ROOT-INJECTION FIELD TRIALS OF MBC-PHOSPHATE IN 1972 FOR DUTCH ELM DISEASE CONTROL

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We are deeply indebted to the National Capital Commission, Ottawa, for their generosity in making available to us student assistants, mature elm trees, heavy equipment to sample the treated elms and a field laboratory and office. We are especially grateful to N. McLaren and J. White of the NCC for their encouragement, assistance and continued interest in the project.

We also thank the many student assistants whose interest, enthusiasm and hard work made possible the production and testing of MBC-P for root injection, the field operations and the bioassay of treated elms.

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Cover photograph shows an overall setup for root injection of a diseased elm 68.0 cm (26.8 in.) dbh, through seven severed roots, employing three root-injection apparatuses.

INTRODUCTION

Recently, research in Dutch elm disease (DED) control has focused mainly on solubilization of benzimidazole fungicides (McWain and Gregory 1971, Buchenauer and Erwin 1971, Kondo *et al.* 1973) and pressure injection directly into the trunk (Gregory *et al.* 1971) or roots of elms (Kondo 1972 and Kondo *et al.* 1973) in an effort to obtain uniform distribution of the chemical compound throughout the tree. McWain and Gregory (1973) reported that benomyl solubilized with hydrochloric acid resulted in the formation of the water-soluble derivative MBC-HCl and that in field trials for control of DED and oak wilt it showed effective disease prevention and promising results for disease therapy. Methyl-2-benzimidazole carbamate hydrochloride (MBC.HCl) was one of five salts of MBC (MBC-phosphate, -nitrate, -sulphate, -hydrochloride, and -sodio derivative) (patent pending) assessed for their potential in DED control in 1971 and reported by Kondo *et al.* (1973). It was concluded on the basis of fungitoxicity and phytotoxicity studies that all five MBC salts showed potential in DED control and had the necessary characteristics for successful pressure injection. However, because of the difficulty in synthesizing an adequate supply of these salts, only MBC-P was selected for the 1971 preliminary root-injection tests (Kondo *et al.* 1973). Extremely good distribution of MBC-P throughout the elms (12 healthy, mature, field-grown elms and one elm with severe symptoms of DED) was obtained. The concentration of the chemical within the elm was shown in laboratory tests to be sufficient to arrest development of *Ceratocystis ulmi* (Buism.) C. Moreau. Healthy treated elms could not be successfully inoculated with *C. ulmi* conidia whereas control trees could. Also, recovery of *C. ulmi* from the diseased elm was greatly reduced after treatment. These results were sufficiently encouraging to warrant a larger field trial involving mainly diseased elms in 1972.

The objectives of this field trial were as follows:

(a) to determine the therapeutic and/or fungicidal value of MBC-P, root injected into naturally infected mature elms showing varying degrees of disease development under field conditions.

(b) to determine the uptake, distribution and persistence of the chemical in large diseased elms under varying field and weather conditions, throughout the growing season.

(c) to root inject infected elms of varying sizes and in some cases to inject only the diseased portions of very large elms.

(d) to obtain information for improvement of the injection apparatus and techniques for efficient operational use.

(e) to collect data on the breakdown of time and costs for the entire operation.

The above information would be of great value in the planning of future root injections in both research and commercial operations.

MATERIALS AND METHODS

Methyl-2-benzimidazole carbamate-phosphate (MBC-P) required in these field trials was prepared as indicated in Figure 1.

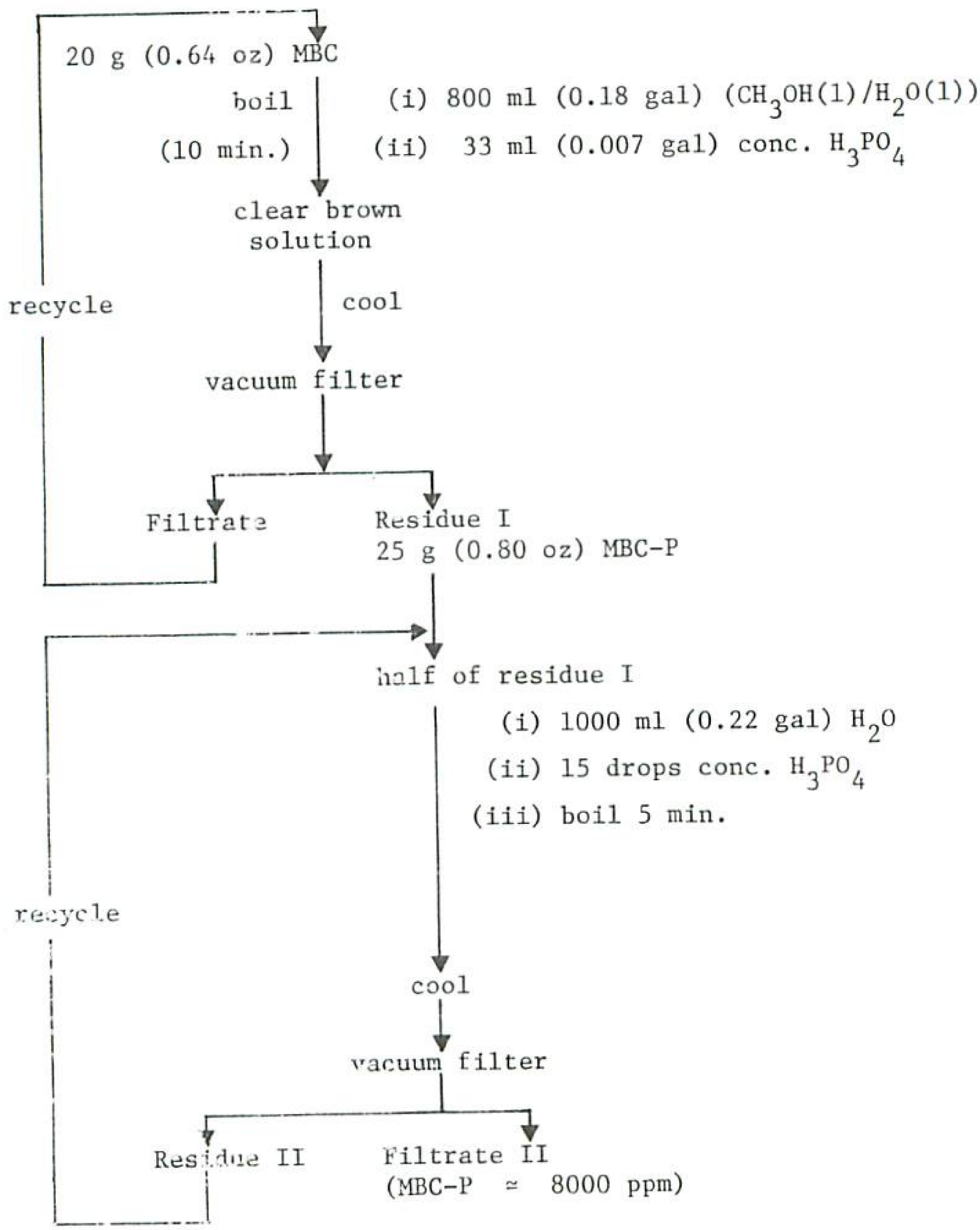

Figure 1. Production of MBC-phosphate concentrate (8000 ppm).

Stock solutions of MBC-P at a concentration of approximately 8000 ppm were diluted to the required concentration of 250 ppm or 750 ppm with distilled water for root injection.

The 80 diseased elms selected for this field trial were located on National Capital Commission property in Ottawa. The majority of the elms treated were high-value, street-grown trees, situated along Island Park Drive, Ottawa River Parkway, Experimental Farm Drive, Queen Elizabeth Driveway, Clemow Avenue and Colonel By Drive. Other locations of treated elms included Hampton Park, Vincent Massey Park, Confederation Heights and the National Arts Centre.

Ten elms known to be infected in 1971, and verified by isolation of *C. ulmi* from twig samples, were root injected after budbreak and prior to full leaf expansion. A continuous search for elms exhibiting DED symptoms was made from mid-June to mid-September. The presence of *C. ulmi* was verified and the trees were immediately root injected with MBC-P solution. Elms were root injected from a minimum of 5 hours to a maximum of 69 hours. In most cases the period of root injection was 48 hours.

An attempt was made to root inject at least one root per major root flare around the base of the elm. For a few of the larger elms (55.5-118.7 cm, or 21.8-46.7 in. dbh) exhibiting early and localized DED symptoms, an attempt was made to treat only the infected portion of the crown. The orientation of the fissures in the outer bark surface of the elm was taken as a rough indication of which roots should be injected to obtain the required coverage of the infected portion of the crown.

Thirty-seven elms treated with MBC-P and 10 untreated, diseased elms (controls) were sampled and bioassayed for presence of the fungitoxicant. The first samples were taken May 25 and the last on November 4, 1972. Of the 37 treated elms sampled, 17 were sampled twice and two were sampled three times during 1972. On the basis of the 1971 preliminary test it was decided that it was unnecessary to sample all the elms prior to root injection to confirm that they were free from compounds which could affect the results of the bioassay.

In order to monitor the concentration and distribution of MBC-P, 0.9- to 1.2-m (3- to 4-ft) branch samples were collected randomly from designated sample stations throughout the crown. This work was done from an aerial bucket truck. Each sample station consisted of a major branch from the main trunk of the elm. The pruning shears were sterilized between samples to prevent contamination. The branch samples were cut into several 15.24- to 20.32-cm (6- to 8-in.) sections, placed in plastic bags along with leaves, flowers and fruit if present, and shipped in styrofoam-lined boxes packed with dry ice to the Great Lakes Forest Research Centre in Sault Ste. Marie for bioassay.

Bioassays of tree parts for presence of the fungitoxicant were carried out with conidia of *Penicillium expansum* Link. and *C. ulmi*. Tissue samples for bioassay, consisting of leaf disks cut with a paper punch, 1-cm (0.39-in.)- long twig sections split longitudinally and leaf and flower buds split longitudinally were randomly obtained from each twig sample. All tissue samples were placed on the surface of the seeded potato-dextrose agar petri plates, incubated 12-48 hours at $22^{o}C$ in the dark and the inhibition zones measured. Controls consisted of known amounts of MBC-P on sterile filter paper disks.

During the entire field trials, records were maintained of the number of man-hours required to excavate suitable roots for injection, and to set up, monitor, refill and finally dismantle the injection apparatus.

In general, information pertinent to future designs of root-injection operations was collected together with information pertinent to research.

RESULTS AND DISCUSSION

The concentration of the stock solution of MBC-P produced according to the procedure in Figure 1 ranged from 8024 to 8857 ppm with a pH range of 2.10-2.15. The pH at 250 and 750 ppm was 3.1 and 2.7, respectively.

The results shown in Table 1 indicate that *P. expansum* may be employed as the bioassay organism as long as checks are maintained periodically with the samples bioassayed against *C. ulmi*. The use of *P. expansum*-seeded bioassay plates has several advantages over *C. ulmi* plates. Large quantities of *P. expansum* bioassay plates can be prepared in half the time required to prepare *C. ulmi* bioassay plates. Also, *P. expansum* bioassay plates can be easily "read" for inhibition approximately 21-24 hours after incubation as opposed to 48 hours for *C. ulmi* plates.

Table 1 Fungitoxicity relationship of MBC-P solutions between *P. expansum* and *C. ulmi*.

Concentration of MBC-P solution (ppm)	Diameter of inhibition zone (mm)[a]			
	Average		Range	
	P. expansum	*C. ulmi*	*P. expansum*	*C. ulmi*
Stock solution	39.6	38.4	35-48	34-44
1000	34.3	37.9	34-40	30-45
750	34.7	33.0	30-39	31-36
500	32.4	33.8	30-36	30-37
250	31.2	28.5	28-35	26-31

[a] Conversion factor, metric to English units: 1 mm = 0.039 in. (approx.)

Bioassays of the tree parts (twigs, leaves and buds) from the 10 control elms for presence of the fungitoxicant with conidia of both *P. expansum* and *C. ulmi* were negative throughout the entire sampling period. This agrees with our 1971 preliminary test results (Kondo *et al.* 1973) that generally, under field conditions, untreated elms are free from compounds which could affect the results of the bioassay.

Table 2 presents a summary of the MBC-P coverage obtained from the 37 root-injected elms which were sampled during 1972 at least 2 weeks after treatment. As expected, the higher the attempted coverage, the higher the observed coverage obtained. In general, actual observed coverage was well below the attempted coverage in all treated elms. It should be kept in mind, however, that the percentage of observed coverage is based solely upon the chemical found in the twigs sampled. Hence, a 0% observed coverage does not necessarily indicate that no chemical can be found within the elm or that no chemical was taken up. It merely means that no trace of the chemical was found in the sampled twigs from the crown of these elms. Bioassay of sectioned MBC-P root-injected elms in our 1971 tests show that the chemical is always found in the sapwood of the trunk and main branches but is not necessarily always found in the smaller twigs. However, when the chemical was detected in the twigs, it was invariably found throughout the length of the tree (roots, trunk and branches) between the root injected and the twig sampled.

Table 2 MBC-P crown coverage of treated elms, as indicated by the presence of the chemical in sampled twigs.

			No. of treated elms sampled					
			% of crown twigs sampled in which the chemical was observed					
Average dbh (cm)[a]	Range dbh (cm)[a]	Attempted coverage (%)	0-10	11-25	26-50	51-75	76-100	Total
37.3	12.1 - 61.5	100	2[b]	1	4	4	1	12
53.2	13.1 - 68.0	75	3	1	4	1	0	9
65.9	34.3 - 98.6	66	5	1	2	1	1	10
61.7	24.3 -118.7	50	0	4	0	1	0	5
22.0	22.0	33	1	0	0	0	0	1

[a] Conversion factor, metric to English units: 1 cm = 0.39 in. (approx.)

[b] Both elms (14.9 and 14.6 cm dbh) had been transplanted 1 year prior to root injection.

One hundred percent MBC-P coverage was obtained in only one of the 37 treated trees sampled, an elm 12.1 cm (4.8 in.) dbh. In general, it was found that better coverage was obtained in elms less than 50 cm (19.7 in.) dbh, and that dbh, total uptake and percentage of observed MBC-P coverage appeared to be interrelated. Hence, only those sampled, treated elms that fell within the following arbitrary percentage of observed MBC-P coverage classes (0-10%, 20-30% and 40-70%) were plotted as shown in Figure 2. A curve was drawn through each of the three groups of points. A few of the points lie some distance from their respective drawn curves. Some factors which could account for this discrepancy are (a) ratio of sapwood to heartwood, (b) amount of injury and "deadwood" in the sapwood area, (c) extent of *C. ulmi* infection, and (d) amount of leaf surface area. However, with the limited data obtained thus far, the drawn curves do give some indication of the amount of chemical solution an elm of given dbh must take up in order to have a given percentage of coverage. For instance, to provide a 40-70% final coverage an elm 80 cm (31.5 in.) dbh will ideally take up approximately 600-700 litres (132-154 gal) of chemical solution whereas an elm 40 cm (15.7 in.) dbh should take up only approximately 300-350 litres (66-77 gal). As additional data of this type are collected, it should be possible to refine these curves and test the validity of this empirical graph.

It was found that the highest concentration of MBC-P in the twigs sampled was approximately 125 ppm. Regardless of the concentration (250 ppm or 750 ppm) of the injected solution or the duration of the injection period, this appeared to be the maximum concentration found in the sampled twigs which were bioassayed. The chemical appeared to move up into the elm crown continually until a maximum chemical distribution was attained approximately 2 weeks after injection. Thereafter, the concentration and distribution of MBC-P remained more or less constant at least until the last sampling in November, 1972.

The results of the bioassay suggest that the orientation of the fissures in the outer bark surface of the elm provides a good indication of which roots should be injected to obtain adequate coverage of at least the infected portion of the crown of very large elms (55.5-118.7 cm or 21.8-46.7 in. dbh). For the fungus to become systemic throughout the host, the spores must follow the vascular system downward from the initial infected portion of the crown before it can spread upward into other healthy portions of the crown. However, with elms root injected specifically to give chemical coverage in only the diseased portions of the crown, any *C. ulmi* spores travelling downward would encounter higher concentrations of MBC-P and hence the spores would not be able to develop.

Dissection of branch samples (collected in November, 1972) from treated elms, which were infected prior to treatment, revealed that appreciable amounts of summer wood had developed over the brown streaks characteristic of DED. Culturing of branch samples from these elms

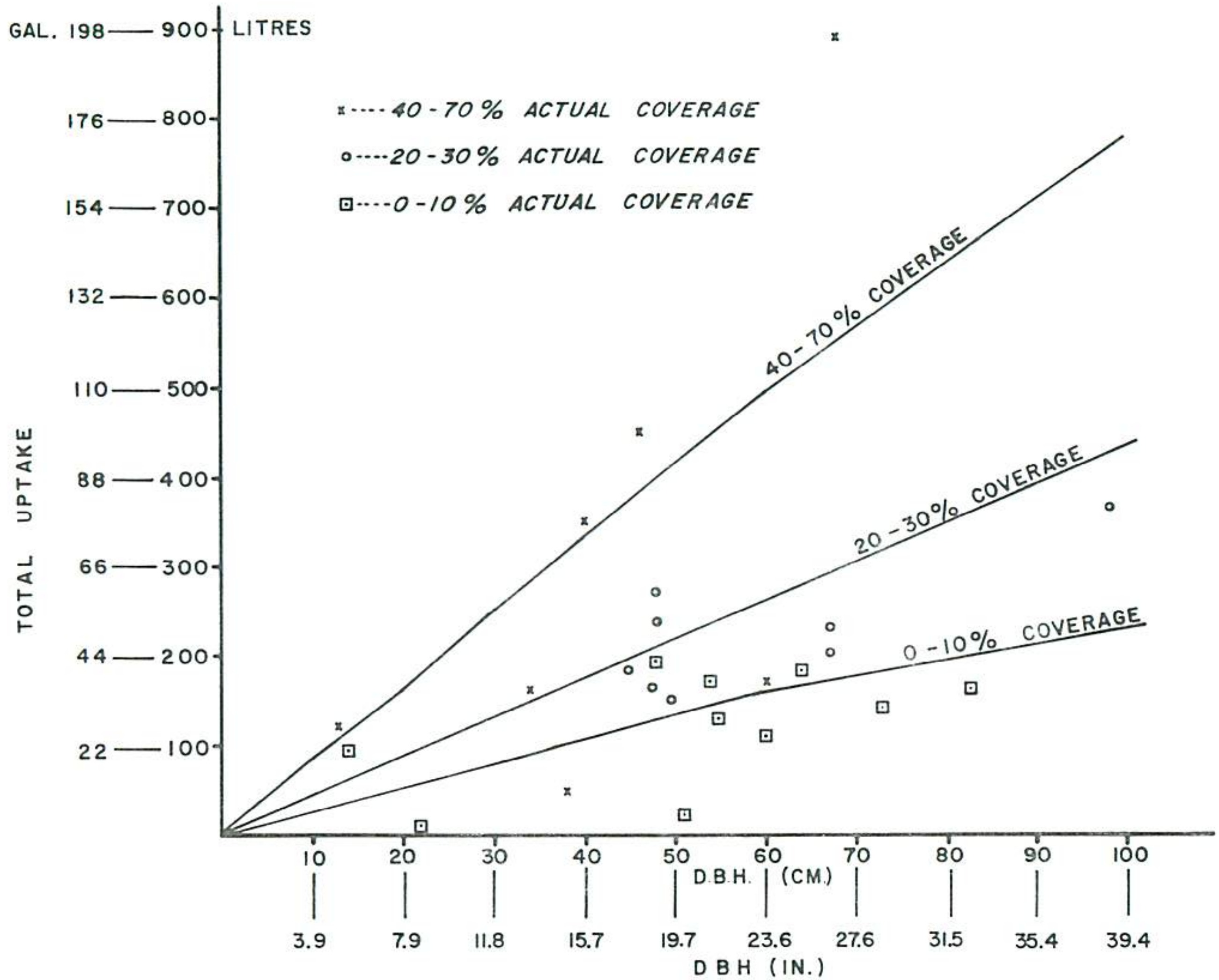

Figure 2. Total uptake of MBC-P solution by dbh for three percentage-coverage classes.

revealed that the incidence of isolation of *C. ulmi* had greatly decreased in comparison with culturing prior to treatment. It should be emphasized that the estimated final concentrations of the chemical introduced into the elms were not designed to eradicate *C. ulmi*, but merely to prevent sporulation and development of the organism. The object is to allow the elm every opportunity to outgrow the fungus (Kondo *et al.* 1973).

In some instances, elms exhibiting early wilt symptoms with very little leaf chlorosis or necrosis appeared to regain leaf turgor in the infected area within a few hours after root injection. This suggests that elms with DED, at least in the initial stages of disease development, suffer a deficit of water owing either to an increased rate of transpiration or to a decrease in water uptake through the roots.

Elms showing only extensive leaf wilt (little or no leaf chlorosis or necrosis and twig dieback) should not be rejected solely on that basis for treatment. A few trees that were rejected as candidates for root injection with MBC-P, because of extensive external DED leaf wilt symptoms (i.e., merely an apparent loss of leaf turgor with little chlorosis or necrosis of the leaves), did not succumb to DED by the end of the summer as expected. The initial symptoms of leaf wilt did not progress as in most cases of elms with DED. One such elm which was root injected in the fall (September 12, 1972) took up enough chemical to suggest that at least 40-70% coverage of the elm was achieved.

With the exception of one tree, which failed to leaf out fully after treatment in the spring of 1972, all trees treated in 1972 were alive at the time of budbreak and leaf expansion in early spring, 1973. Also, no chemical phytotoxicity was observed in any of the 80 trees treated with MBC-P. Four of the untreated, diseased trees (controls) died during the same period and were removed. It was apparent that the spread of disease symptoms throughout the elm was arrested after treatment. However, those parts of treated elms showing extensive symptoms of DED (wilt, chlorosis and necrosis of leaves and dieback of twigs) at the time of treatment continued to deteriorate along with some of the adjacent apparently healthy branches, but symptoms of DED did not spread extensively into the remaining healthy portions of the crown. This suggests that the disease is more widespread than is apparent from external symptoms and once a portion of the tree is physiologically destined to dieback, the treatment is unable to reverse the process.

Observations in 1972 suggest that the most successful treatment of diseased elms resulted when root injection with MBC-P was carried out as soon as disease symptoms were visible and at least before the fungus became systemic. An elm infected for at least 1 year, where the fungus has already become systemic, may or may not leaf out the following spring. Normally, elms which do leaf out produce only sparse foliage and show extensive twig dieback. Treatment of these elms during budbreak or early leaf expansion does not drastically improve their condition or appearance. However, other elms also infected the year prior to treatment, in which the fungus was localized and not systemic, developed in a normal fashion except for death of the infected branch. By midsummer, no external symptoms of DED were evident. Thus it is suggested that infected elms should be root injected as soon as disease symptoms become evident, in order to provide optimum conditions for arresting the further development of the disease and to maintain the aesthetic appearance of the elm. Delaying root injection merely results in more extensive loss of portions of the crown as the infection progresses at a rapid rate.

Since all diseased elms treated in 1972, with the exception of one tree, were alive at time of leaf fall and since MBC-P coverage of sampled, treated elms varied from 0 to 100%, the question then remains, what is adequate chemical coverage to arrest further development of the disease? It would appear, at least at present, that something less than 100% coverage is more than adequate. However, in order to determine an acceptable coverage range, much more data must be collected, and treated, diseased elms must be monitored for a number of years. Also, elms with low initial coverage may be retreated to obtain adequate disease control.

Elms root injected in 1972 ranged in dbh from 12.1 to 124.0 cm (4.8-48.8 in.) and in height from 6.2 to 36.5 m (20.3-119.7 ft). The dbh measurements were grouped into 5-cm (2.0-in.) classes to facilitate comparisons. Figure 3 shows the dbh class distribution of the treated, diseased elms. It is suggested that root-injection operations in most urban situations would involve mainly elms between 22.5 and 62.5 cm (8.8 and 24.6 in.) dbh. This would be of significance in estimating the amount of chemical, equipment and manpower required for treatment of elms under similar urban conditions. It should be emphasized again that these treated elms were chosen from a population of originally healthy elms as they became infected naturally during the course of the field trial. Therefore, Figure 3 also shows the dbh class distribution of elms which became infected during the growing season.

Of the 80 elms root injected, 11 were treated with 750 ppm and 69 treated with 250 ppm MBC-P solution. The average volume uptake of trees injected during the trial was 202.01 litres (44.4 gal). However, trees treated with 250 ppm MBC-P solution received an average of 54.32 g (1.7 oz) of the chemical compound.

The total volume uptake over a 48-hour period varied from 5 litres (1.1 gal) for a diseased elm 22.0 cm (8.7 in.) dbh exhibiting extensive wilt and dieback to 145.0 litres (31.9 gal) for a comparable elm exhibiting only slight disease symptoms. The greatest single uptake obtained in the trial was 892 litres (196.2 gal) during a 47-hour period with an elm 68 cm (26.8 in.) dbh with only slight DED symptoms.

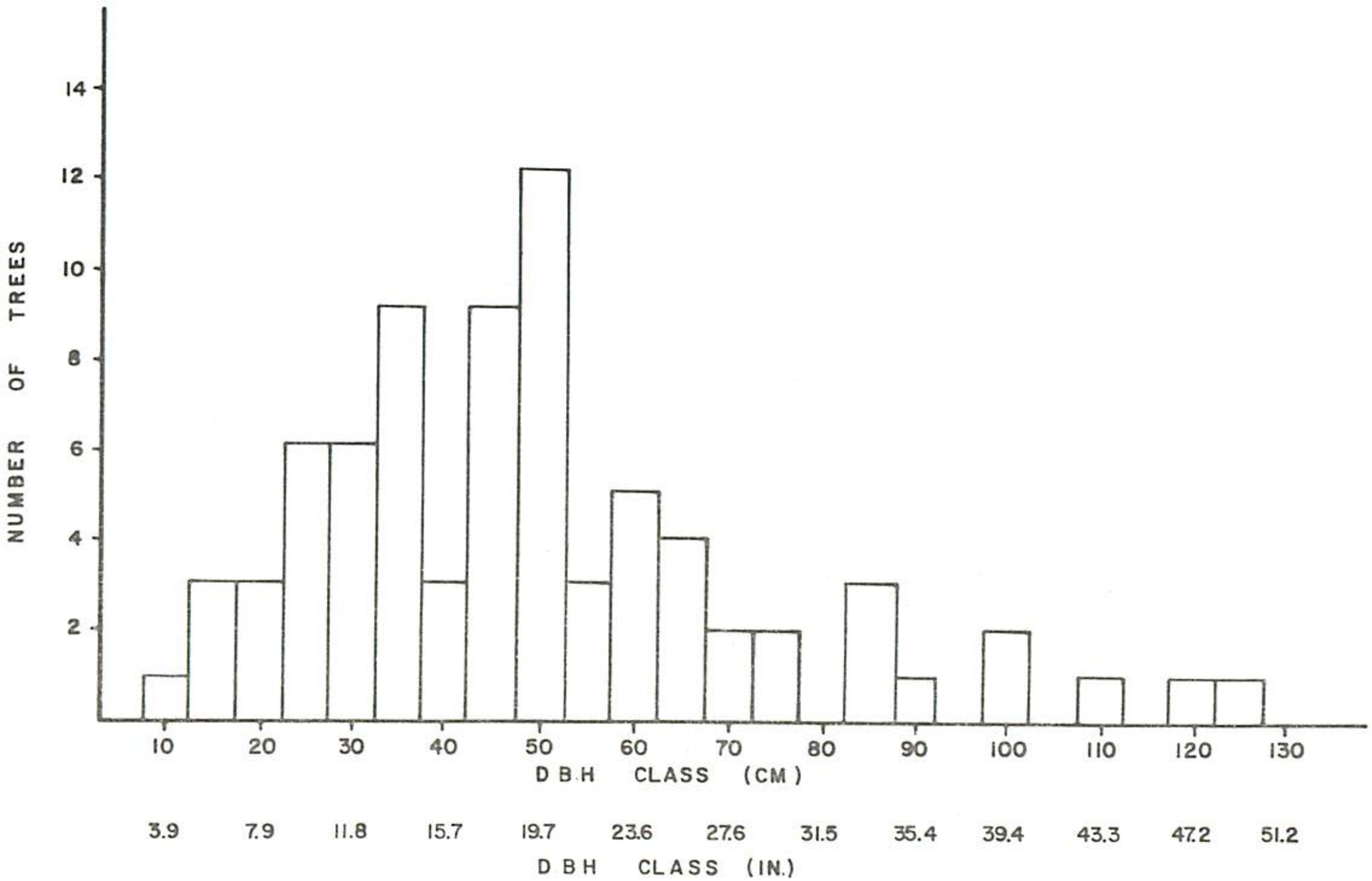

Figure 3. Distribution of treated, diseased elms by dbh class.

In order to compare uptake rates of MBC-P solution, the volume of uptake was calculated as litres per hour, per square centimeter of the injected root surface area. Figure 4 presents the average uptake rates for each dbh class. There appears to be a general increase in the average uptake rate with an increase in dbh of the elm. However, other factors such as weather, site condition and degree of disease also greatly affect uptake rates. In general it was found that total volume uptake of chemical solution through root injection was very low for elms growing on very wet sites.

From the data presented in Figure 5 a monthly variation in the rate of uptake is evident. There appears to be a general increase in the uptake rate from May until September. However, an unexplained decrease in the uptake rate occurred during August. Analysis of the data collected on the weather and site and the condition of elms injected during August failed to show a reason for this decrease. It is suggested that this August decrease in uptake may be due to the normal physiological condition of elms at this time of year.

The data in Figure 6 indicate that the average radial distance from the tree trunk to a root of suitable diameter for root injection increased with tree size up to a point. In general, with elms larger than 45 cm (17.7 in.) dbh the average radial distance is greatly affected by factors other than the elm size. The further root excavation progressed from the elm, the greater was the probability of encountering obstructions, such as sidewalks, pavement or buried debris. These obstructions prevented the continuation of excavation of a particular root and necessitated the selection of an alternative root, branching from the original root.

Since an attempt was made to select at least one root per major root flare, the total number of roots injected per elm increased with the elm size. The largest number of roots injected was 12 for an elm 124.0 cm (48.8 in.) dbh. This required four complete root-injection setups because of the low capacity (45 litres, or 9.9 gal) of the reservoir with respect to the possible maximum uptake rate. For instance, under ideal conditions, one of the elms (65.0 cm dbh, or 25.6 in.) had an uptake rate of 34.0 litres (7.5 gal) per hour over a 9.5-hour period. Therefore, a much larger reservoir should be employed for commercial operations to minimize the time and cost of refills.

The average number of man-hours required for root injection was 11.05. Root excavation consumed most of the man-hours in the root-injection operations. As can be seen from Table 3, on the average, 7.23 man-hours were required for root excavation. This represents 65% of the total man-hour input. Average excavation times increased with tree size. This is directly related to the increase in the number of roots excavated with larger trees. Excavation time was greatest on heavy, compact sites and on light sites where the roots were buried deeply by land fill. Trees buried deeply in land fill should not be

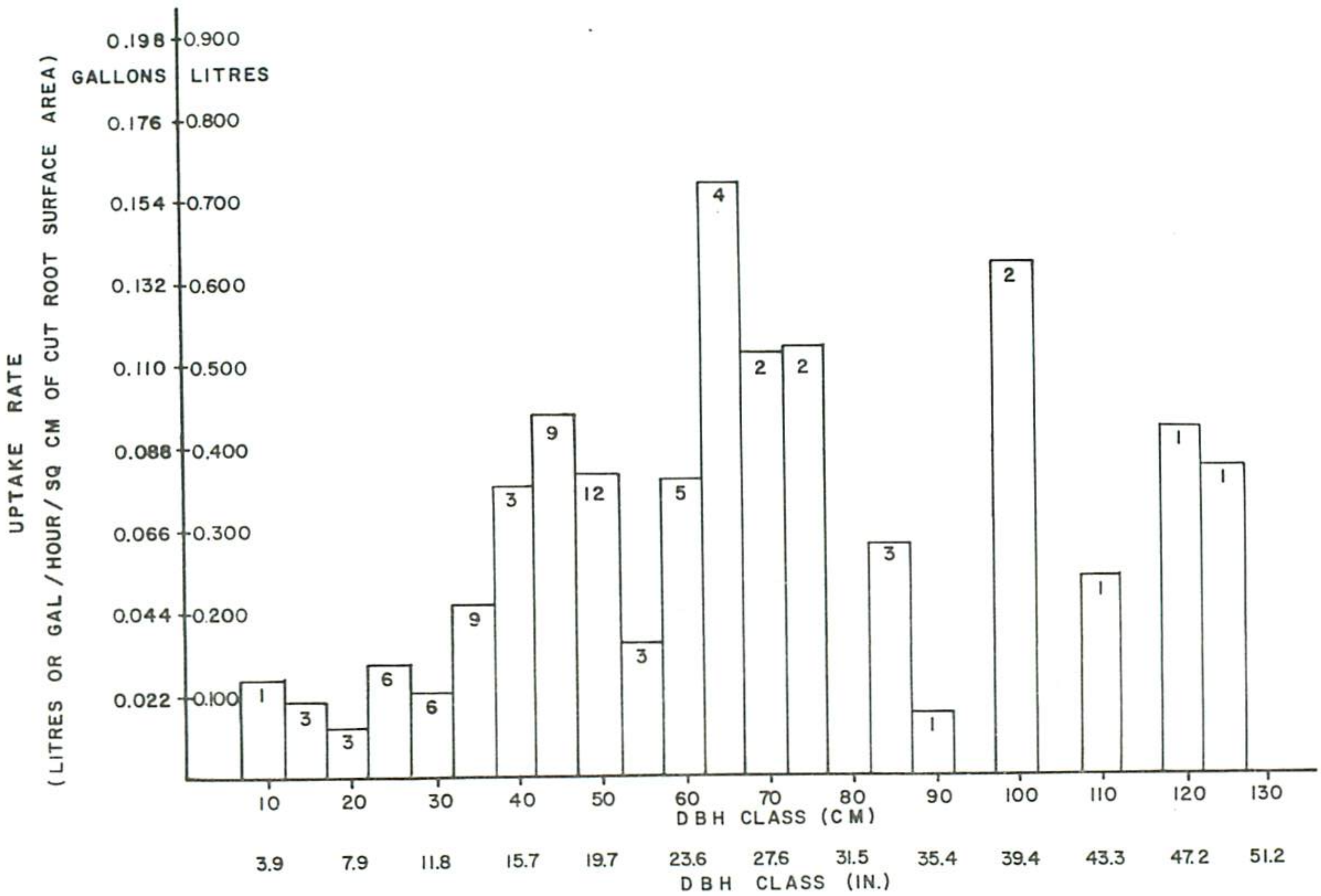

Figure 4. Average uptake rate by dbh class of treated, diseased elms. (Numbers on bars refer to numbers of samples.)

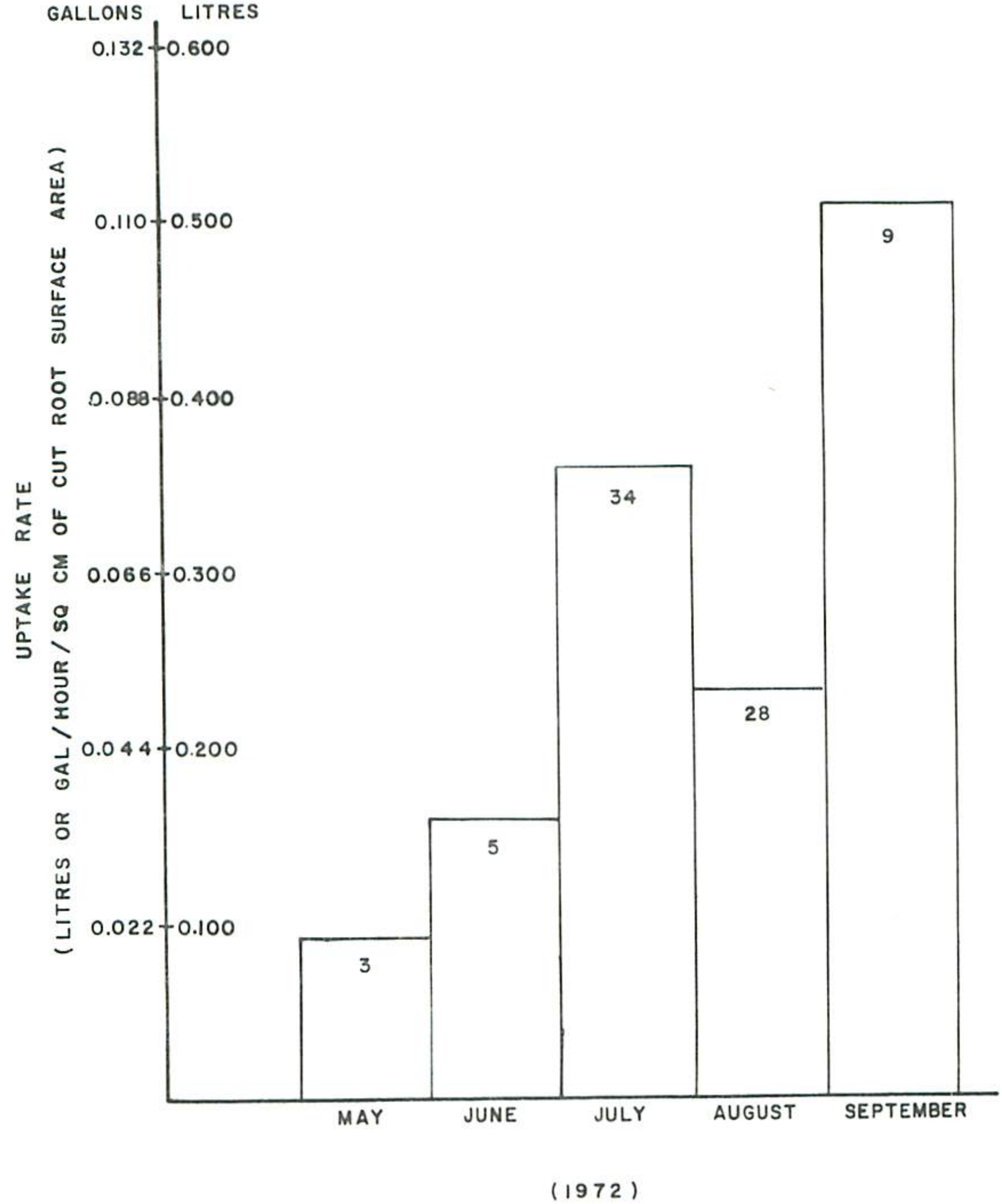

Figure 5. Average monthly uptake rates of diseased, treated elms. (Numbers on bars refer to numbers of samples.)

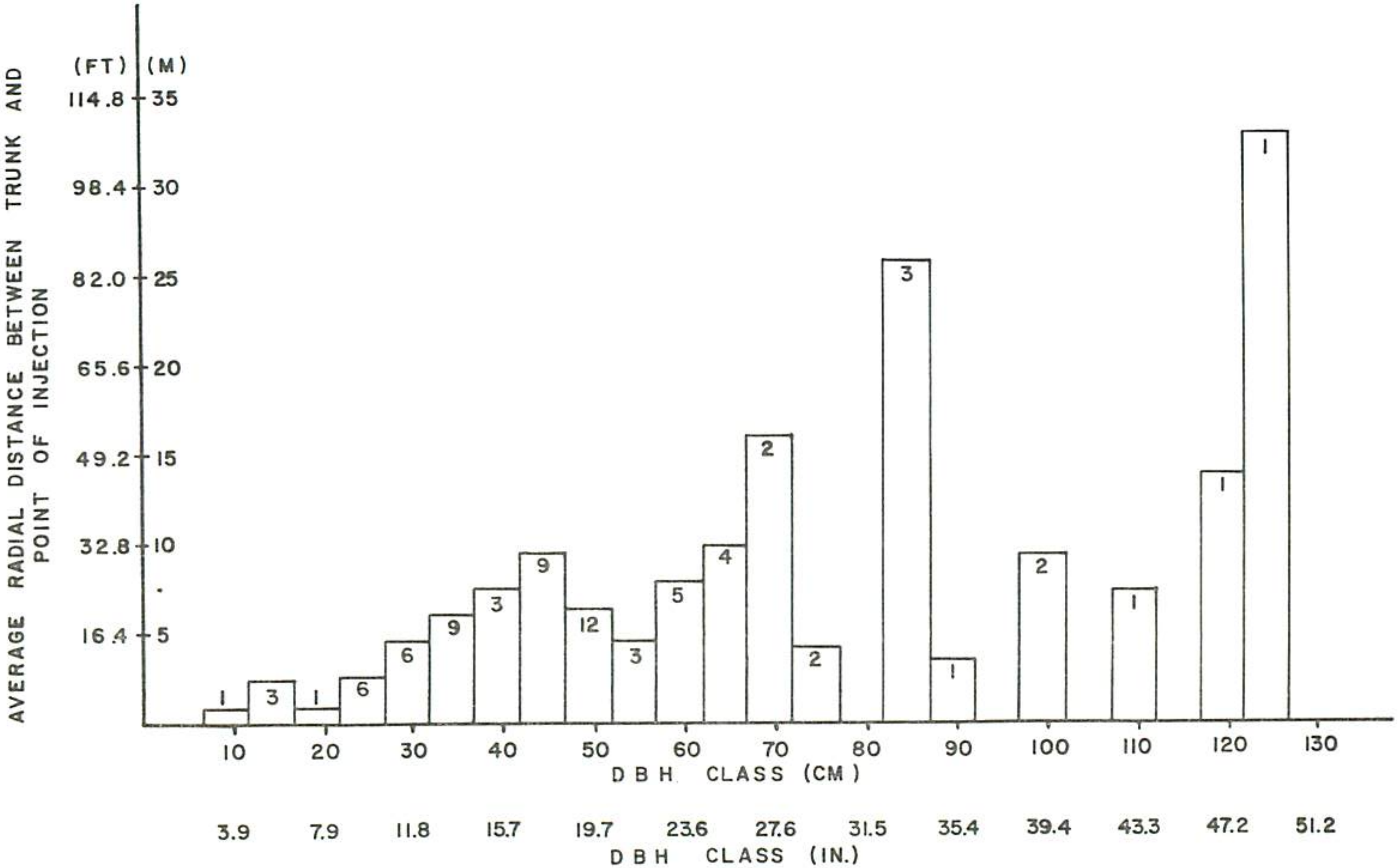

Figure 6. Average radial distance from diseased elm trunk to point of root injection. (Numbers on bars refer to number of samples.)

root injected unless they are of high value and growing vigorously. Excavation in these situations is extremely time consuming and expensive unless some mechanical technique which does not damage the roots can be used.

Table 3 Cost analysis for MBC-P root injection

Item	Average amount used per tree	Total cost per item ($)	Percentage of overall cost (%)
Distilled water	217.27 1 (47.86 gal)	40.63	43.84
MBC-P (250 ppm)	54.32 g (1.74 oz)	16.61	17.92
Excavation time	7.23 man-hours	21.69	23.40
Setup time	1.94 man-hours	5.82	6.28
Dismantling and measurement time	1.38 man-hours	4.14	4.47
Refill time	0.50 man-hours	1.50	1.62
Nitrogen gas	1,586.00 1 (56.00 cu. ft)	2.29	2.47
Injection apparatus[a]			
Total cost per tree		$92.68	100.00%

[a] Cost of the apparatus has not been included since the apparatus can be used for many trees.

The average cost of root injection was $92.68 per tree. Root excavation represents 23.40% of the total cost of root injection. The total man-hour input represents 35.77% of the total cost of root injection. As a result, 64.23% of the total cost was attributable to the cost of materials. The most expensive material was distilled water which, by itself, was 43.84% of the total cost of root injection. It can be seen from these data that a substitute for distilled water such as possibly ordinary tap water or filtered and/or deionized water would greatly reduce the operating costs.

SUMMARY

1. *P. expansum* may be employed as the bioassay organism as long as checks are maintained periodically with samples bioassayed against *C. ulmi*.

2. Under field conditions untreated elms are free from compounds which could affect the results of the bioassay.

3. In general, it was found that better distribution of the chemical was obtained with elms less than 50 cm (19.7 in.) dbh and that the total uptake, dbh and percentage of observed MBC-P coverage appeared to be interrelated. An empirical graph was obtained from data collected to give some indication of the amount of chemical solution an elm of given dbh must take up if a given percentage of coverage is to be achieved.

4. Regardless of the concentration (250 ppm or 750 ppm) of MBC-P solution or the duration of the injection period, 125 ppm was the maximum concentration found in twigs sampled approximately 2 weeks after injection.

5. Orientation of the bark fissures provides a good indication of the major roots which should be root injected to cover a specific portion of the elm crown.

6. Appreciable amounts of summer wood had developed over the brown streaks characteristic of DED in treated, diseased elms and the incidence of isolation of *C. ulmi* had greatly decreased after root injection with MBC-P.

7. Further confirmation of the importance of weather, site and disease condition upon rate of uptake of chemical through severed roots was obtained.

8. With the exception of one tree, which failed to leaf out fully after treatment in the spring of 1972, all trees treated in 1972 were alive at the time of budbreak and leaf expansion in early spring, 1973. Also, no chemical phytotoxicity was observed in any of the 80 elms treated. Four of the 10 untreated, diseased elms (controls) died during the same period and were removed.

9. Observations in 1972 suggest that the most successful treatment of diseased elms was obtained when they were root injected with MBC-P, as soon as DED symptoms became visible and at least before the fungus became systemic.

10. The average volume uptake of MBC-P solution of trees injected during the trial was 202.01 litres (44.4 gal) (an average of 54.32 g (1.7 oz) of MBC-P for elms treated with 250 ppm of MBC-P).

11. The average cost of root injection was $92.68 per elm. Root excavation represented 23.40 percent of the total cost of root injection. The most expensive material was distilled water, representing 43.84 percent of the total cost of treatment.

12. Useful data were collected on selection and excavation of roots and the operational capability of the root-injection technique and apparatus. These data will be useful for future root-injection operations.

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