of controlling cambium or xylem insects by the injection of insecticides that are non-toxic to the tree has been explored to some extent, but without any marked success to date. In pathological work, however, chemotherapy has been applied successfully by the injection method in the treatment of vascular pathogens and virus diseases of trees (15). Another recent development is the injection of chemicals to facilitate bark removal from trees to be used for pulpwood, and to extend the length of the peeling season (10). This treatment is now used on a commercial scale in some places. One of the most successful applications of injection methods to date has been in the diagnosis and treatment of nutrient deficiencies in fruit trees. The work of Roach (11, 12) and his associates at the East Malling Research Station presents an outstanding example of this technique. For diagnostic purposes, injection provides much better control than the application of chemicals or fertilizers to the ground by ordinary methods.

With the exception of this brief summary of the objects of tree injection, no review will be attempted of the rather extensive literature that has accumulated on the subject. Reviews and bibliographies covering most of the work up to the past decade have already been presented by several authors (4, 7, 11, 13).

The experiments described in the present paper constituted one phase of a study on a condition of birch known as 'dieback', and the principal object was

1. Contribution No. 16, Division of Forest Biology, Science Service, Department of Agriculture of Canada, Ottawa.
2. Dominion Entomological Laboratory, Fredericton, N.B.

to test the injection method as a tool for use in diagnosing the causes of the malady. This unhealthy condition of white birch, *Betula papyrifera* March., and yellow birch, *Betula Lutea* Michx. f., which was first noticed in New Brunswick in the 1930's, was characterized in its initial stages by small, chlorotic and often curled foliage in the top of the crown. These symptoms were followed by the death of the twigs and branches, which generally progressed from the top to the base of the crown. Dieback became very prevalent in the Maritime Provinces and birch in all types of stands was affected. The condition of birch in 1943, when the present experiments were initiated, was described by Balch, Morris, and Barter (1). Most of the merchantable birch in New Brunswick is now dead and in recent years the areas affected in other provinces have greatly increased (3).

The factor, or factors, primarily responsible for dieback are not known. The bronze birch borer, *Agilus anxius* Gory, a buprestid which mines in the cambium region, is almost invariably found in trees that are seriously affected. Trees with initial symptoms of dieback are sometimes found, however, without evidence of borer attack. This has led to the supposition that predisposing factors of a physiological, or perhaps pathological, nature are involved, but the role of such factors has not yet been demonstrated effectively.

In attempting to determine the factors responsible, it was considered that the injection of three types of chemicals might provide useful information: nutrient solutions to determine whether initial stages of dieback are associated with nutrient deficiencies; insecticides to kill the borer without killing the tree, and thus to determine whether dieback can progress to advanced stages in the absence of borer injury; tree-killing poisons to determine the effect on borer populations. These three aspects of the injection work will be discussed separately.

The experiments were conducted near Fredericton, New Brunswick, in 1943 and 1944. They constituted only a part-time project and were discontinued after 1944 because of the pressure of other projects and because dieback had reached such an advanced stage in this area that suitable trees showing initial symptoms were not available. The results are therefore preliminary and are presented mainly because of their possible use to investigators who may be contemplating similar diagnostic studies in areas where dieback is more recent.

TYPE OF TREES EMPLOYED

Experimentation with tree injection is complicated by many variables, some of which are difficult to control, or even to measure. Among them are the species, size, age, form and physiological condition of the tree, the season, the weather, the method of injection, the amount and concentration of the injected chemical, and the distribution of the chemical inside the tree. In order to minimize the variables presented by the tree itself, the experiments were largely restricted to one species, white birch, and to trees of uniform size. It was considered that once the appropriate methods and chemicals had been determined, it would not be difficult to work out the required quantities for trees of larger size. The use of fairly small trees for preliminary work also reduced

the time involved in sectioning and analysing treated trees, and thus permitted a larger number of experiments to be completed.

Most of the work was carried out in a stand of almost pure white birch in which the trees were between 40 and 50 years of age. Healthy trees were available for the experiments in 1943, as well as trees with initial symptoms of dieback. Some yellow birch trees of the same size were injected with nutrient solutions. They occurred in a mixedwood stand with white birch and with softwoods. In this stand the yellow birch showed various degrees of dieback and it was possible to find a few trees in which dieback was still in its early stages. Both stands originated after fire.

Co-dominant trees were selected for injection. It was found that the tolerance of the trees to injected chemicals depended largely on crown size, and for this reason an effort was made to select the trees on the basis of equal crown size, rather than equal diameter. The diameter at breast height varied from 3.0 to 5.5 inches, but for most of the selected trees it was near to 4.0 inches. The mean height of the selected tree was 33 feet, the lower 22 feet being bare stem and the top 11 feet representing the crown height. Crown width, which was measured as the mean rather than the extreme width, averaged 4.2 feet.

All trees selected for injection or for control purposes were numbered and described. Height and crown size were estimated by standing a long, graduated pole against the tree. On trees that were subsequently felled for examination, these estimations were checked by actual measurement. The condition of the crown, with particular reference to symptoms of dieback, was described in detail and, when necessary, sketches were made on cross-section paper to show the location and extent of crown injury. Separate records were kept for each tree and included the original description, the injection particulars, the records of subsequent examinations, the cross-sectional drawings of trees that were felled to trace the path of dye movements, and the condition of borer larvae in trees that were injected with insecticides. Square-lined note paper was used for this purpose so that any required drawings could be made to scale in the field. This system proved efficacious for both field and office use. In analysing the data, the tree record sheets could first be grouped according to the method of injection for analysing the distributional diagrams, and then according to the chemical used for determining tolerance limits and effect upon the trees.

In the course of the experiments, 266 trees were selected and described. Of this number, 179 trees were injected with various chemicals and the others selected for purposes of control, were either not treated or were injected with water only.

METHODS OF INJECTION

Many methods for introducing chemicals into trees have been proposed and, in some cases, patented, by earlier investigators. The method that should be used depends very largely upon the purpose of the injections. In nutrition studies and chemotherapy it has been found that the injection of an aqueous

solution by means of one or more shallow holes bored in the trunk is usually satisfactory. In dark beetle control, where it is intended not to save the tree but to secure distribution of the chemical in the peripheral part of the stem, the tree is generally girdled and the chemical applied in a metal or rubber trough encircling the tree at the girdle. The poisoning of trees for silvicultural purposes alone has been accomplished very simply by one or more auger holes, in which a paper cartridge containing the solid chemical is introduced, or by the use of a special instrument which makes an incision and injects a concen-

FIGURE 1
Bottle and siphon in position for the injection of white birch, and cross-section of stem showing size of injection hole. Tree is about 4 inches in diameter.

trated solution at the same time. In brief, the introduction of a chemical, such as a nutrient or tree poison, that is intended for the foliage can be accomplished by very simple methods; the impregnation of the peripheral part of the stem or of the entire xylem requires more time-consuming methods and often involves girdling.

There is considerable controversy in the literature as to the necessity for making the injection holes under water in order to exclude air from the vessels

of the xylem. After careful tests on fruit trees, Roach (11) concluded: "The attempt to exclude air from the injection hole complicates the actual operation so greatly that for its justification the rate of injection or the thoroughness of permeation would have to be at least doubled . . . comparative tests were carried out sufficiently carefully to reveal differences of this magnitude if they existed, but in none was a definite advantage secured by excluding air." In the present experiments with birch trees, no under-water injections were used because no difficulty was experienced in securing fairly rapid introduction of the solutions by ordinary methods. In all instances, however, the equipment was prepared in advance so that only a few seconds elapsed between the boring of the hole and the introduction of the solution. It has been shown that the same holes cannot be used for repeated injections (5).

The equipment used for the injections of birch was very simple and consisted mainly of a supply of siphons and of a 1- and 2-litre bottle (Fig. 1).. Rubber corks were found satisfactory for connecting the siphons to the injection holes and leaking did not occur so long as the bit was kept sharp so that the edges of the hole were smooth. A $\frac{1}{2}$ -inch bit was used and the holes were drilled about 2 feet above the ground. When the injection was completed, the holes were plugged with either grafting wax or mastic compound.

Rumboldt (13) has studied the rate of intake in relation to meteorological factors and to the concentration of the solution. Roach (11) concluded that soil moisture is a major factor influencing the rate of intake. In the present work no detailed study was made of such factors, but general observations support the conclusion that available moisture in the soil is important. In periods of dry weather, the 1-litre bottles were often empty in 3 hours, but after heavy or prolonged rainfall it sometimes required 24 hours; under the former conditions the existence of a negative pressure in the trunk was often indicated by the initiation of siphonic action of its own accord when the siphons were attached to the tree.

Different numbers and arrangements of injection holes were tested, particularly for the purpose of determining whether it would be possible to secure distribution in the region occupied by the borer without girdling or similar techniques fatal to the tree. For convenient reference, these methods will be represented by the following abbreviations:

1 R.H.—One hole, bored radially into the stem to a depth of about 1 inch (Fig. 1, 2).

2 R.H.—Two such holes, on opposite sides of the stem (Fig. 2).

1 D.H.—One hole, bored diametrically through the stem; in some cases one end of the hole was plugged with a rubber cork and in others the bit was stopped just before it broke through the bark (Fig. 2).

2 D.H.—Two such holes, at right angles to each other.

1 T.H.—One hole bored tangentially through the outer part of the xylem; usually the bit was stopped before it broke through the bark.

2 T.H.—Two such holes, on opposite sides of the stem (Fig. 2).

In addition to these methods, a few experiments were tried with two basically different methods that, to the writer's knowledge, had not previously been tried. In one of these, which will be called the root method, a shallow

root with a diameter of $\frac{1}{2}$ -inch was exposed about 3 or 4 feet from the base of the tree. The bottle of solution was placed on the ground near the root, the root was severed with a sharp knife, and the rubber cork at the end of the siphon was quickly coupled to the root by a piece of rubber tubing. It was considered that this method, if successful, might prove less injurious to the tree than holes bored in the stem. The other method, which will be called the bark method, was also an attempt to make the injection without severance of the xylem vessels. The bottle and siphon were hung on the stem, as in the ordinary injection, but the hole was made with a cork-borer and extended only through the cortex and the phloem to the surface of the xylem. The cork at the end of the siphon was coupled to a short length of rubber tubing, the other end of which was placed flat on the bark around the outside of the hole and made fast by a liberal application of grafting wax.

The work was started in June, after the leaves of birch were fully expanded, and, with the exception of the injection of insecticides in the month of August, the great majority of the experiments were carried out in June and July.

DISTRIBUTION OF INJECTED CHEMICALS

Patent blue and acid fuchsin were found to be satisfactory for studying distribution in the stem and in the crown, patent blue being effective in slightly smaller quantities. Two grams of either one, injected as a 0.1 or 0.2 per cent solution, were sufficient to colour conspicuously the whole crown. Both are non-toxic to birch; a few weeks after injection the colour gradually faded from the leaves and in the following season the trees appeared completely normal. Certain chemicals injected for other purposes, particularly nicotine alkaloid and copper sulphate, were also found to stain the xylem and hence to provide additional data on distribution. With the exception of potassium cyanide, which will be discussed later, these chemicals were found to be distributed in the same manner as the stains. Nutrients or other chemicals, when injected in quantities greater than the tree will tolerate, cause visible injury to the leaves and thus provide further information on distribution in the crown. Some investigators have used chemical analysis to determine quantitatively the deposition of the injected chemical in various parts of the tree. In the present work only visual evidence was used and the possibility exists, therefore, that quantities of stain or of other chemicals too small to cause visible staining were present in other parts of the tree.

Data on distribution in the stem were obtained for 34 white birch, which were injected principally with patent blue, acid fuchsin, or nicotine alkaloid, and subsequently felled and sectioned at 5-foot intervals. Some typical patterns obtained by different methods of injection are shown in Figure 2. In these drawings the cross-sections from each tree have been oriented, insofar as possible, to represent conditions in a straight-grained tree in which the xylem vessels are vertical. Actually, most of the injected trees appeared to have a spiral grain, which was invariably clockwise, and spirals of as

much as 45 degrees in 5 feet of height were not uncommon. Most of the trees used in these experiments had no visible heartwood.

From a comparison of the cross-sectional drawings for the 34 trees, the following conclusions are derived:

1. Tangential distribution in the xylem of white birch is rapid; by the 1 R.H. method, encirclement inside the xylem was nearly always completed at a point 10 feet above the injection hole, and by the 2 R.H. or 1 D.H. methods it was often completed at 5 feet. It would naturally be assumed, and this was supported by observations on the crown, that once

FIGURE 2
Some typical distributional patterns (shaded areas) made by chemicals injected into the xylem of white birch, showing the injection point and successive 5-foot intervals. The bark is not shown.

xylem encirclement is completed the chemical will reach all sides of the crown. For the type of trees used in the present work, therefore, the 1 R.H. method was satisfactory for the injection of nutrient solutions and other chemicals intended for the foliage.

2. Distribution of the injected chemical in the peripheral xylem rings, adjacent to the cambium, was poor. Horizontal diffusion generally occurred in a tangential and inward direction, so that encirclement of the xylem was most often completed at a point about midway between the cambium and the centre of the tree. In the crown of the tree, there was a tendency for the outer rings to become stained again and smaller branches sometimes had the complete xylem stained. This tendency is reflected in the following figures, which show what percentage of the surface of the xylem was stained at each level by the 2 T.H. method: injection holes, 60; 5 feet, 40; 10 feet, 35; 15 feet, 25; 20 feet, 40; 25 feet, 50. These figures are the means for 15 trees. Variability from tree to tree was very high with regard to both the percentages and the patterns of the areas stained. Distribution in the periphery by the R.H. or D.H. methods would appear to be useless for the injection of insecticides to kill borers in the cambium region of the stem, and it seems doubtful whether even the 2 T.H. method would be highly effective.

The distribution of potassium cyanide was very different from that of all the other chemicals tested. It ascends the tree in the cambium region, rapidly disappearing from all but the periphery of the xylem, and often stains the cortex. This unusual behaviour has been observed before and has led some investigators to believe that it ascends in gaseous form. It would appear to offer possibilities for the control of cambium insects, at least in its method of distribution.

The root injection method did not prove feasible. Even in the driest weather, only a few cubic centimetres of solution could be induced to enter a birch tree through cut roots, and this reached a maximum height of 15 feet in the stem. In no instances did chemicals injected in the roots reach the crown. The path of conduction in the stem was again about midway between the cambium and the pith. The bark method was also unsuccessful and the small amount of solution which entered by this method was deposited in a narrow strip extending about 1 foot above and 1 foot below the injection point. This tends to support the results of the experiments described above, showing that conduction at the surface of the xylem is very poor. Severance of some of the xylem vessels of the stem appears to be necessary for satisfactory injection of chemicals into birch trees. Neither the root nor the bark method, however, was tested for the injection of cyanide. Nor was it determined whether the use of under-water techniques to exclude air from the holes would have changed the pattern of conduction or given better distribution in the outer xylem.

Dyes sometimes appeared in the leaves in less than one day after injection and the entire crown was usually coloured in one to three days. When overdoses of other chemicals were injected, the first signs of injury to the leaves were usually apparent in one to three days, but it was often two weeks or longer before the injury reached its maximum. As to vertical distribution in the crown, stained or injured foliage nearly always appeared first at the base and progressed towards the top. When insufficient dye or only slight overdoses of chemicals were used, the effects were usually confined to the

base of the crown. This suggests that nutrient or water deficiencies would first affect the top of the crown, with results perhaps similar to those of dieback. Distribution in the lateral plane of the crown was nearly always complete, even when only the base of the crown was affected.

NUTRIENT SOLUTIONS

Experiments to test the injection method for use in the detection of possible nutrient deficiencies were carried out on 75 trees, excluding the control trees. All injections were made in the stem, generally by the 1 R.H. method, and in this preliminary work only salts containing the three principal growth elements, nitrogen, potassium, and phosphorus, were tested. The injections were all made in June or July of 1943 but observations on the treated trees and on the controls were continued to the fall of 1944. The following salts were injected:

Sodium nitrate, NaNO_3

Potassium nitrate, KNO_3

Urea, $\text{CO}(\text{NH}_2)_2$

Potassium chloride, KCl

Potassium dihydrogen phosphate, KH_2PO_4

In the injection of any chemical that is not designed to kill the tree, the first step is to determine what quantity and concentration of the chemical will be tolerated without foliage or cambium injury. Leaf injury is the factor that generally limits the dosage, and the three types of leaf injury described by Roach (11) on fruit trees were observed on birch. Small overdoses of any chemical usually resulted in the marginal type of leaf injury, the dead areas along the leaf margins subsequently falling off and leaving the leaf irregular in outline, but not killing it. The interveinal type of injury was the most common type on birch and resulted from somewhat greater overdoses than those causing marginal injury. If interveinal injury was at all severe the whole leaf dropped, often before it was completely brown. The veinal type of injury was caused only by very severe overdoses which often killed the small twigs as well as the leaves. When leaf injury was severe some of the branches died; others put out a new crop of leaves in the same summer or in the following spring. Hence, the permanent damage suffered by the tree cannot be accurately determined until the year after injection, particularly when the injury occurs rather late in the summer with the result that new foliage does not appear until the next year.

To determine the maximum toleration limits for the above salts, 45 healthy trees were selected. They were mostly white birch, but enough yellow birch were used to show that the two species are about equal in their tolerance to injected nutrient salts. In the first experiments, the weight of salt was varied from 3 to 10 grams per tree, in solution concentrations of 0.25 and 0.50 per cent. No injury resulted, suggesting that birch would tolerate greater quantities and concentrations than those recommended for fruit trees. The amounts were then varied between 20 and 110 grams per tree and the concentration between 2 and 8 per cent. These experiments on toleration were sufficiently extensive to permit the following conclusions concerning the salts that were tested:

1. Crown injury is related to the weight of the nutrient salt injected rather than to its concentration; increasing the concentration of the solution from 2 per cent to 8 per cent does not increase the leaf injury resulting from a given weight of salt. This conclusion is at variance with the results reported by horticulturists, who found that concentration is very important. Roach (11), for instance, recommends concentrations of 0.25 to 0.50 per cent. This can be explained by the fact that in apple trees there are often only a few feet of bare stem or branch between the injection point and the leaves; in the injected birch trees there are generally over 20 feet, so that the solution was probably greatly diluted in the stem before reaching the leaves.

2. The concentration of solution is limited, however, by the undesirable effects of high concentrations on the cambium. When the concentration of the injected salts was over 4 per cent, a narrow strip of cambium was often killed to a height of 3 or 4 feet above the injection point.

3. Tolerance is related to the weight of the salt employed, not to the relative content of the growth element. For example, birch trees are about equally tolerant to sodium nitrate and to urea and therefore, because of the greater nitrogen content of urea, it permits the introduction of more nitrogen than does sodium or potassium nitrate.

4. When two salts, such as urea and potassium dihydrogen phosphate, are mixed in order to provide all three growth elements to the same tree, tolerance depends upon the weight of the mixture and the tree is about equally tolerant to a given weight of mixture as it is to the same weight of one of the component salts. Hence, the amount of any one element that can be introduced in such mixtures is less than can be introduced by using a single salt.

5. The maximum dosage tolerated without foliage injury is extremely variable and cannot be predetermined with accuracy. In dry weather, when the solution is taken up rapidly, the tree is more susceptible to injury than it is under conditions of adequate soil moisture. Variability is also high from tree to tree, even when the trees are selected on the basis of equal crown size. For example, 5 trees, each injected on the same date with 108 grams of an equal mixture of urea and potassium dihydrogen phosphate, suffered respectively 85, 5, 30, 0, and 75 per cent crown injury.

6. Under ordinary weather conditions, the type of birch trees used in the present work will tolerate without foliage injury, injections of 40 grams of any one of the nutrient salts listed above, or a 40 gram mixture of urea and potassium dihydrogen phosphate.

After the tolerance limits were determined, 20 white birch and 10 yellow birch were selected for injection and carefully described, along with an equal number of controls of each species. All these trees showed early stages of dieback in the tops of the crowns, generally represented by dwarfed, chlorotic foliage, and, in some cases, a few dead twigs. Some of these trees were treated with urea, some with potassium dihydrogen phosphate, some with an equal mixture of the two, and a few with sodium nitrate. After injection in July, 1943, periodic observations were made on the injected

trees and on the controls during the remainder of the 1943 season and during 1944. In the fall of 1944, increment cores were taken from two sides of each tree at breast height, and also from the healthy trees used in the study of tolerance. With the aid of a microscope, the annual growth rings were measured for 1943 and 1944, and for the five years preceding 1943.

The maximum tolerated amounts of salts containing the three common growth elements failed to bring about any improvement in the appearance of trees with weak foliage or with other early symptoms of dieback, either in the year of injection or in the following year. In most instances the condition of the tree was the same or somewhat worse in the year after injection, as was also true of the control trees. Similarly, no significant improvement in radial growth was found in either the healthy or decadent trees during the year of injection or the following year, as compared with the growth of the control trees.

Although the number of decadent trees involved in the experiment was small, the indicated conclusion is that deficiencies of nitrogen, potassium, or phosphorus, are not initially responsible for dieback, or else that the maximum quantities of these elements that can safely be introduced at one time are insufficient to have any apparent effect on the foliage or on radial growth. To test the latter possibility, it is possible that fertilization by means of crowbar holes in the ground would be necessary. On the other hand, Roach (12) has more recently devised a very simple method for making interveinal leaf injections for diagnostic purposes. If this method were found applicable to birch, it would provide a rapid means for checking the present conclusions and for extending the experiments to include the many "trace" elements that are important to tree vigour.

INSECTICIDES

It has been shown above that satisfactory distribution of injected chemicals was not obtained in the cambium region, even when the 2 T.H. method was used. Some injections were made by this method, however, to determine the tolerance of birch to a few of the common insecticides and to see whether tolerated quantities would be sufficient to kill borer larvae at points in the stem where the chemical contacted the cambium. The insecticides used were "Nico Fume" (40 per cent nicotine alkaloid), "Black Leaf 40" (40 per cent nicotine sulphate), sodium selenate, and potassium cyanide. Sodium selenate was tried because of its use as a systemic insecticide in greenhouse work, where it is sometimes mixed with the soil in which the plants are grown.

Tolerance tests were first made on 23 healthy white birch. These trees proved to be surprisingly tolerant to nicotine, especially in the form of the alkaloid. The quantity of "Nico Fume" per tree was varied from 10 cc. to 200 cc. caused slight foliage injury but 180 cc. could be injected with safety. Observations on the trees in 1944 and increment cores taken in the fall of 1944 showed that no effects on appearance or growth occurred in the year following injection. Nicotine sulphate was more toxic, and only 100 cc.

of "Black Leaf 40" could be injected with safety. Both of these insecticides were diluted with water to make a 4 per cent solution, since higher concentrations were injurious to the cambium. Sodium selenate was extremely toxic to birch; injections of 1 gram or more killed the trees very quickly, and even 0.1 gram caused crown injury up to 30 per cent. The highest concentration tested was a 1 per cent aqueous solution and this did not injure the cambium, directly even at the injection point. Only four trees were injected with potassium cyanide; the smallest amount used, 4.5 grams in a 0.3 per cent solution, killed the cambium on all sides of the tree from the injection point to the top of the crown.

In order to determine the effect of injected insecticides on larvae of the bronze birch borer, it was desired to have a supply of trees that were attacked throughout the length of the stem, so that the effect of possible dilution of the chemical inside the tree could be determined at different heights. Work in progress on the borer by G. W. Barter showed that girdled trees are subject to this type of stem attack. White birch trees were therefore girdled close to the ground in the early spring of 1943, the girdle extending about $\frac{1}{2}$ inch into the xylem. Of these trees, 23 were injected with nicotine alkaloid, 6 with nicotine sulphate, and 10 with sodium selenate. An equal number of girdled control trees was available. The injections were made in August, after most of the borer larvae had emerged from the eggs and started to feed in the cambium region.

In October, 1943, half of the controls and half of the treated trees were felled and the stems were cut into 5-foot bolts. The bark was peeled from each bolt, the borer larvae were counted and recorded as living or dead, and the lengths of the larval galleries were measured. Unfortunately for the purposes of the experiment, it was found that practically 100 per cent of the larvae were dead at all levels in the stem both in the injected trees and in the controls. During the first summer after girdling, it appears that the birch trees used in this study were still too vigorous to permit successful development of the larvae. Most of the larvae had not developed beyond the first instar in these trees and the galleries varied from less than 1 inch to a maximum of 4 inches in length. Gallery length was very variable and there was no significant difference between treated trees and controls. The girdled trees had a completely normal appearance during 1943, but in the fall the leaves were observed to turn colour and drop a little earlier than those on ungirdled trees; this has also been observed by Baldwin (2).

Defoliating insects were uncommon on the birch on these plots in 1943 so there was no opportunity to observe the effects of injected nicotine on insects feeding on the leaves.

The rest of the trees were left standing until the fall of 1944 to determine whether conditions would be more suitable for larval development during the second season after girdling. They were then felled and peeled. In 1944 many of the trees remained quite normal in appearance and some put out a crop of foliage again in 1945; in others the appearance of the crown deteriorated during 1944 and the trees were dead by the end of the season. The mortality of the borer larvae that attacked these trees in

1944 was extremely variable, depending upon the condition of the individual tree, and ranged from 0 to nearly 100 per cent. There was no significant difference in mortality between the injected trees and the controls, showing that the insecticide probably had no effect during the year after injection. This was anticipated because it seemed doubtful whether enough insecticide would remain in the wood to be effective during the second season; moreover, the addition of the new growth ring would remove the site of feeding away from the original outer ring.

To summarize the results of these experiments, it is clear that there are several difficulties to be overcome before insecticides can be injected with success to control the bronze birch borer and thus to permit the study of the progress of dieback in the absence of borer injury. In the first place, it is very difficult, or perhaps impossible, to secure satisfactory distribution of the chemical in the peripheral region without using girdling or similar injection methods fatal to the tree. It also seems probable that injections would have to be repeated at least once a year; since each injection requires new boring holes, the effect on the strength of the stem would become rather serious. A technique for determining the effect of the chemical on the insect at different heights in the stem is also required; the present experiments have shown that the use of girdling to induce attack is not satisfactory. Another unknown factor is the possible effect of insecticides on any other organism that may be associated with dieback.

Because of its unique method of distribution, potassium cyanide is perhaps worthy of further tests in more dilute concentrations than those tested in these experiments. Possibilities are also offered by certain fluorine and phosphorus compounds that have recently been synthesized and found very effective as systematic insecticides, suitable for internal therapy in plants (9). On the other hand, DDT, which was not generally available when the present work was conducted, has recently been tested externally on birch for preventing attack by the bronze birch borer (14). As it was found possible to prevent attack by the use of two external applications per season, this method offers much better possibilities for immediate diagnostic work than does tree injection.

TREE POISONS

The injection of poisons to kill trees was not a major object of the present work, since practical methods for poisoning trees in silvicultural improvement work have already been devised. It is believed that poisoning could be used to advantage in birch stands, where the practice of logging by economic selection methods leaves many defective and decadent trees that occupy growing space and encourage borer populations. In the course of the present experiments, a number of trees were killed by the injection of overdoses of nutrients or insecticides. The examination of these trees provided some information on the attack and survival of the birch borer in trees killed by chemicals, and it is with this aspect of tree poisoning that the present section is concerned.

To augment the data provided by these trees, 12 trees were injected

with lethal quantities of copper sulphate. It was found that 50 grams of copper sulphate, injected as a 5 per cent aqueous solution, was sufficient to kill all the foliage in a few days. However, powdered copper sulphate when packed dry into holes in the xylem proved ineffective. As much as 300 grams per tree were injected without injury to the foliage, and subsequent sectioning of the stem showed that the chemical had ascended to a height of only 15 feet above the injection holes. The distribution obtained by the solid injections was similar to that of the liquid injections in that the chemical tended to leave the circumferential rings and form a pattern about half-way between the periphery and the pith. As a matter of interest, places were found in these trees where larvae of the birch borer had tunnelled through wood stained by copper sulphate without apparent harm to the larvae. In trees killed by potassium cyanide, narrow strips of living cambium were also found in which larvae had completed development and avoided contact with cambium that had been killed by the cyanide by making a zig-zag gallery pattern within the living strip.

Seventeen trees that were injected in 1943 with overdoses of copper sulphate or of nutrients and insecticides were felled and peeled in the fall of 1944, and the following conclusions formed:

1. When the foliage was killed rapidly, i.e., within a week or so after injection, the cambium died during the same summer. *Agrilus* attack was usually absent, especially if the trees were injected in late May or early June. If the trees were injected late in June the cambium did not die until late in the summer and there was usually some borer attack, the larvae being killed by the dying of the cambium in late summer. In trees injected in late July or in August, the cambium did not die until early the following year and there was no borer attack either season.

2. When the foliage was killed more slowly, becoming completely brown only after 6 or 8 weeks, the cambium in the upper part of the stem died in the fall of the same year; that in the lower part the following spring or summer. Such trees were usually attacked quite heavily, the larvae in the upper stem being killed by the dying of the cambium; those in the lower stem sometimes maturing and emerging the following spring. The attack was usually more severe in the upper part of the stem, however, and many more larvae were trapped than were able to mature.

3. When the foliage was only partly killed and the crown did not die entirely until the second summer, the cambium did not die until the second fall or the third spring. There was generally heavy attack during the second season and most of the larvae were able to mature and to emerge the third season.

It is clear from these observations that by a proper application and timing of tree poisons, it is possible either to prevent borer attack in the poisoned trees or to poison in such a way that large number of borer larvae are trapped and destroyed. The injected chemical does not act as a repellent or an insecticide, but the desired results are obtained as a result of the close relationship that exists between the attack and survival of *Agrilus* and the condition of the tree. The rapid tangential distribution of chemicals injected

into birch makes it possible to poison the entire crown rapidly by very simple methods. It is recommended that poisons more toxic than copper sulphate be used, however, and particularly poisons that can be introduced successfully in the dry state, or in mixture with calcium chloride, by the use of small paper cartridges.

SUMMARY

1. Experiments were carried out in New Brunswick in 1943 and 1944 to determine whether tree injection techniques would be useful for diagnostic purposes in determining the causes of birch dieback. Injection techniques were tested for three purposes: (1) The injection of nutrient solutions to determine whether initial symptoms of dieback are associated with nutrient deficiencies. (2) The injection of insecticides to kill larvae of *Agrilus anxius* Gory without killing the tree, and thus to determine whether dieback can progress to advanced stages in the absence of borer damage. (3) The injection of tree poisons to determine the survival of borer populations in killed trees.

2. The tolerance of birch to injected chemicals is a function of crown size. To minimize this variable, the trees were selected on the basis of equal crown size and had a crown height of approximately 11 feet, mean crown width of 4.2 feet, diameter of 4 inches, and height of 33 feet. Of the 266 trees selected and described, 179 were injected and the others served as control trees.

3. As an injected chemical was transported up through the xylem, lateral distribution was tangentially inwards. Injection through one shallow radial hole ensured encirclement inside the xylem within a height of 10 feet and consequent distribution throughout the crown, and was therefore satisfactory for injections of nutrients or tree poisons. However, because the tangential distribution was also inwards, the use of two tangential holes in the outer part of the xylem failed to provide for adequate distribution of insecticides in the cambium region where *Agrilus* larvae are usually found. It was found that solutions could not be injected through the roots, or through holes that failed to penetrate the xylem of the stem.

4. The tolerance of white and yellow birch to nutrient salts was related to the weight of salt injected; the concentration of the solution did not affect the leaf injury resulting from over-doses, but concentrations over 4 per cent often killed a strip of cambium above the injection hole. The maximum tolerated quantity of a salt was very variable, being influenced both by weather and by the condition of the individual tree. About 40 grams of a salt containing nitrogen, potassium, or phosphorus, was the maximum that could be injected with safety in dry weather. Trees showing initial symptoms of dieback were injected with the maximum tolerated quantity of these salts but no improvement in foliage colour or in radial growth could be detected during the year of injection or the following year. It is concluded either that these amounts are too small to affect the crown or that deficiency in the three common growth elements is not a factor in dieback. It is suggested that methods of interveinal leaf injection would provide a more

rapid and satisfactory method for testing these conclusions and for extending the study to include a more complete list of elements.

5. Birch was found to be rather tolerant to nicotine but very intolerant to cyanide. With the exception of cyanide, which has an unusual method of distribution in the stem, difficulties in technique remain to be overcome in securing satisfactory distribution of insecticides in the peripheral region where *Agrilus* larvae are found. Satisfactory techniques for determining the effect of the insecticide on the border at different levels in the stem are also wanting; girdling induces stem attack but natural mortality is very high. Although new systematic insecticides offer possibilities, it is suggested that external applications of DDT are likely to present a more practical method for preventing borer attack in diagnostic work.

6. A close relationship exists between the survival of borer larvae and the condition of the tree. As a result of this relationship it is possible to poison trees in silvicultural stand improvement in such a way that borer attack and multiplication are prevented, or to make the poisoned trees serve as trap trees.

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