ASPECTS OF THE FEEDING BEHAVIOR OF *SCOLYTUS MULTISTRIATUS*, THE PRIMARY VECTOR OF DUTCH ELM DISEASE, AND A CRITICAL EVALUATION OF PRESENT CHEMICAL CONTROL MEASURES

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THE PRIMARY VECTOR OF
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Sampling of untreated elms revealed that S. multistriatus feeds preferentially in the whole upper tree region. Feeding attack increased linearily from the bottom to the top. Physical twig crotch characteristics were investigated with respect to vector feeding. The angle size between the main and lateral member of a twig crotch had no influence on feeding. There was, however, a significant association between a more rounded twig crotch base and lateral feeding which reportedly results in more successful inoculations than central feeding.

Twig samples were collected from 3 height strata of helicopter- and mist blower-sprayed elm trees and then subjected to bioassays with Scolytus multistriatus, and to chemical analysis for bark residues. Of five helicopter-applied methoxychlor formulations, a 12.5% formulation containing Dacagin as anti-drift material gave the best protection. However, significant methoxychlor carry-over from mist blower applications in previous years heavily interfered with an objective evaluation of the various helicopter treatments. Carry-over from helicopter treatments appeared to be negligible. A comparison of ground and aerial application definitely favored the mist blower which gave between 80 and 90% protection during the first 10 weeks after application. Tree height was

the only factor which affected effectiveness of the mist blower. The helicopter, although favoring tree-top coverage, gave insufficient protection, never exceeding 30% in any of the sampled height strata. The two responses measured in the bioassays, mortality and 'failure to score xylem', were well correlated except in bioassays of twig crotches with fresh surface deposits.

Tree kill in the helicopter and mist blower treatment areas over a 2-year period appeared to be independent of the degree of protection, as was indicated by the bioassay results. This discrepancy was explained by differential vector pressure and root-graft transmission in the various treatment areas.

To objectively monitor quality and quantity of spray coverage throughout the crown region, a sampling system was developed employing 3-dimensional cube-like sampling devices. The helicopter gave highly discrete spray patterns. Dacagin shifted the droplet size spectrum to the larger sizes, while straight emulsions showed higher dispersion. Aerial application resulted in poor 3-dimensional coverage of the sample cubes, which suggested that less than half the total bark or twig crotch area received spray. Also, dosages applied to each tree (ca. 2 quarts 12.5% spray) were found to be too small to provide necessary protection. The mist blower created finely-dispersed, almost continuous spray deposits which often exceeded the levels needed for protection, and 3-dimensional coverage was superior, even in the upper tree region.

Correlations of beetle response (mortality) with bark residues yielded a lower LC_{50} value for the mist blower- and lab-treated-twigs than for helicopter-treated twigs. This was explained by the difference in droplet size spectrum.

Contact toxicity of fresh methoxychlor emulsion deposits was greater than solution deposits. The addition of Dacagin to the emulsion reduced contact toxicity significantly. Contact with weathered surface deposits caused little mortality and had no measurable impact on feeding response. Also, the physical structure of methoxychlor surface deposits was documented and related to contact toxicity.

Methoxychlor deposits on elm bark proved to be quite persistent with a decomposition rate of around 70% after one year. Dacagin delayed weathering, even when added in small quantities to the formulation. Displacement of methoxychlor residue from the point of deposition to other sites on an elm branch was not observed.

The Dutch elm disease control program on the campus of Michigan State University was considerably more successful than that of surrounding communities between 1958 and 1972. Elm die-back increased since 1968 when the mist blower-DDT-methoxychlor program was replaced by a helicopter-methoxychlor program. However, increasing vector pressure over the past few years contributed to the growing number of diseased elms on the campus.

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To Crystal and
my Parents

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INTRODUCTION

Dutch elm disease is an insect-borne fungus disease and is characterized by the close interaction of 3 organisms: the vector (or vectors), primarily scolytids; the pathogen (an ascomycete fungus), and almost all the members in the genus Ulmus. When dealing with control strategies of a vector-borne disease one has to understand the whole complex of the problem. Any change in one of the 3 interacting components will alter the performance of the whole disease system.

Assuming that the amount of inoculum carried by a given population is directly related to vector density, the performance of the disease system is determined by (a) the host tree density, and (b) the vector density. Indirectly, the vector provides for new breeding sites by inoculating healthy elms which subsequently become attractive for breeding. If undisturbed (no control measures), the cycle of inoculation and beetle infestation will continue until a threshold host tree density is reached; then the disease will turn from the epidemic to the endemic stage. In other words, a minimum host tree density is a prerequisite for the perpetuation of Dutch elm disease. Since the European elm bark beetle, the most successful vector, has a certain flight range, it determines the threshold density. In other words, this density is reached when the mean distance between host trees will exceed the flight range of the most successful vector. In areas where Dutch elm disease has occurred for the past forty years, such a threshold

density might be present already. For all practical purposes, if one were to wait until the balanced state of a threshold density were reached in a given area, the loss of the majority of all elms would be the probable result. One can imagine that the elm density in which the disease would become endemic would be very low considering the maximum flight range of 4 miles of Scolytus multistriatus Marsham (Wolfenbarger et al., 1939). Therefore, in order to protect and save high density urban elms, the disease transmission cycle has to be interrupted. The problem of disease prevention presents itself in the simple question: How can optimum control be achieved knowing the biological features of all three interacting organisms? A simple answer would be to eliminate (eradicate) one of the three components. For instance, the elimination of the elm from the American scene would certainly solve the problem. This would be, of course, self-defeating since the overall goal is to save the American elm, one of the most ideal and common shade trees in the Northeast of the United States. Thus, besides resistance breeding of elms, control can come only from the manipulation of either the fungus component or the vector population per se or some combination thereof. Figure 1 schematically shows the interaction of the pathogen, the vector and the host tree in the disease cycle. The shaded circles stand for dying elms, the unshaded circle is a healthy elm. A vector emerging from a diseased tree will either go directly to another breeding site or feed in the twig crotches of a healthy tree. Eventually this feeding portion of the population will look for a breeding site. During the feeding process, the healthy tree becomes inoculated with the fungus, will eventually die and provide new breeding sites for the vector population.

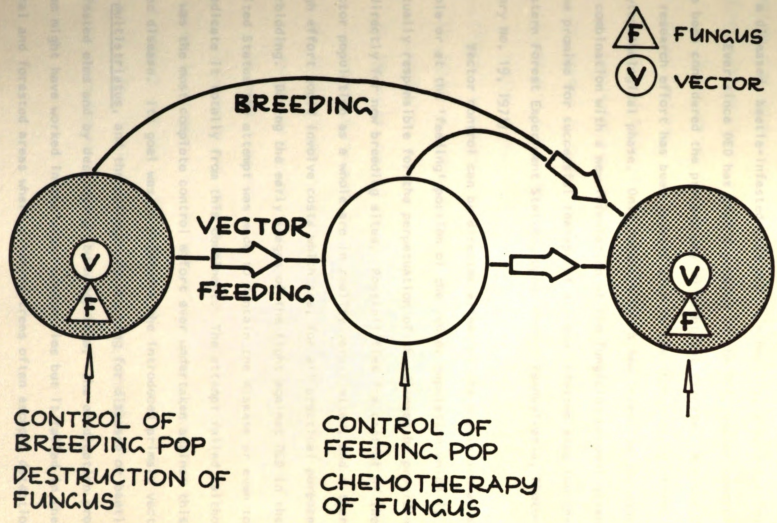


Figure 1. Dutch elm disease cycle: interaction of vector, fungus and host tree (unshaded circle: healthy elm; shaded circle: diseased elm).

In this way pertetuation of the disease is guaranteed. The small arrows depict the types of control and protective measures which can be applied to a diseased, beetle-infested tree and to a healthy, vigorous tree.

Ever since DED has been a problem in the USA, vector control has been considered the primary way to fight the disease. A great deal of research effort has been devoted also to the chemotherapy of the phytopathological phase. Only recently has a new injection technique in combination with a new formulation of the fungicide benomyl given some promise for successful therapy of already infested elms (Northeastern Forest Experiment Station, Upper Darby, Pennsylvania, Photostory No. 19, 1971).

Vector control can be directed either at the population as a whole or at the 'feeding' portion of the vector population which is actually responsible for the perpetuation of the disease by providing indirectly for new breeding sites. Possibilities for control of the vector population as a whole are in reality very limited, and a thorough effort would involve costs which are, for all practical purposes, forbidding. During the early stages of the fight against DED in the United States the attempt was made to contain the disease or even to eradicate it totally from this continent. The attempt failed, although it was the most complete control effort ever undertaken against this tree disease. Its goal was to eliminate the introduced primary vector, S. multistriatus, and the pathogen by scouting for diseased or beetle-infested elms and by destroying (burning) them. The eradication program might have worked in the urban communities but it failed in the rural and forested areas where diseased trees often escaped detection and necessary destruction. The woodlands surrounding cities were and are, therefore, still a great pool for new vector populations. A

community might have an effective tree removal, sanitation and elm tree protection program, but the continuous supply of thriving vector populations from neighboring woodlands is always a threat to the success of well-planned control programs in the urban communities.

Vector control can be practiced along the following lines:

A. Direct Control:

1) Control of breeding population - detection and destruction of beetle-infested elms, trapping of beetles.

2) Control of feeding population - prevention of disease transmission during feeding process through protective spraying of healthy elms.

B. Indirect Control:

1) Intensive tree care (sanitation) to keep elms in healthy, vigorous condition.

2) Detection, removal and destruction of possible breeding sites, such as dying elms, broken limbs, and so on.

3) Silvicultural control: regulation and manipulation of elm tree density to a point where the probability of disease occurrence is greatly reduced.

Integration and combination of all of the mentioned control measures is necessary in order to provide a well-rounded control system. The research reported in this thesis dealt with questions concerning control of the "feeding" population of S. multistriatus to prevent disease transmission in healthy elm trees.

I. Aspects of the Feeding Behavior of Scolytus multistriatus Marsham.

The location of a feeding site (inoculation point) on the tree region determines the probability of infection and also possible recovery. Inoculations were far more effective when close to the main trunk than at the outer branches (Zentmyer et al., 1946). This means that for adequate elm tree protection one has to take into account not only where the vector feeds on the tree but also possible hazard regions of infection during the period of susceptibility. Therefore, a valid evaluation of elm protection systems must be based solidly on a complete understanding of the behavior of both the vector and the pathogen. Not all vectors emerging from a diseased tree make successful inoculations. Some go directly to a breeding site and never get involved in direct disease transmission. The current aim of protective spraying is to prevent inoculation by killing the vector at the feeding site, the 1-5 year old twig crotches. Their post-emergence flight carries part of the beetles into the canopy of healthy elm trees where they commence feeding. Wolfenbarger and Buchanan (1939) and Whitten (1958) found most of the feeding scars in the upper and peripheral part of the tree; beyond that no information is available as to regions of preference for feeding within the tree crown. In order to maximize protection against feeding, it is necessary to know where feeding takes place primarily.

The chemical aspect of stimulation for feeding has received considerable attention and several phenolics have been identified and verified as such by bioassays (Baker and Norris, 1967). Physical stimuli, such as twig crotch angle and certain bark characteristics, were believed by Meyer and Norris (1964) to influence the feeding attack. The potential of physical characteristics as a basis for elm resistance

breeding warranted additional research on factors determining selection of feeding sites by beetles.

II. Evaluation of Methoxychlor Formulations and Application Techniques Against Feeding by Scolytus multistriatus Marsham.

Ever since Scolytus multistriatus and Scolytus scolytus (in Europe) were established as the main vectors of DED, spraying of elm trees has been used extensively to prevent feeding by these vectors. The questionable value of spraying was recognized as early as 1931 by Fransen: materials then available lacked the persistence to be effective during the period of transmission, and application techniques were not available which would provide for complete coverage of all feeding sites on a tree. Initially, arsenicals were recommended; later with the development of the organochlorines, DDT became available and was successfully used for prevention of twig crotch feeding (Becker, 1946; Plumb, 1950). Fall and Spring applications before the leaves appeared were almost equally effective because of the long persistence of DDT (Whitten, 1949, 1960). During recent years, concern over environmental pollution made the continued use of DDT in urban areas increasingly difficult. Trials with many other insecticides such as Lindane, malathion, etc., proved that DDT was still superior in all respects. During the 1960's, DDT was phased out of most urban DED spray programs and systemic insecticides received considerable attention for controlling DED vectors (Norris 1959; Peacock,1967). Unfortunately, no systemic proved effective enough to replace DDT. Although earlier tests did not favor methoxychlor as a possible alternative to DDT, investigations by Becker (1956) and Wooten (1962) established methoxychlor as a substitute for DDT. What made methoxychlor so appealing was its low mammalian toxicity and environmental safety. Serious problems arose, however,

with timing of the applications during the dormant season. It was generally assumed that the lower persistence (Norris, 1961; Wallner and Leeling, 1968) of methoxychlor would render fall applications ineffective. Consequently only spring applications were recommended. This put great pressure on extensive spray programs because not enough manpower or equipment were available to complete the spraying in time before bud break. Applications made from the ground, either with the hydraulic sprayer or mist blower, were naturally slow and expensive, so cheaper ways were sought.

Wallner and Leeling (1968) pioneered the use of the helicopter for elm tree spraying. Aerial application proved to be cheaper and much faster once the pilot became familiar with the location of the elm trees in an area. However, the effectiveness of helicopter spraying in comparison with mist blower application remained in doubt, and further experimental evidence was needed. The Dutch elm disease symposium in Delaware, Ohio (Peacock, 1967) placed research priority on methoxychlor formulation studies and application techniques. In 1968, Wallner et al. (1969) compared and evaluated several methoxychlor formulations which were applied by helicopter to the campus elms of MSU. This study was continued and expanded during 1969 and the following years. The performance of formulations and application techniques was evaluated by bioassays with S. multistriatus and chemical analysis of bark residues. The results are reported in this dissertation.

III. Monitoring of Spray Coverage on Elm Trees.

Two factors made a valid comparison of formulations and application techniques difficult in 1969 and 1970. One was the great variability in bark residues and the other was carry-over from previous applications. A more objective way was sought to study the overall coverage

of an elm tree when the material was applied either by helicopter or mist blower. A sampling method was developed which permitted study of spray patterns, actual spray deposition and 3-dimensional coverage.

IV. Insecticidal and Decomposition Properties of Methoxychlor Formulations.

Extraction and quantification of bark deposits have frequently been used to test the performance (coverage and persistence) of spray applications against DED vectors (Matthysse et al., 1954; Thompson, 1965; Wallner and Leeling, 1968). Feeding trials with S. multistriatus served the same purpose (Doane, 1958, 1962; Norris, 1960). Only a few workers attempted to correlate beetle response with residue levels in twig crotches (Miller, 1951; Matthysse et al., 1954). Information of this kind was lacking for methoxychlor. In order to establish residue levels at which control is sufficient, beetle response (mortality) on field-collected twig crotches was correlated with the respective residue values. LC_{50} values were also determined in bioassays with lab-treated twig crotches.

During the search for a feeding site on a treated elm tree, S. multistriatus comes in contact with surface deposits of methoxychlor. Although the contact toxicity of methoxychlor is known to be low (Cuthbert et al., 1970), it was of interest to compare contact toxicity of various methoxychlor formulations. Several experiments were conducted to investigate the effect of contact with methoxychlor residues on feeding behavior. The toxicity and decomposition rates of a compound are largely dependent on its deposit structure. Therefore, crystallization patterns on elm bark were documented for various formulations and an attempt was made to point out possible relationships between contact toxicity, weathering and type of surface residue. Decomposition rate is, to a great extent, predetermined by the makeup of the formulation

(Hoskins, 1962). Certain additives have the ability to increase persistence. This possibility was researched with a formulation which contained a drift-control agent.

V. Dutch Elm Disease Control on the Campus of Michigan State University: 1958-71.

The history of DED and control measures on the campus was documented for the period since 1958. The successful elm protection program at Michigan State University is cited as an example of excellent cooperation between the Grounds and Maintenance Department and the Department of Entomology.

LITERATURE REVIEW

(A) History and Development of Dutch Elm Disease in Europe

The name Dutch elm disease implies incorrectly that the origin of this fungus disease is Holland. Actually, elm die-back was observed independently in several areas of Europe at about the same time. Spierenburg (1921) discovered the disease in Holland while Guyot (1921) described similar disease symptoms on elms from Northern France and a report by Georgescu and Orenski (1957) mentions that the disease had been found in Rumania as early as 1910. The question as to the origin of the disease cannot be answered conclusively.

The region of origin is believed to be the Far East, in particular, China. The manner in which it was introduced into Europe is not quite certain. During the past century travelling Chinese tradesmen who roamed Europe used baskets made of branches of their own native elm, Ulmus parvifolia. Some of these branches might have been infested with the disease agent (Heybroek, personal communication). A further reason to suspect China as the place or origin is the fact that the Chinese elm, U. parvifolia, is rather resistant or tolerant to the disease. This low level of susceptibility can best be explained by a long evolutionary parallel development of both host tree and disease. The Dutch elm disease problem might be analogous to the chestnut blight caused by Endothia parasitica, a fungus, (definitely of Asiatic origin) in the sense that the Chinese chestnut is also resistant to the blight, whereas other

chestnut species are very susceptible. The actual cause of the disease was unknown until Schwarz (1922) isolated a fungus from diseased elm wood which she later described as Graphium ulmi, Ascomycetae. Schwarz speculated that the fungus entered the tree through the leaves and considerable controversy developed over the cause of the elm die-back. Some attributed the disease to unfavorable conditions in the soil (Pape, 1924) while others held frequent moisture changes in the soil responsible (Hoestermann, Noack, 1925). The large European elm bark beetle, Scolytus scolytus F. and the smaller European elm bark beetle Scolytus multistriatus Marsham, together with other Scolytids, were also suggested as the primary causes of Dutch elm disease (Malaquin, 1923; Knop, 1928; Kaiser, 1931).

The question about the cause of the disease was finally resolved by Wollenweber (1927) and Buisman (1928) who both proved Graphium ulmi Schwarz to be the pathogen. Schwarz (1922) described only the imperfect stage of Graphium ulmi; but in 1932 Buisman found the perfect stage of the disease agent and named it Ceratostomella ulmi (Schwarz) Buisman. A further name change to Ophiostoma ulmi (Buisman) Nannf. was made by Nannfeldt (Melin and Nannfeldt, 1934). A revision of the group by Moreau (1952) and Hunt (1956) yielded the name, Ceratocystis ulmi (Buisman) C. Moreau, which is now commonly accepted.

Even though the causal organism was isolated and identified, the method of the transmission from one host to another still remained unsolved. Wollenweber and Stapp (1928) mentioned birds and beetles as possible vectors. However, the first evidence of a causal vector was obtained by Wollenweber (1929) who observed C. ulmi in the galleries of S. scolytus in diseased elm wood. Subsequent research in Holland (Betrem, 1929; Roepke, 1930; Fransen, 1931), in Germany (Prell, 1930) and in

other European countries, verified S. scolytus and S. multistriatus as the main vectors of the disease.

Using S. scolytus, Fransen (1931) obtained experimental proof that the fungus is transmitted during the feeding process shortly after emergence. In fungus-infested elms, C. ulmi fruits in galleries of S. scolytus and S. multistriatus and will form coremia (fruiting bodies). The sticky spores adhere to the body integument or are carried internally in the digestive tract of emerging beetles (Betrem, 1929). Both the larger and the smaller European elm bark beetle have basically the same feeding behavior. After emerging they feed in the young twig crotches of healthy elms and introduce fungus spores into the vascular system of the tree.

(B) Introduction of Dutch Elm Disease into the United States and Present Status

Both European elm bark beetles were probably introduced in the United States; however, only S. multistriatus could establish itself. The first record of its occurrence in this country, Massachusetts, dates back to 1909 (Chapman, 1910). Not until 1930 were the first cases of Dutch elm disease reported from Cleveland and Cincinnati, Ohio. These localized and relatively small infestations were soon eradicated. In the early summer of 1933, the disease appeared in New Jersey. It was soon discovered that all of these recent infestations were caused by diseased elm burl logs shipped from France to New York and from there to several veneer factories in the country. Many of these logs were also infested with the 2 European scolytid vectors, S. scolytus and S. multistriatus. Federal quarantine No. 70 from October, 1933, restricted the further importation of elm logs into the United States (Beattie, 1934). This regulatory action came too late to prevent the

establishment of the disease and its main vector, S. multistriatus. S. multistriatus was soon joined by the native elm bark beetle, Hylurgopinus rufipes (Eichh.), as an important vector, especially in areas with lower winter temperatures (Collins, 1936, 1941; Finnegan, 1957).

The distribution of the introduced vector S. multistriatus and the disease pathogen covers at the present almost the entire range of the American elm, Ulmus americana. As of 1970 infestations were found in all states except Arizona, Florida and Montana (Barger and Hock, 1971). It is common belief that the disease will eventually spread to wherever elms grow in North America.

(C) Hosts in Europe and North America

Practically all of the species in the genus Ulmus are susceptible to varying degrees to C. ulmi. Asiatic species in general and the Chinese elm, Ulmus parvifolia, and the Siberian elm, Ulmus pumila L. show considerable resistance if not immunity (Buisman, 1931, 1933, 1934). Similarly, S. multistriatus attacks all the elm species within its range in Europe, in the United States and Canada. In the United States this beetle has never been reported from hosts other than from members in the genus Ulmus (Wallace, 1940), although in Europe it has been recorded from aspen, plum and ash (Escherich, 1923). According to Becker and Mankowsky (1965) there was little or no significant difference between feeding response on Ulmus americana and other elm species, including the fungus-resistant Chinese and Siberian elm.

(D) Other Vectors in North America

The problem of other possible vectors of Dutch elm disease besides S. multistriatus received early attention. Readio (1935) stated that all the insects associated with elm trees must be suspected as vectors. These are the native elm bark beetle, H. rufipes, the curculionids,

Magdalis ssp., the cerambycid, Saperda tridentata, buprestids, cossids, and even leaf feeders, such as the chrysomelid elm leaf beetle, cankerworms, tent caterpillars and several sapsuckers. Significant disease transmission was suspected by the bark and wood borers, particularly by H. rufipes (Clinton and McCormick, 1935). Even certain Acarina were believed to be capable vectors (Jacob, 1934).

Collins (1936) performed several transmission experiments on small elm trees with insects which emerged from or fed on diseased elms. Only S. multistriatus and H. rufipes proved to be successful vectors and no infections occurred with the wood borers Saperda tridentata, Magdalis armicollis and M. barbita, or with the leaf feeder Galerucella xanthomelaena, Chrysomelidae. Goeden and Norris (1963) failed also to establish Magdalis ssp. as vectors.

Collins (1941) isolated C. ulmi from several wood-boring species which developed in diseased wood. Based upon the total number of emerging insect species from several logs the following percentage carried fungus spores:

Scolytus multistriatus	6.9, 5.8, 7.7, 5.7%
Hylurgopinus rufipes	4.3, 2.4, 3.3, 0.7%
Xylobiops basilare, Xylosandrus germanus, Magdalis armicollis and others	small % with spores

The same author cautioned against underestimating H. rufipes as a vector of Dutch elm disease, especially in areas where the European elm bark beetle is absent or cannot establish itself because of low winter temperatures. The geographical distribution of the indigenous elm bark beetle follows closely the natural range of its host trees, Ulmus ssp. Finnegan (1957) also believed that H. rufipes deserved more attention considering the fast spread of Dutch elm disease in Quebec where it is the only vector. A detailed description of the biology is given by

Kaston and Riggs (1937) and Kaston (1939). Like S. multistriatus, H. rufipes is a secondary pest and oviposits only in sickly or dying elms. Before constructing galleries, H. rufipes makes feeding tunnels in healthy elms which rarely reach the xylem. The adults hibernate in bark tunnels which penetrate deeper into the bark than do the feeding tunnels. It is commonly assumed that the disease is transmitted during the construction of these tunnels (Kaston and Riggs, 1938).

Disease spread does not depend solely on vectors as Verrall and Graham (1935) pointed out. The pathogen itself can pass from a diseased tree to a healthy tree via root-grafts. This can occur in situations where elms grow close together as in roadside-plantings. The authors mentioned as evidence for root-graft transmission the fact that dyes passed readily from one tree to the other via the root-grafts, and further pathogenic discolorations in the vessels of the roots reached into the root system of neighboring trees.

(E) Dutch Elm Disease Pathogen: Ceratocystis ulmi (Buisman) C. Moreau

The literature on the phytopathological phase of Dutch elm disease is very extensive and will not be reviewed here since Elgersma (1969) gave a comprehensive review of the subject matter. Instead key references will be given. Vectors introduce fungus spores into the conductive tissue while feeding on the host tree. After inoculation, the infection spreads rapidly as passively moving spores in the sap stream. Invasion of the vessels by C. ulmi results in a brownish discoloration of the vessel walls. The fungus moves then through pits into adjoining vessels and finally causes complete obstruction of vessels through the formation of gums and tyloses. The water and nutrient supply to leaves is interrupted, and this results in wilting and yellowing of

the leaves (Pomerleau, 1968; Buisman, 1932b). It was also suggested that toxin formation alone or in conjunction with tylosis and gummosis is responsible for the pathogenicity of C. ulmi (Zentmyer, 1942, 1946). A recent study by Landis (1969) supports this conclusion. Wollenweber (1929) found the fungus to be vertically distributed, mostly in the xylem part of the youngest growth ring. Banfield (1968) investigated seasonal difference in movement and distribution of spores in the conductive system of elms and found that they moved only a few centimeters away from the inoculation point between September and April. Susceptibility to infection reached a maximum during early July because of the rapid movement of the yeast-like spores in the spring wood vessels (Parker, 1941; Banfield, 1968; Pomerleau, 1965). The location of the inoculation point determines the rate of development of disease symptoms. Trunk inoculations were far more effective than inoculations in the upper branches and twigs (Zentmyer et al., 1946). The number of spores entering the vessel system was definitely correlated with symptom development: the higher the spore load the higher the proportion of leaves wilting. Multiple inoculations produced a higher percentage of disease incidence and also a greater percentage of leaves wilting, than did a single inoculation with the same number of spores (Zentmyer et al., 1946). C. ulmi was cultured from 6-8% of feeding scars made by naturally infested S. multistriatus. Transmission experiments with artificially infested beetles yielded 40-50% C. ulmi cultures. The fungus became established in many feeding scars without leading to further disease development, which indicates that many inoculations due to feeding never lead to true infection (Parker et al., 1941). The pattern of feeding in the twig crotch seems to influence effective inoculation.

According to Ouellette (1962), wounds along the lateral part of the twig crotch showed a higher true infection rate than did injuries in the center of the crotch.

(F) Biology of Scolytus Multistriatus in Michigan

Readio (1935) and Wallace (1940) gave a detailed description of the life cycle and breeding habits of S. multistriatus in the United States. The adults, strongly attracted to recently killed elms or dying elm branches, breed in the trunk portion, the main branches and also the smaller twigs if their diameter exceeds 2.5 cm. The female bores into the bark and excavates a nuptial chamber, after which the maternal gallery is constructed upward along the grain. The female carves egg niches in both sides of the gallery and lays an average of 68.5 eggs (Wallace, 1940). The males help in the construction and cleaning of the egg gallery and the developing larvae make their own tunnels perpendicular to the maternal gallery. The larvae go through 5 moults. Following the last larval instar, they leave the cambium region and bore into the outer bark region where pupation occurs. Depending on geographical area and temperature conditions in a given year, S. multistriatus will have 1, 1½ or 2 generations per year. Fox (1958) investigated the emergence pattern of the smaller European elm bark beetle and concluded that there are 2 complete generations per year in northern Michigan. The first peak occurs in late May or early June and the second population surge takes place between late July and late August. In the northern part of the lower peninsula Truchan (1970) found that the survival of the majority of the second generation was low. This suggests that there may be 1½ to 2 generations per year under Michigan conditions. This knowledge is very crucial for the proper

timing of elm tree protection. After emergence a fair portion of a population will feed in the twig crotches of healthy elm trees before breeding places are found. During this feeding process the healthy elm becomes infested provided the beetle was contaminated with spores of C. ulmi.

(G) Feeding and Breeding Behavior of Scolytus Multistriatus

The peculiar feeding behavior of S. multistriatus is the main cause for the effective transmission of Dutch elm disease in the United States. When the disease arrived in the United States, the basic mechanism of the vector-pathogen-host interaction of this disease problem had been elucidated by European workers. Fransen (1931) was the first to link disease transmission with the twig crotch feeding of elm scolytids. When research on S. multistriatus as a vector of Dutch elm disease started in the United States, the following scientific evidence was available from previous European work (Readio, 1935):

1. Beetles are attracted to dying elms for breeding purposes.
2. C. ulmi produces coremia in the galleries of S. multistriatus.
3. Emerging beetles carry the gelatinous spores externally and internally.
4. Freshly emerged beetles exhibit some sort of "maturation feeding" which can last up to 7-11 days. During this post-emergent feeding in young twig crotches of healthy elms the tree is inoculated with the fungus.

Since this feeding is the point where infection occurs, the factors influencing it were of particular interest. Collins (1938) studied feeding injuries on 500 elm trees, but he was unable to find a correlation between the number of feeding scars and the diameter or age of

the attacked twigs. Wolfenbarger and Buchanan (1939) found that the smaller, lateral member of the injured crotch varied from current season's growth to 3-year old twigs and the age of the larger twigs ranged from 1-5 years. In addition, they gave a detailed description of the actual feeding injury. The adults of both sexes bore into the center or into the somewhat lateral part of the twig crotch where they make holes up to 5 mm in length and about 2 mm in width.

The attacked crotch reacts with extensive callus formation around the scar. If the injury was too severe and the scar too deep, then the lateral twig often broke off completely. These two authors characterized borings in the twig crotches as actual "feeding" and not simply a search for a breeding place. They obtained this conclusion from experiments where feeding beetles lived 7.6 ± 2.6 (SE) days as opposed to 2.2 ± 0.4 days when not feeding. Beetles of each sex of S. multistriatus were confined in glass ball jars together with fresh elm twigs and logs. The average number of feeding injuries per beetle after 7-14 days were 0.91 for males and 1.03 for females. Log injuries were considerably lower with 0.13 for males and 0.33 for females.

The question 'if and how' S. multistriatus is attracted to a healthy elm tree for feeding purposes received wide research attention. Upon examination of a rather extensive elm tree population, Collins (1938) was unable to discern a regular pattern of feeding among elm trees. Some trees had numerous feeding wounds, whereas adjacent trees had few, thus indicating that injuries within the elm population were erratic and clumped. Collins (1938) concluded therefore that 'centers of attraction' are responsible for this erratic but clumped feeding pattern. Wolfenbarger and Jones (1943) distinguished between feeding

near the dispersion point (beetle productive tree) and feeding near the convergence point (beetle attractive tree), but in terms of feeding , they attributed more importance to the dispersion point. From field observations they concluded that beetles attack twig crotches near dispersion points regardless of the immediate proximity of breeding material. The feeding injuries decreased as the distance from the dispersion point increased. From these field-collected data, Wolfenbarger and Jones (1943) calculated a maximum flight distance of about 4 miles. However, it has been demonstrated that wind forces are able to aid the dispersal of small insects such as scolytids, to places which lie far outside the natural flight range (Felt, 1935). The importance of wind drift for the dispersal of S. multistriatus and local outbreaks of the disease were demonstrated in Connecticut and other states by releasing balloons and following their flight path (Felt, 1937). Wallace (1940) attributed dispersal beyond the natural flight range to transportation of beetle-infested material. Of interest is his statement that under ordinary conditions, flight range of S. multistriatus is limited to about one-quarter of a mile. Field observations taken in 1939 indicated that only a small percentage of adults feed on elm twigs at any time. Feeding is by no means obligatory for successful breeding since freshly emerged beetles commenced to construct brood galleries within 24 hours when given the opportunity (Wallace, 1940). More recent research indicated that the search for a feeding site is more random and that healthy elms have no olfactory attractiveness to S. multistriatus. Unlike many other scolytids, S. multistriatus exhibits no positive anemotactic response; that is, flight after emergence is initially not directed against the wind (Meyer and Norris, 1964). The emerging beetles fly into the canopy

of nearby trees and if they encounter a specific feeding stimulant in the bark, they start to feed. Repellents in the bark of other tree species or lack of the attractive compounds reinitiate flight until a healthy elm is found. These feeding stimulants were extracted from bark of U. americana and characterized as p-Hydroxybenzaldehyde and other phenolics (Baker and Norris, 1967; Baker et al., 1968). Norris, at the Dutch Elm Disease Symposium in Delaware, Ohio, in 1967 held that the total feeding response of S. multistriatus involves physical as well as chemical characteristics of the bark. Meyer and Norris (1964) pointed out that crotches with more acute angles and rough bark are preferred over those with less acute angles and smooth bark but no data were given to substantiate the above statement. Dixon (1964) placed more emphasis for the induction of a feeding response on thigmotactic stimulation. Attack response on logs of several unrelated species, including elm, seemed to favor bark with many fissures. In cage experiments twig crotches from pear and elm were almost equally attacked. Dixon (1964) concluded that thigmotactic stimuli, bark fissures in the trunk region and twig crotches on the smaller branches, were more conducive to attack than chemical stimuli.

With respect to effective control of feeding beetles it is necessary to know about the pattern of feeding and the distribution of feeding injuries within an elm tree. Wolfenbarger and Buchanan (1939) found most of the scars in the upper and outer parts of the crown. Whitten (1958) stated also that feeding occurs predominantly at the periphery of the top portion of the tree. The total number of twig crotches increases approximately at the same rate as does the diameter of the tree (Wolfenbarger, 1940). However, tree location has an overriding influence

on the total number of twig crotches. Therefore, the above author could not give a general formula for computing the number of twig crotches based upon the diameter of a given tree.

While no direct attraction seems to be involved during the post-emergent flight to a feeding place, S. multistriatus responds strongly to dying or weakened elm trees as possible breeding places. Chemical changes in the tree during the process of dying, or during extreme stress situations such as drought render the tree very attractive to bark beetle attack. This seems to initiate a strong olfactory response in the beetle (Meyer and Norris, 1967). Even pruning wounds on otherwise healthy elms will attract beetles due to the volatility of degradation products in the wound (Peacock, 1967). Hart et al. (1967) reported an increase in elm die-back caused by C. ulmi on trees which had been trimmed during the previous summer. The authors explained this as an obviously increased attractiveness which was due to the strong odor exuding from trimming wounds and the weakening of trees by heavy pruning. The beetles infested the trees in their attempt to breed near the pruning wounds. Goeden and Norris (1964) theorized that the "lag between cutting and initial attack by beetles represented the necessary time for the production of the volatile, attractive degradation products." According to Meyer and Norris (1967), elm logs were attractive to beetles for a period of 18 hours to 16 days after cutting, with a peak attractiveness on the 11th day. They attributed the decline in attractiveness to a decrease in the amount of the olfactory stimuli exuding from the bark. Further attractancy studies with logs plus females, versus logs alone, and logs plus males revealed that significantly more beetles were attracted to the logs with females. However, the high correlation between the number of

attacking beetles (regardless of sex) and the total number of attracted beetles led them to conclude that the attractancy had its origin in the decaying tree tissue and not in beetles per se. This conclusion is in contrast to the secondary beetle-associated attraction demonstrated by several researchers in other scolytids (Anderson, 1948; Wood, 1962). Martin (1935, 1938, 1946) investigated effects of sunlight, phloem condition and moisture content of phloem on infestation rate by the beetle. Most of the beetles entered during the first 4 weeks after cutting and the total infestation period did not last longer than 10 weeks. Phloem older than 4 weeks had lost its attractiveness to the beetle almost entirely. Moisture content did not appear to influence the extent of the infestation unless it was associated with the phloem condition. Also, logs exposed to sunlight were preferred over logs in the shade.

(H) Control Measures

1. Population Control

When the first Dutch elm disease cases were reported from Ohio in 1931, the seriousness of the problem was realized and, since the infested area was small and localized, diseased trees were eradicated. May (1931) outlined eradication plans and asked for state, federal and private cooperation to initiate a survey for Dutch elm disease infections. In this paper he gave practical instructions for the removal and burning of all diseased trees. Felt (1934a,b) stated that the control of the disease is "largely an entomological one, since the most feasible method of checking this disease, so far as known at present, is by reducing the abundance of the carrier." But he recognized that locating and destroying diseased trees failed to check spread because of several difficulties involved. Scouting for infested trees was relatively

simple in urban areas, but in rural areas and in woodlands, detection and removal were difficult. Above all, the control costs were too high, and generally state and federal funds were inadequate to support an effective eradication program. The following time table for control measures was advocated by Felt (1934 b) and he urged that a shift of emphasis be made from tree removal to sanitation and other protective measures:

Winter (until April): cut and burn sickly or dead trees or parts thereof (removal and sanitation)

May: spray against feeding of 1st beetle generation with lead arsenate or Bordeaux mixture; adjust treatment to control leaf feeders.

August (mid): spray against feeding of 2nd beetle generation

April to November: fertilize weak trees to increase vigor

The primary objective at that time was the elimination of the disease in the United States, although discouraging reports about the progress of the eradication program became more frequent (Worthley, 1935, 1936). Two statements from 1934 expressed the pessimism of many people who were involved at that time in this large-scale control program:

"We must learn to live with the disease" (Felt, 1934 b)
"Only a scientific miracle can save the American elm"
(Dr. O. N. Lining in Felt, 1934 b)

The basic avenues for combatting this problem were already laid out during the incipient stages of disease spread.

Removal and subsequent destruction of diseased and beetle-infested elm trees is as important today as it was 40 years ago when the disease started in Ohio. Usually the infested trees were burned, a practice

which is still recommended. Sanitation, that is, keeping the tree in good health by pruning wilting or dead branches, requires active and well-trained personnel (Felt, 1934 b). After pruning, the wounds should be painted with coal tar creosote to prevent infection by the beetle (Parks, 1936). Recently, the effectiveness of pruning was questioned by Hart (1970). Although removal of the diseased portion of the elm tree led to complete recovery in 19 out of 84 attempted cases, he concluded from this low success ratio (only 23%) and the high pruning costs that this control method is impractical.

Measures such as increasing or maintaining the vigor of the tree by fertilization or spraying against insect defoliators were considered useful in preventing successful colonization by C. ulmi (Felt, 1934 b). The possibility of using trap logs of the two Dutch elm disease vectors, S. multistriatus and H. rufipes, was investigated by Whitten and Baker (1939). In 1935 the tests included felled trees, chemically killed trees, girdled trees and elm logs lying and standing on the ground. Of these the felled trees were most attractive to Scolytus and Hylurgopinus. Further trap log studies in 1937 and 1938 revealed that the trees killed with sodium chlorate were most attractive. The authors did not draw any conclusions with regard to the control potential of elm-wood traps. To prevent emergence of bark beetles from elm wood, Becker (1946, 1947) applied DDT and DDD sprays. A 5% oil solution of DDT gave 81 - 8% control while the wettable powder and the emulsion sprays were less effective. DDD sprays were not effective. Applications of DDT and DDD sprays to prevent scolytid infestations on elm logs were more successful when No. 2 fuel oil solutions were used. Prevention of infestation ranged from 99.7% to 100% for DDT treatments at concentrations

of 0.0625% to 5.0% of actual material as compared to the untreated control. No. 2 fuel oil alone gave 100% prevention while orthodichlorobenzene when mixed 1:8 with No. 2 fuel oil also gave complete prevention. Thirty-six chemicals were introduced by Himelick and Neely (1961) into the sapstream of living elms in order to prevent vector breeding. Sodium arsenite (applied in ax frills) gave complete control in healthy trees and 97% prevention when trees were in the early stages of infection. Sodium iodide was somewhat less effective, but because of its lower mammalian toxicity it should be preferred over sodium arsenite (Himelick and Neely, 1963).

Over recent years the interest in alternatives to chemical control has established a firm place for biological control in many control programs. The development of new, more selective pesticides made it possible to integrate such contrasting concepts as chemical and biological control. Today, the term integrated control comprises all possible control measures, used in combination and adjusted to each other, in order to bring about optimal control of the target organism. Bosch and Stern (1962) summarized the literature on this new control concept and gave a critical evaluation of the subject. Chapter 17 in Insect-Pest Management and Control (Subcommittee on Insect Pests, National Academy of Sciences, 1969) is a more recent and complete discussion of this topic. Dutch elm disease is an example where only the combined use of several control strategies will lead to the desired goal of reduction of disease incidence. Biological control has been neglected for the control of Dutch elm disease vectors for many years. In 1964, a braconid wasp, Dendrosoter protuberans, a native parasite of Scolytus multistriatus was shipped from France to the U. S. Forest Service Laboratory at Delaware, Ohio. Further shipments went to Michigan State University at

East Lansing, Michigan, in 1965. Valek (1967) and Truchan (1970) reviewed the literature dealing with the natural enemies of S. multistriatus and discussed the potential of the indigenous and introduced natural enemies in terms of population control.

Early prominence was given to resistant or immune elm species or elm varieties. Buisman (1931, 1932, 1934) started a worldwide collection of elm species in Holland. The Asiatic species, in particular the Chinese elm, U. parvifolia, and the Siberian elm, U. pumila L. and its variety Ulmus pumila pinnato-ramosa Henry showed high degrees of resistance.

Resistance breeding began in 1928 in the Netherlands (Westerdijk, 1928). The first resistant clone was released in 1937, but it was susceptible to another fungus, the coral spot canker, Nectria cinnabarina (Tode x Fr.) Fr. The next clone from 1948, though resistant to Dutch elm disease and the coral spot fungus, did not meet requirements in respect to growth rate and form. In 1961 the 'Commelin' elm was released. It had less resistance but it had the desired growth and shape characteristics (Heybroek, 1961). The highly resistant 'Groeneveld' elm appeared 2 years later but this clone had a limited growth rate (Heybroek, 1963).

In the United States, resistance breeding programs were not initiated until recently. Selection programs are under way at several locations. Neither in Europe nor in the U.S.A. did possibilities of resistance breeding against vector feeding receive much attention.

Heybroek (1969) gave the following reasons why breeding for vector-resistant elms was not further investigated: (a) there are too many vectors involved, (b) forcing beetle attack is too difficult, and

(c) disease resistance mechanisms are readily available in elms.

The question of elm resistance to C. ulmi was fully reviewed by Bingham et al. (1971). The recent discovery of a new, more aggressive strain of C. ulmi from southern England indicated the possibility that already released resistant elm clones might again be threatened by the disease (Holmes et al., 1972).

The newer developments in insect control might find useful application in the fight against Dutch elm disease vectors. The potential of sex attractants (pheromones) given off by virgin females when they enter an elm log for breeding is under scrutiny at present at the U. S. Forest Service Laboratory in Delaware, Ohio, and at the University of Syracuse, New York. A sex attractant could be used effectively in connection with a sterilant.

2. Chemotherapy against fungus

A whole generation of pathologists has worked on the chemotherapy of DED infections. It is mostly a problem of getting the active material into a soluble state and introducing it into the conductive tissue of the tree. The extensive literature will not be reviewed here. Mention will be made only that a new tree injection system in combination with an effective fungicide showed promise in curing infected elm trees (Northeastern Forest Experiment Station, Upper Darby, Pennsylvania, Photostory No. 19, 1971).

3. Control of vector feeding

The effectiveness of spraying to prevent S. multistriatus feeding seemed doubtful before the new organic insecticides arrived. Fransen (1931) considered spraying as useless, but Felt and Bromley (1938) observed a reduction in the intensity and depth of feeding scars on small

potted elm trees, after they were treated with lead arsenate and a sticker. They spraying equipment at that time was not satisfactory to cover a mature tree sufficiently and guarantee protection. Thus, the effectiveness of spraying was largely a problem of finding the right material and the proper application equipment. With the discovery of the persistent broad-spectrum insecticide DDT and progress in spray equipment technology, it became feasible to obtain long-lasting protection against vector feeding. Becker (1946, 1947) evaluated several concentrations of DDT ranging from 0.0625% to 5% applied as emulsions or wettable powders against S. multistriatus. Materials were applied by means of a compressed air-sprayer to branches in full foliage. Lasting protection was obtained with the higher concentrations, but the disadvantage was their phytotoxicity to neighboring trees. Feeding trials indicated that emulsions were more effective than wettable powder formulations. Preliminary experiments with a mist blower (Buffalo turbine blower) resulted in good coverage of the lower portion of 60-65 ft. elms while the upper part had little coverage. Concentrations of up to 15% were used either in the form of oil solutions or water emulsions and best coverage was obtained when there was no wind. Dimond et al. (1949) also experimented with DDT formulations (0.5, 1.0 and 2.0 lbs per gallon). In field tests in 1947, up to 1 gallon was applied per tree. Feeding tests showed little control in top and center portions of the treated trees but it was concluded that the dosage per tree was too low. The following year an average of 2.6 gallons was used per tree. Good control, also in the upper tree region, was obtained as a consequence of the increased amount of spray per tree. The Elm Bark Beetle Conference (Scanlon, 1948) discussed elm protection in detail. Enough evidence

was accumulated to prove that only prefoliar sprays gave satisfactory protection. Further, it was felt that 100% coverage was necessary since one feeding vector might be enough to kill the tree.

Dimond et al. (1948) attempted to give exact guidelines for the amount of spray for a given tree and calculated a regression between dosage and diameter of the tree. Because of the poor correlation between diameter and volume of the crown region, a regression of dosage on diameter was not very useful. Later recommendations included the statement to "spray each elm thoroughly on an individual basis."

Commercial DDT formulations were found to be too injurious for trees. Therefore, special formulations were developed which were not phytotoxic and which provided persistent deposits (Whitten, 1949). The following formulations were recommended:

FORMULATION A: 16 pounds of technical DDT dissolved in $2\frac{1}{4}$ gallons benzene and 1 gallon Velsicol AR-50 (mono- and dimethylnaphtalenes) plus 1 pint Triton X-100 (arakylpolyetheralcohol) as emulsifier

FORMULATION B: 16 pounds of technical DDT dissolved in 4 gallons of xylene plus 1 pint Triton X-100 as emulsifier

The above emulsifiable concentrates were diluted with water to make 100 gallons of spray emulsion in each case. Formula A and B were suggested for hydraulic sprayers only. About 25-30 gallons spray were recommended for an average 50-foot elm tree.

FORMULATION C: 20 pounds of technical DDT dissolved in 5 gallons of xylene and $2\frac{1}{2}$ gallons Acme white oil plus $1\frac{1}{4}$ pint Triton X-100 as emulsifier

Formula C was recommended only for mist blower application. The concentrate was diluted with water to make 20 gallons of spray. Two or

three gallons was the average amount of spray used for a 50 foot elm tree. The first treatment was applied shortly before the appearance of elm flowers and leaves while the second application was usually 2-3 months later with the same formulation but only half as concentrated. The purpose of the second treatment was to control defoliators and vectors of the phloem necrosis.

Mist blower equipment had the following specifications: output of 6000+ cubic feet of air per minute at a velocity of 120 miles per hour. One to four gallons of spray concentrate were injected into the air stream at 40 pounds of pressure. Plumb (1950) used mist blowers which were modified No. 12 Bean-Vapo dusters. Output was 8400 cubic feet of air per minute at a speed of 140 miles per hour. Four nozzles injected roughly 1 gallon of spray into the air stream per minute. The formulations used were 12.5% DDT emulsions and were similar to Formula C of Whitten (1949) as suggested for mist blower application. The dosage per tree was calculated on the basis of diameter. A 10-inch tree received 1 gallon, and for each additional 5 inches, another gallon was added. The average dosage per tree in these field experiments amounted to 4.2 gallons of spray. Over a 2 year period 9% of the sprayed trees became diseased, whereas 39% of the trees in the unsprayed plots fell victim to DED. With respect to an additional application it appeared that 2 treatments (dormant and summer application) did not reduce disease incidence more than that which was achieved with 1 dormant application alone.

Matthysse et al. (1954) conducted bark beetle assays and chemical analysis to evaluate prefoliar DDT treatments. Emulsions did not weather as fast as suspensions probably due to better penetration into the bark. A 27.5% DDT concentrate (xylene, Triton X-100) was diluted with water to

make a 13.7% emulsion for the mist blower application and a 2% emulsion for the hydraulic sprayer. Samples were taken from the lower portion of the tree (up to 50 ft.) and from the upper portion (50-70 ft.). The lower portion was usually overdosed whereas above 50 ft. the coverage was adequate initially, but weathering reduced the deposits to inadequate levels within 10 weeks. Shell #7 horticultural oil added to the formulation for the mist blower seemed to favor tree top deposits. Kerosene solution of the same concentration applied by mist blower was not superior to the emulsion. Xylene emulsions caused the least foliar injuries, but use of kerosene and #7 oil increased phytotoxicity. The disease incidence in the treated plots was low with only 1 tree lost over a period of 3 years. However, 29 out of 369 check trees became diseased during the same period. Miller (1951) compared the toxicity of several organic insecticides to H. rufipes and S. multistriatus. Twigs were dipped in 0.05%, 0.25%, 1.0% emulsions (xylene, Triton X-100) of DDT, dieldrin, lindane, parathion, chlordane, toxaphene and methoxychlor. Emulsions (0.05%) of the respective insecticides caused 100% mortality to feeding S. multistriatus after 3 days weathering but methoxychlor caused only 90% mortality. After 5 weeks weathering, the mortality was highest for dieldrin with 95%, next were DDT and lindane with 85% and methoxychlor was low with 45% mortality. A general correlation of bark beetle data and chemical analysis indicated that good protection was guaranteed with 40 micrograms of DDT/cm^2. Deposits below 20 micrograms of DDT/cm^2 gave only poor or no protection against feeding. Somewhat modified DDT formulations for (A) hydraulic sprayer and for (B) mist blower were recommended by Whitten (1958):

	A	B	
DDT	32	26	(percent by
xylol	59	48	weight)
emulsifier (Triton X-100)	2	2	
acetone	7		
white oil		24	

The acetone in formula (A) was added to improve the DDT holding qualities at low temperatures (below 50° F). The white oil in formula (B) slowed down the volatility of the DDT-xylol solution and prevented crystallization of DDT before the spray mist reached the tree tops. The emulsifiable concentrates were diluted so that 100 gallons of hydraulic spray contain 16 lbs. of DDT and 100 gallons of mist blower spray contain 100 lbs. of DDT. The same author stated also that methoxychlor is as effective as DDT and less toxic to birds, but DDT is cheaper. The drawbacks of the continued use of DDT became apparent and people involved in shade tree protection looked for alternatives. Doane (1958) reported on the residual behavior of four insecticides as compared to DDT. Small elm trees (10-12 ft.) were sprayed until run-off with emulsions of varying concentrations of DDT, dieldrin, heptachlor, lindane and malathion. Twig samples were bioassayed 4, 8, and 13 weeks after spraying. DDT (1% and 2%) was most effective with respect to beetle mortality and prevention of feeding scars. Dieldrin (1%) caused high beetle mortality but this was not reflected in a low incidence of feeding scars. Lindane (1%) killed 60% of the beetles after 13 weeks and was comparable to DDT (1% and 2%) in the prevention of xylem scoring. Doane (1962) compared 4 organophosphate insecticides and methoxychlor with DDT. Again, DDT was superior, but although methoxychlor caused only low mortality the feeding response (xylem scoring) was comparatively weaker. Touhey and Bray (1961) experimented with 42 chemicals and investigated their potential as feeding

repellents for S. multistriatus. Decanoic acid and 3 other chemicals showed considerable bark beetle repellency when applied to the twig crotch. Combinations of each of these 4 chemicals with DDT were more effective in reducing twig crotch injuries than was DDT alone.

Fall applications of DDT-xylene emulsions and DDT-xylene-white oil emulsions gave the same protection for the ensuing season as did pre-foliar spring applications on the basis of feeding assays of treated twigs and by chemical analysis of residues on bark. However, the second formulation with the white oil seemed to favor higher deposits (Thompson, 1965).

The ability of systemic insecticides to prevent feeding by S. multistriatus was investigated first by Al-Azawi and Casida (1958). The uptake of organophosphate systemic insecticides, such as demeton, dimefox, Thimet, Chipman-6199, but cut elm branches and their toxicity to feeding European elm bark beetles was investigated in the lab. Of those listed, Chipman-6199 was least toxic to S. multistriatus but when it was implanted into the trunk of 40 ft. mature elm trees, it was readily translocated in the foliage and persisted there for a long time. In 48 hour feeding trials the mortality reached 86% 16 days after implantation of the systemic and fell to 56% after 81 days. In other field experiments, 20 ft. elm trees in forest situations were treated with Chipman-6199 implanted into the trunk. Scolytus beetles artificially infested with C. ulmi were caged for a week on branches of treated and control trees. No disease incidence occurred in the treated plot and feeding was reduced by 52.7% from the control. The mean scar length for the control trees was 5.7 mm, but for the treated trees only 1.5mm (Al-Azawi and Norris, 1959; Al-Azawi et al., 1961). Great hopes for eventual control of S. multistriatus were connected with the new organophosphate chemical, Bidrin, suggested

for stem injections (Shell Chemical Company Instruction Manual, 1964). Norris (1959) injected 20 grams technical Bidrin per 2 inch d.b.h. and obtained protection for 21 days. Similar encouraging results were reported by English and Hartstirn (1962), however, phytotoxic effects were noticed at levels slightly above the concentrations necessary to kill the beetle. In 1965, extensive field experiments were performed under the supervision of the Northeastern Forest Experiment Station, Delaware, Ohio. Michigan State University participated in this effort and conducted several field trials. Butcher et. al. (1966) tested Bidrin and other candidate systemics and concluded that their control potential was not promising. Repeated injections (3-4) of Bidrin resulted in a girdling effect and scattered phytotoxicity on the foliage (Lamdin, 1969). Williams (1969) suggested that the disease-susceptible period in southern latitudes where 3 beetle generations develop might last longer than in northern areas; therefore,control with shortlived systemic insecticides must be modified to provide longer protection against feeding.

Increasing public concern about the continued use of DDT and all its side effects, which came to light in the early 1960's, called for an effective substitute in DED control programs. The only material which seemed to offer the desired insecticidal and residual properties and which had low toxicity to mammals and birds, was methoxyclor. Although Miller (1951) found the residual life of methoxychlor to be too short, more recent reports indicate that methoxychlor could be just as effective as DDT when applied in mid-April before bud break (Whitten, 1958, 1960; Norris, 1961; Hafstadt and Reynolds, 1961; Wooten, 1962). Typical methoxychlor concentrates had 25% active ingredient, 2% inert material plus emulsifier with the remaining 75% xylene. The mist blower sprays were

diluted with water to 12.5% emulsions and the hydraulic sprays were used as 2% emulsions (Wallner and Hart, 1968).

In the Dutch elm disease conference in 1967 in Delaware, Ohio, the possible alternatives to DDT were discussed in detail. The major problems with methoxychlor were that to be effective it must be applied in the spring and tree tops are difficult to cover sufficiently with the available spray equipment. The conference called in particular for further research in the area of application technology and for intensified tests with new methoxychlor formulations. Experimental aerial spraying of elms with the helicopter was reported by several participants in this meeting (Peacock, 1967). Wallner and Leeling (1969) found methoxychlor deposits (quantified by gas-chromatographic analysis) to be 11 to 25 times higher on helicopter sprayed trees than on mist blower sprayed trees. In the latter experiments a Bell G-2 helicopter was utilized with an output rate of 7 gallons per acre and with a 50 ft. swath width. Deposits were not consistent for 1966 and 1967, probably due to different boom length, nozzle types and flight height above tree tops. Emulsions of 6.25% and 12.5% methoxychlor concentrations gave comparable deposits with the helicopter. The average amount of 12.5% spray per tree was 1 gallon for the helicopter and twice as much for the mist blower. During mist blower as well as helicopter spraying a substantial amount of spray will drift and contaminate a larger area. Wallner et al. (1969) investigated the problem of spray drift during helicopter application and followed the movement and fate of methoxychlor in the soil and in water on the campus of Michigan State University. Four methoxychlor formulations, which had the same amount of active material but differed in ingredients, were compared. The longest lasting deposits were produced by a 12.5% emulsion

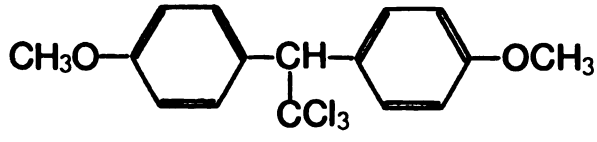
plus Dacagin[1)] (a polymeric gelling agent) which was added to prevent excessive drift (4.2 pounds per 100 gallons of 12.5% spray). Dacagin appeared to produce more uniform and somewhat larger droplets and significantly prevented spray drift.

Low spring temperatures might delay a spraying operation considerably. Epstein (1969) added methanol (30%) to a 12.5% methoxychlor emulsion in order to depress the freezing point of the formulation to -7.77° C. Deposits appeared to be comparable to those produced by a straight 12.5% emulsion.

Field tests in Wisconsin (Barger, 1970) where helicopter and mist blower were used showed higher mortality and less xylem scoring in the mist blower plots. Although the results of the bioassays were in favor of the mist blower treatment, the disease incidence in both helicopter and mist blower plots did not differ. For future programs Barger (1970) suggested that saturation treatment (spraying of all trees in a given area) may not be essential for controlling disease incidence. Using only disease incidence as a valid criterion, he stated that helicopter application achieved the same result as the mist blower spraying.

(1) Methoxychlor: Properties and Performance

The chemical synthesis of methoxychlor was accomplished in 1893 (Elbs) but its insecticidal properties were not discovered until the time of growing interest in analogues of DDT (Läuger et al., 1944). The chemical structure is:



1,1,1 - trichloro - 2,2 - bis (p-methoxyphenyl) ethane

1) Diamond Alkali Co.

The use of methoxychlor for the past 25 years was actually very limited partly due to the great popularity of DDT, which is much cheaper to manufacture. The physical properties of methoxychlor are also similar to DDT, with an extremely low solubility in water, a melting point of 88° C and a low vapor pressure (Metcalf, 1955). UV radiation, heat and oxidation cause only slow degradation to non-insecticidal, more polar components.

Methoxychlor and DDT differ significantly in their toxicity to mammals. The oral LD_{50} for methoxychlor is larger than 6000 mg per kg in rats while DDT has an oral LD_{50} of around 250. Fish, on the other hand, are very sensitive to methoxychlor. The qualitative degradation of methoxychlor within the physiological system of insects and mammals has not received much attention until recently. The elucidation of the degradative pathway of methoxychlor in organisms by Kapoor et al. (1970) established this compound as a highly biodegradable relative of DDT (see Metcalf, et al., 1972). Consequently, high biodegradability and low mammalian toxicity make this insecticide a good choice for spray programs in urban communities (for instance, for Dutch elm disease control).

(J) Structure and Toxicity of Insecticidal Deposits

Three major steps are involved from the time the toxicant is applied until it exerts its action in the target organism. Step one is concerned with the actual application of the insecticide on a surface. The next step is the transfer of the deposit to the target insect and is frequently referred to in the literature as 'pick-up'. The deposit is taken up either on contact in the form of a small crystal or orally during the feeding process. The penetration of the toxic substance to the site of action within the insect is then the final step. Barlow

and Hadaway (1952) and also Hoskins (1962) have reviewed and treated this subject in detail. Only the key points necessary for the understanding of the matter are repeated here. The structure of the pesticidal deposit depends on several factors of which the most important are:

1. technology of the spray equipment (e.g. aerial or ground application, nozzle size and type, line pressure, speed during application, etc.).
2. formulation (dust, suspension, emulsion or solution; concentration; additives such as stickers or anti-drift materials).
3. type of supporting surface (foliage, bark, soil, brick wall, etc.).
4. climatic conditions during time of application (temperature, humidity, precipitation, air movement, etc.).

Decomposition properties and contact toxicity on a given surface depend to a high degree on the structure of the pesticidal deposit and the environmental conditions. The pesticidal deposit can be looked upon as a physical system whose behavior is controlled by: (a) the formulation employed, (b) the supporting surface, and (c) the surrounding atmosphere (Hoskins, 1962).

The underlying question is which formulation gives an optimum effect on a given surface. On an absorbant surface, such as wood or bark, penetration into the substrate varies with the type of formulation. In emulsions or solutions the toxicant is dissolved in the carrier and penetrates with the solvent. As for suspensions, the active ingredient stays on the surface and only the carrier penetrates. The type of deposit created by an emulsion or a solution is highly influenced by the volatility of the solvent (Hoskins, 1962). Schmitz and Goette (1948) looked at the depth of penetration achieved by various DDT solutions. Blocks of poplar wood were treated with DDT solutions (200 mg/square foot). The percentage recovery of DDT at several depths is given in the following

table for 2 solvents:

Solvent	% Recovery	Depth of Layer (mm)
Kerosene	30%	0.03
	60%	0.15
	74%	0.92
Xylene	38%	0.03
	73%	0.15

Thus, highly volatile solvents such as xylene resulted in less penetration due to fast evaporation from the surface.

The physical state of the insecticide on a particular surface is very important in regard to toxicity (Barlow and Hadaway, 1952). Upon evaporation of the solvent a mixture of crystals, supersaturated and supercooled droplets are formed. Sometimes complete crystallization can be induced by mechanical stimuli (e.g. a crawling insect) as is the case with DDT. A reduction in toxicity goes usually hand in hand with complete crystallization. Also, the size of individual crystals has a bearing on 'pick-up' and therefore on contact toxicity. Stickers, such as glue, gelatin, casein and flour, increased persistence of the deposit but made good 'pick-up' more difficult. This is, of course, especially a problem where contact toxicity is more important than oral toxicity. Therefore, Barlow and Hadaway (1952) suggested that a balance must be found between the availability of a deposit to the insect and resistance to weathering.

The problem of bark deposit structure and toxicity to bark beetles on pines was fully reviewed and dealt with in a bulletin by R. L. Lyon (1965). Although he worked primarily with Ips confusus (Lec.) on ponderosa pine, generalizations for other bark beetles and tree species are valid.

The deposit structure on and in the bark is related to toxicity. Two types of deposits can be distinguished as to location and also structure: (a) surface deposits on the bark (important for contact toxicity), and (b) tissue deposits within the bark (important for oral toxicity).

Solutions and emulsions mainly form tissue deposits. Since variability in the crystallization pattern of surface deposits on conifer bark was too great, Lyon (1965) suggested using uniform fiber board panels to study the toxicity of surface deposits. Lyon's study bore out that tissue deposits are more toxic to emerging beetles than are surface deposits; in addition, deposits within the bark are protected from weathering and are selective in the sense that other insects crawling on the bark surface do not come in contact with the insecticide. Lindane proved to be the universal chemical against bark beetles for 'pre-attack' protection sprays and for control of the breeding population. Solutions with their high degree of penetration were more effective than emulsions and suspensions resulted in the least degree of protection. At higher concentrations, however, emulsions were as effective as solutions.

The life span of insecticidal deposits is often a problem and greater persistence is desired in many cases. Certain PCB compounds (polychlorinated biphenyls) act as vapor-depressing agents when applied with the insecticide (Sullivan and Hornstein, 1953; Sullivan et al., 1955). Crystallization of surface deposits never occurred when formulated with a PCB agent. Aroclor 5460 [1] reduced decomposition in many instances where it was added to the basic formulation. Nielsen (1959) was able to extend the effectiveness of a 12.5% BHC spray with Aroclor 5460 against Dutch elm disease. Over a period of 2 months a BHC spray alone decomposed very rapidly. The suspicion that PCB's are biologically active

1) Monsanto Chemical Co.

and that they can be concentrated in the food chain of ecological systems made further use impossible. Stickers, although their primary purpose is different, also have the ability to retard decomposition by forming a coating over the deposit (Barlow and Hadaway, 1952). Dacagin, a thickening agent, was thought to slow degradation of methoxychlor deposits on elm bark (Wallner et al., 1969).

(K) Spray Technology

The many kinds of spraying equipment differ widely in the pattern, density and structure of deposits they produce with a given formulation. Before the advent of DDT, it was necessary to enhance the action of the few available insecticides with careful improvements in the formulations and spray equipment. The new organic insecticides, especially DDT, were at first too powerful to make application, formulation and deposit structure studies necessary. This attitude changed as resistance developed in a number of pest populations and tolerance levels were established for residues on agricultural commodities (Hoskins, 1952).

Basically, two types of application techniques must be considered here for Dutch elm disease control: (a) aerial application by helicopter or aircraft, and (b) ground application by mist blower or hydraulic sprayer.

These two basic types differ not only in the way the spray is applied (from the air or from the ground) but also in many other respects such as spray pattern, degree of coverage and pesticidal drift. Aerial application usually gives a discrete spray pattern. The density and size of droplets depend mainly on nozzle design, spray pressure, formulation and speed during application. It is common knowledge that the toxicity for a given amount of insecticide increases as droplets become finer

(Hoskins, 1952). LV and ULV technology employ devices which break up the concentrated spray into extremely fine droplets which are subject naturally to enormous drift (Courshee, 1959; Yates, 1960). Drift is one of the major problems of aerial application; it can be defined as the portion of spray that is moved away from the target area by wind or other meteorological factors (Maksymiuk, 1971b). Many factors contribute to the drift problem including the formulation itself. Special spray equipment in conjunction with certain formulations will help to reduce drift. Reducing the spray pressure and aircraft speed during application will result in coarser droplets and subsequently in reduced drift. The new Microfoil spray system for helicopters appears to be a significant development in this direction. According to Kirch (1971) this spray system produces patterns of large uniform droplet size without the addition of adjuvants. Maksymiuk (1971b) lists as further potential factors which contribute to the drift problem application methods and meteorological conditions, especially air currents. Thickeners have been added to formulations to increase viscosity and droplet size. If the spray becomes too viscous the coverage of the target plant will become very poor. Maksymiuk (1971b) concludes that a desirable drop size spectrum with little drift hazards will be accomplished only by spray equipment design and formulation research.

Ware et al. (1969) compared the spray drift of aerial application versus mist blower in cotton fields. The mist blower caused more drift for two reasons: (a) smaller droplet size and (b) initial horizontal velocity in a moving air stream. Butcher et al. (1966) reported significant drift of methoxychlor spray to the lee side during mist blower spraying of elms at low wind speeds (between 2-5 mph). Spray applied to elm

trees by helicopter drifted excessively at a wind speed of 3 mph. When Dacagin was added to the formulation, drift was greatly reduced (Wallner et al., 1969). Filter paper was successfully employed in this study to trap drifting spray.

For the assessment of spray deposits on target and non-target areas, Maksymiuk (1971a) added fluorescent dyes to the formulation. The amount of deposits and the spray pattern were determined directly from cards or aluminum plates. Oil sensitive cards also found considerable use for the immediate assessment of deposits. However, they can be utilized only for oil-based sprays.

(L) Bioassay

A bioassay is, by definition, a quantitative appraisal of biologically active substances (such as insecticides) by the amount of their action on living organisms (such as S. multistriatus) under standardized conditions. However, the conditions under which bioassays of treated elm twigs with S. multistriatus were conducted by different researchers were surprisingly variable. This absence of standardization naturally prohibits direct comparisons of the results of these assays. The common technique for feeding trials was the 1-gallon container with treated elm twigs. A certain number of S. multistriatus beetles was introduced into the container together with the twigs. After a day or longer, the feeding response, mortality, or other responses were measured. The following table summarizes the conditions of the bioassays performed by several researchers and is taken from an unpublished manuscript by Lincoln (1968). The number of beetles and the number of twig crotches per jar varied greatly; it is conceivable that these two factors have a significant influence on feeding and other responses. The conditions of the twigs

Table 1. Comparison of bioassay procedures used by investigators in evaluating the effectiveness of insecticides for controlling S. multistriatus.

Investigator	Exposure Period	Temperature	Relative Humidity	Beetles Per Jar	Crotches Per Jar
Whitten (1945)	until feeding stopped in checks	--	--	100	50-100
Matthysse, Miller, and Thompson (1954)	up to 72 hrs.	74 F.	60%	--	--
Al-Azawi and Casida (1958)	48 hrs.	--	--	5-10 per vial	2 treated sections
Doane (1958)	24 and 48 hrs.	72 and 48 F.	--	25	35
Norris (1960)	24 hrs. 48 hrs.	-- --	-- --	10 10	75 75
Doane (1962)	24, 48, 60, and 72 hrs.	--	--	12-18	60 $\pm$ 12
Butcher, Ilnitzky, and Shaddy (1966)	until last beetle died	room temp.	--	5	one 6 in. long twig

to be assayed, especially moisture content, freshness, and previous storage conditions were poorly documented or absent. Information about the light conditions during the feeding trials is also lacking for most assays. Another factor which is of significance for a standardized bioassay is the uniformity of the bioassay material. In the 1-gallon container each beetle has the possibility to move around freely and come in close contact with the residue. Cut ends of twigs are very attractive to the beetles and they will readily feed there. Under natural conditions, the twig crotches are the points of feeding and infection and a precise assessment of the deposits at these points is necessary. It has been argued that the 1-gallon jar approximates natural conditions since the overall effect of the insecticide (contact and oral toxicity) is measured. Of major interest however is not the overall effect alone but the response at the feeding sites. Lincoln (1968) pointed out these and other disadvantages of the 1-gallon jar technique and proposed a new bioassay technique with one beetle caged in a small perforated brass cylinder in each individual crotch. Barger et al. (1971) illustrated and described this new bioassay technique in a recent publication. With this method the beetle is forced to feed in a specific twig crotch, instead of anywhere on the twigs. Also, the movement of the beetle is confined to the area of one twig crotch instead of the whole 1-gallon jar. Finally the confinement in one twig crotch allows for uninterrupted feeding.

The condition (freshness, moisture content) of the bioassay twigs is also critical for a uniform feeding response. Due to the large number of bioassays twigs had to be stored, sometimes for several weeks, in a freezer at -10° C. Barger et al. (1971) looked at the possible

effects of pre-storage treatments and prolonged storage on the feeding response. Quick-freezing of systemically treated elm twigs in liquid nitrogen, freezing in dry ice, or no pre-freezing at all before storage at -10° C up to 6 months did not influence the feeding response. Also, the toxicity of the systemic dicrotophos did not change appreciably over the total storage period.

The responses most commonly measured were beetle mortality and xylem scores. Al-Azawi and Norris (1959) stressed the importance of scar length for effective inoculation, based on experimental evidence. According to these authors, scar lengths above 3mm are conducive to successful transmission of the fungus. Butcher et al. (1966) recorded average survival time, number of hours the longest survivor lived, and grams of frass produced during bioassay.

Ideally, contact toxicity is determined by the surface which will be the solid support for the deposit in a true situation. For various reasons researchers use artificial surfaces such as filter paper, glass plates, etc. Since the structure of the deposit and, therefore, its toxicity is partly influenced by the underlying surface, inferences drawn from the test surface to the real surface on which the insecticide will be applied eventually are questionable. Lyon (1965) intended to compare the toxicity of bark deposits to bark beetles but because of the difficult task of obtaining uniform deposit patterns, he chose fiber board squares with absorption qualities similar to bark.

Cuthbert et al. (1970) tested S. multistriatus and its introduced parasite Dendrosoter protuberans for contact toxicity of DDT and methoxychlor on treated filter paper in plastic petri-dishes. There was no real difference between the EC_{50} for the parasite and the beetle. However, DDT was 100 times more toxic than methoxychlor.

I. Aspects of the Feeding Behavior of Scolytus multistriatus Marsham.

Introduction

It was felt that only a thorough understanding of the feeding behavior of S. multistriatus could give the needed basis for elm tree protection and for successful prevention of infection. A facultative type of behavior feeding serves probably only to extend the life span of the insect until the search for a breeding site is successful. Of primary interest was the question: Does the feeding attack on elm trees follow a certain, consistent pattern? The literature offered little information on this question other than statements by Wolfenbarger and Buchanan (1939) and Whitten (1958) that feeding occurred primarily in the upper, outer portion of the crown region.

For this purpose, 6 elms were selected to study the regional distribution of feeding injuries along a vertical and horizontal gradient. One can not emphasize enough the importance of this knowledge for the proper choice of application techniques.

Although the chemical aspects of feeding stimulation have received considerable attention (Baker and Norris, 1967), the importance of physical characteristics of the twig crotch to induction of feeding was only speculated upon (Meyer and Norris, 1964). Certain characteristics of the twig crotch, such as the angle between the 2 lateral branches, formation of the crotch base, position of the feeding scar, roughness of bark, etc., were recorded from large twig samples from 2 trees. It was hoped that certain characteristics were associated with feeding

injuries. The idea was that this type of information could be useful in an eventual elm breeding program which sought to develop an elm with physical twig crotch characteristics which are not favored by S. multistriatus. However, as Heybroek (1969) pointed out, an elm resistant to S. multistriatus would still be attacked by other vectors.

Materials and Methods

Six elm trees, designated A to F, were selected in the Lansing area and sampled at 3 height levels: 5, 10, and 15 meters. These elms had not been sprayed over the previous 5 years, and in this respect the natural feeding pattern was not disturbed. Tree height ranged from 15 to 18 meters. Samples of 50-70 twig crotches were taken at random from 5 points in each height level: from 4 compass points and the center (Figure 2). A lightweight aluminum pole pruner was used to sample elm twigs at the respective height levels which permitted sampling tree heights up to 16 m. Total number of twig crotches and number of feeding injuries were recorded from each sample. The number of scars in places other than twig crotches (mainly in leaf axils) was not included in the figure for total attack. Also, the position of branches at the 3 height levels was noted as follows: (a) those pointing upwards, (b) those which were horizontal, and (c) those which were pointing downwards. The percent feeding attack was computed for each sample point and the mean attack for each height level represents an average of the percentage figures from the 5 sample points (Table 2).

It was suspected that there might be differences in the feeding attack (or feeding preference) not only between height levels but also between vertical subdivisions of the tree region. To explore that possibility, each of the 6 sample trees, A to F, was subdivided into

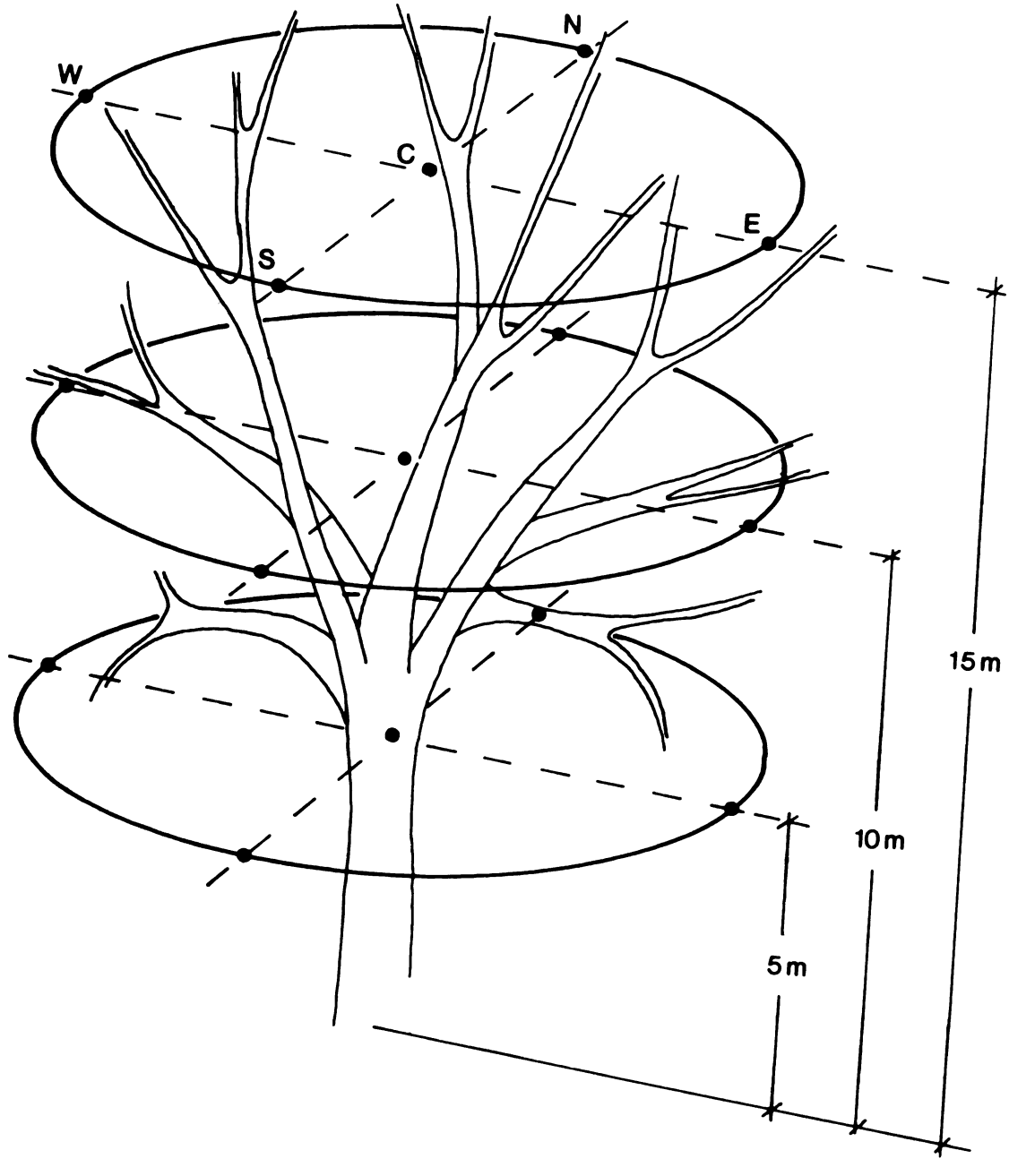


Figure 2. Sampling scheme for mature elm trees: location of sample points in three height levels.

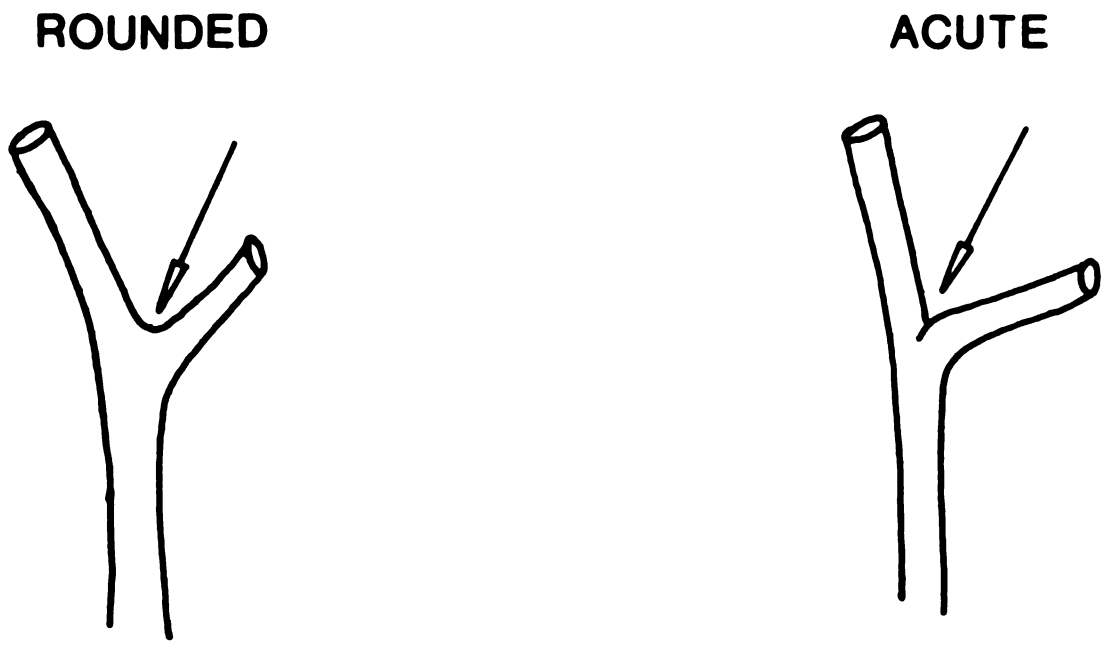


Figure 3. Type of twig crotch base.

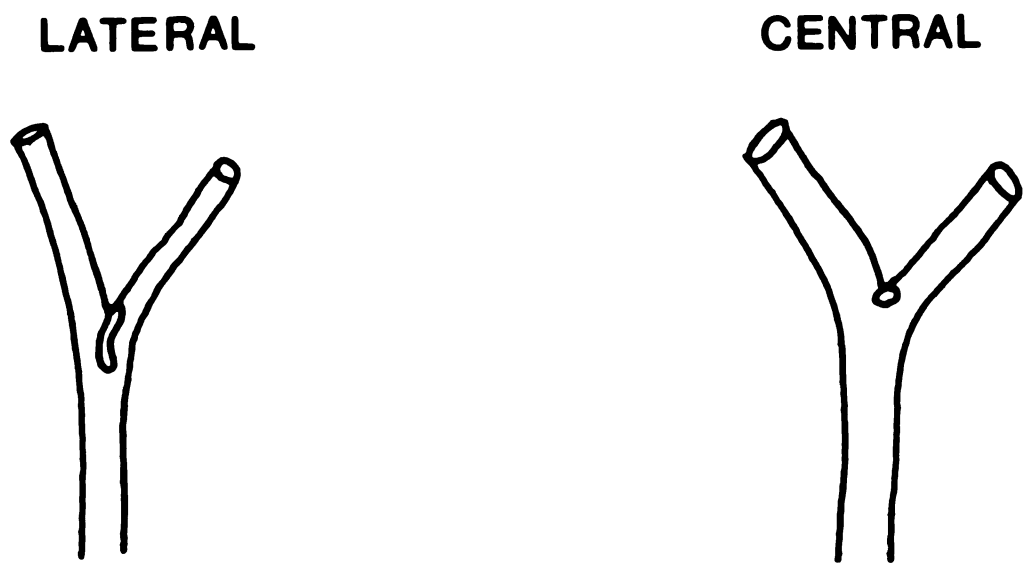


Figure 4. Type of twig crotch feeding by S. multistriatus.

5 sections: N, S, E, W, and C(Center) (Figure 2). In order to demonstrate the consistency of the horizontal feeding pattern over the 3 height levels the twig-crotch injury data (Table 2) for each tree were subjected to Friedman's ANOVA according to rank (Siegel, 1956, p. 166). A X^2 value was computed for each sample tree and compared with the tabulated value for significance (Table N in Siegel, 1956). Tree A was deleted from this analysis because of the low attack rate at all levels.

In order to study the association of certain morphological features of twig crotches to feeding, part of the samples from tree B and D were analyzed and the following characteristics recorded:

1. Angle between the 2 lateral members of crotch; it was measured by placing the twig crotch on polar coordinate graph paper (accurate to 5^{o}).
2. Crotch base: either rounded or acute (Figure 3).
3. Roughness of bark: either rough or smooth.
4. Injuries by vector feeding.
5. Position of feeding scar: either lateral or central (Figure 4).

300 twig crotches were analyzed in this fashion from tree B, 100 from each height level. A total of 400 twig crotches were analyzed from tree D; 100 from the bottom level and 150 from the center and top level respectively. Characteristic 3, roughness of bark, was discarded for the statistical analysis because of the difficulty in making a distinction between smooth and rough bark.

Several t-tests were performed on the twig-crotch angle data to test the hypothesis that the mean angles of attacked and unattacked twig crotches were not significantly different. No t value was calculated for tree B at the lowest height level because the number of attacked

Table 2. Percent feeding attack at 5 sample points in 3 height levels.

Tree	Height Level	N	S	E	W	C	Mean Attack	Attacked Leaf Axils
A	5							
	10	6.3		4.6		3.6	2.9	
	15	3.7	1.9	10.0	5.3	1.8	4.5	
B	5	10.0	5.6	13.6	9.7	7.4	9.3	
	10	50.7	30.4	37.5	21.6	33.9	34.8	
	15	75.7	48.0	65.3	71.9	49.5	62.1	
C	5	50.9	8.1	31.4	16.4	13.4	24.0	
	10	85.7	88.5	84.7	77.6	84.4	84.2	11
	15	96.4	83.9	95.6	94.2	93.1	92.6	32
D	5	18.2	32.8	8.2	17.5	12.5	17.8	
	10	96.0	91.0	80.3	46.5	94.4	81.7	
	15	100.0	47.5	97.3	91.1	79.3	83.0	6
E	5	53.4	22.4	40.6	16.4	3.4	27.2	
	10	90.8	51.2	98.0	20.7	65.5	65.2	6
	15	73.3	78.8	99.0	70.7	86.9	81.7	3
F	5				1.9		0.4	
	10	29.2	38.3	40.4	27.7	3.3	27.8	3
	15	54.7	84.7	75.6	32.9	67.2	63.0	

crotches was too small.

It was necessary to ensure that the percentage of attacked crotches was not too high, since, in a sample with a high proportion of injured crotches, possible differences of angle means could have been masked.

Combinations of type of twig-crotch base and type of feeding were tested for their independence in a 2 x 2 contingency table. The combinations were round-lateral, round-central, acute-lateral and acute-central.

Results and Discussion

(A) Regional Distribution of Feeding Injuries Along a Vertical Gradient

The mean percentage feeding (Table 2, column 8) at each height level was plotted for trees A to F respectively in Figure 5. The relationship between height level and amount of feeding appeared to be linear for low and high vector pressure. As most of the available twig crotches in the top level become attacked, newly arriving vectors have difficulty finding suitable feeding sites and move down into the middle level of the crown region. This explains the departure from the linear relationship between elevation and feeding in tree C and D.

Although some of these trees, especially tree C, D and E, had a tremendous number of feeding injuries in the upper tree region, their vigor and good health were surprising. This high attack rate reflected the thriving vector population in the community of Lansing where Dutch elm disease control measures were dropped several years ago.

In trees where the attack rate exceeded 80%, the beetle vectors occasionally resorted to feeding in leaf axils. This was especially noticeable on trees D, C, and E, but feeding wounds of this kind were also present on tree F where the mean attack at height level 2 (10m)

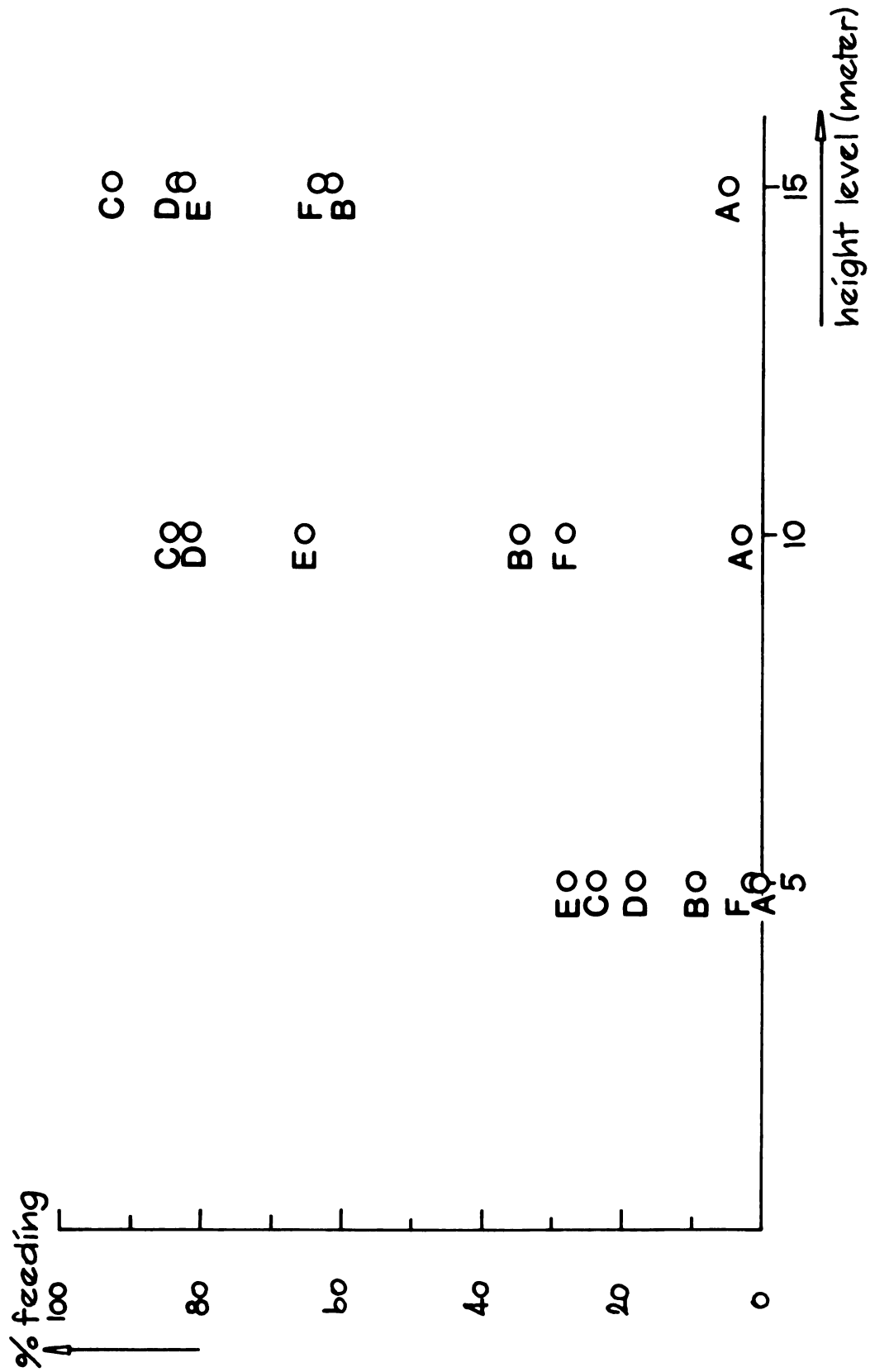


Figure 5. Twig crotch feeding by S. multistriatus on 6 untreated elms.

was lower at 27.8%. No information could be found in the literature about the importance of feeding in leaf axils with regard to effective disease transmission. Twig crotches were sometimes attacked more than once, even those with old scarred callus tissue, particularly in samples with a great number of feeding injuries.

The attempt to determine the influence of branch position on the amount of feeding was unsuccessful. In general the branches in the top region pointed upwards, were more or less horizontal in the middle region, and hung straight down in the bottom portion of the tree. Not only is the morphological variation of twig characteristics great, but also location (isolated tree or in a stand) has a lot to do with the morphology of the twigs. Some elms have long stringy twigs which are very flexible and with few crotches (tree E and F) while others had many crotches and are more sturdy in appearance. Location of the feeding wound, on the upper or on the bottom side of the twig, showed no pattern regardless of the position of a branch.

(B) Regional Distribution of Feeding Injuries Along a Horizontal Gradient

Friedman's ANOVA by rank gave the results listed in Table 3 below.

Table 3. Comparison of feeding attack in 4 cardinal quarters and center section of elm trees using Friedman's ANOVA.

Tree	X_r^2	Significance	Highest Feeding in Quarter of Tree
B	7.47	0.20	N, E
C	3.67	n. s.	--
D	3.00	n. s.	--
E	7.47	0.20	E
F	6.50	0.20	--

$X_r^2 = 7.78$ ($\alpha = 0.10$, df = 4)
$X_r^2 = 5.99$ ($\alpha = 0.20$, df = 4)

Only trees B and E had greater differences in feeding attack between the five sample points N, S, E, W and C (Center) consistently over all 3 height levels. In tree B the percentage of feeding injuries was lowest in the S section, but considerably higher in the N and E sections. Tree E had the least feeding in the W section and the highest number of injuries in the E section.

Initially it was suspected that the center section at the lower height levels 2 and 3 would be the region with the fewest number of feeding scars because of a possible vector preference for the periphery of the crown. However, this was not the case as this analysis proved.

Uneven distribution of feeding wounds in the 5 vertical sections of the tree (N, S, E, W, C) was probably not the result of preferential feeding in any one of these 5 regions. Rather, the proximity of a beetle-producing elm tree to a particular quarter of the above sample trees might have caused this difference in attack.

The question of a possible difference in the overall feeding pattern on elm trees in a closed stand and on road-side elms was also raised. Sample trees A, B, C and D were in closed stands together with other tree species while trees E and F were part of a road-side planting. The vertical and horizontal distribution of feeding injuries in these 2 groups of trees did not seem to be different; however, both road-side elms showed differences in the number of feeding injuries in the 5 sections of the tree. Whether this is a general trend among road-side trees can only be speculated upon since the sample was too small.

(C) Association of Physical Twig Crotch Characteristics With Feeding

1. Angle of twig crotch and feeding

There was no relation between crotch angle and feeding. None of

Table 4. T-comparisons of angle means of attacked and unattacked twig crotches.

Tree	Height Level		N	Mean Angle	S	S_x	$t_{calc.}$
B	5	att.	5	85.00	7.07	3.16	
		unatt.	95				not calc.
		total	100	87.25			
	10	att.	32	84.06	14.89	2.63	
		unatt.	68	82.43	12.38	1.50	0.575
		total	100	82.95			
	15	att.	32	81.72	17.67	3.12	
		unatt.	68	85.74	16.58	2.01	1.110
		total	100	84.45			
D	5	att.	18	63.89	11.58	2.72	
		unatt.	82	64.70	11.56	1.28	0.266
		total	100	64.55			
	10	att.	120	66.53	10.39	0.94	
		unatt.	30	67.33	11.80	2.15	0.364
		total	150	66.82			
	15	att.	118	68.52	11.25	1.04	
		unatt.	32	71.41	11.38	2.01	1.277
		total	150	69.13			

$t = 1.980$ ($\alpha = 0.05$, df = 98)

$t = 1.960$ ($\alpha = 0.05$, df = 148)

the calculated t-values exceeded the table values (Table 4). Of interest is the fact that the angle means of the height level samples for tree B, with 87.25°, 82.95°, and 84.45° were considerably higher than the means for tree D, with 64.55°, 66.82°, and 69.13°. The great difference in angle means between these two trees is an example of the morphological variability one can find among elm trees.

2. Association of round or acute crotch base with type of feeding: lateral or central

Ouellette (1962) noted that lateral feeding resulted in a higher infection rate than did central feeding. Presumably during lateral feeding the beetle vector with the spores glued to its body establishes better contact with the conductive tissue.

Type of Feeding	Twig Crotch Base		
	Round	Acute	Total
Lateral	124	60	184
Central	83	72	155
Total	207	132	399

$X^2_{calc} = 6.78$

$X^2_{tab} = 3.84$
($\alpha = 0.05$, df = 1)

The contingency table suggested that twig crotches with a more rounded base were more likely associated with lateral feeding than with central feeding. The association of these 2 characteristics was significant at $\alpha = 0.05$.

These findings suggest that trees with a higher proportion of twig crotches with a rounded base are more likely to be infested because of the preferred lateral type of feeding in these crotches.

Summary and Conclusions

Extensive sampling of untreated elm trees revealed that S. multistriatus feed preferentially in the whole upper tree region. Feeding attack rate increased linearly from the bottom to the top. Differences in attack in vertical sections of a tree were observed but were believed to be related to the close proximity of a beetle-producing tree to a particular quarter of a sample tree. Several physical twig crotch characteristics were investigated in respect to their association with vector feeding. The size of the angle between the main and lateral member of a twig crotch had no influence on feeding. There was, however, a significant association between a more rounded twig crotch base and lateral feeding. Lateral feeding reportedly results in more successful inoculations than does central feeding. These findings pointed out a possible resistance mechanism against feeding by S. multistriatus.

II. Evaluation of Methoxychlor Formulations and Application Techniques

Introduction

The protective spraying against the feeding of S. multistriatus has been heralded by many as the main way to prevent Dutch elm disease transmission. But before the availability of organic insecticides, in particular DDT, 'pre-attack' spraying was considered to be of doubtful value; first, because of the nature of the available materials and, second, because of the lack of an adequate spray technology at the time (Fransen, 1931). DDT had to be phased out of all Dutch elm disease programs in the urban communities in the mid-1960's. There was never any doubt about the superior performance of DDT applications against the feeding of S. multistriatus (Becker, 1946; Whitten, 1958; Doane, 1958, 1962). However, when environmental considerations forced the replacement of DDT with other insecticides, there were few materials which could substitute satisfactorily. DDT's long persistence and its toxicity to S. multistriatus made it very valuable for Dutch elm disease control programs. Methoxychlor, also called methoxy-DDT, emerged in the 1960's as the only promising replacement for DDT (Wooten, 1962; Barger, 1971) although earlier reports did not favor it as a possible DDT substitute (Matthysse et al., 1954). Recent work dealing with the behavior of methoxychlor in ecological systems indicated that this compound is highly biodegradable (Metcalf et al., 1972). This feature, and low mammalian toxicity make methoxychlor a good choice for applications in urban communities.

Elm tree protection requires very thorough spray coverage since

the feeding sites, or twig crotches, are distributed over the whole crown region. The tree tops deserve the most attention because feeding was observed to be heaviest in the upper crown region (Wolfenbarger and Buchanan, 1939; see also section I of this thesis).

The problem of ground applications with the mist blower or with hydraulic equipment was often one of insufficient tree top coverage, especially on tall trees (more than 18-20 m high). Wallner and Leeling (1968) pioneered the use of helicopter applications for Dutch elm disease control and reported superior coverage of the tree tops.

The Dutch elm disease conference in 1967 (Peacock, 1967) outlined two important research areas: (a) formulation studies with methoxychlor and (b) research on application techniques. One reason why the switch from DDT to methoxychlor put a stop to many community control programs was the high cost of this insecticide. Increasing labor costs and the expensive, slow ground applications contributed to the decision of communities to discontinue control programs. Aerial spraying of elms had major cost advantages over ground applications which were related to speed of the spraying operation. Also, the amount of insecticide added to the environment by the helicopter was considerably less than by ground equipment. However, the value of aerial spraying for elm protection needed further experimental support.

Part of the work reported here followed a formulation study initiated by Wallner et al. (1969) on the elm population of Michigan State University. In 1969 and 1970 ground and aerial applications were evaluated by feeding trials and residue analysis.

Materials and Methods

(A) Spray Applications 1969

For study purposes, the campus of Michigan State University, with an elm tree population of circa 1600, was divided into 5 areas (Figure 6). Each area contained at least 100 elms. The spray plot arrangement was the same as for 1968 (Wallner et al., 1969).

Treatment I: Michlin Chemical Co. 'MZ-2' Elm Spray
25% methoxychlor
36.5% xylene
36.5% petroleum distillates
2% inert ingredients and emulsifier

Treatment II: Standard Oil Elm Spray 'M'
25% methoxychlor
65% xylene
8% paraffinic white oil
2% inert ingredients and emulsifier

Treatment III: Michlin Chemical Co. 'MA-2' Elm Spray
25% methoxychlor
67% xylene
6% paraffinic white oil
2% inert ingredients and emulsifier

Treatment IV: Michlin Chemical Co. 'MX-2' Elm Spray
25% methoxychlor
73% xylene
2% inert ingredients and emulsifier

Two pounds of Dacagin[1)] (86.9% polysaccharide-gum mixture, 13.1% inert ingredients) were added to 100 gallons of 12.5% emulsion in order to prevent excessive spray drift. The rate was reduced since nozzle clogging occurred during the 1968 application.

Treatment V: Amoco Elm Spray
25% methoxychlor
73% xylene
2% inert ingredients and emulsifier

All the above concentrates were mixed with water to give 12.5% emulsions. In the middle of April, all 5 areas were sprayed with a Bell G-2 helicopter which was equipped with a 30 ft. boom and 59 D-6 nozzles

1) Diamond Alkali Co.

Figure 7: DCD TREATMENT AREAS 1994-98

MICHIGAN STATE UNIVERSITY
N
Figure 6 DED TREATY AREAS 1968-69

(Figures 8, 9). Flight speed during application was above 20 miles per hour and the flight height above tree tops ranged between 15-20 ft. The helicopter delivered 7 gallons per acre in a 50 ft. swath at a line pressure of 45 psi. The wind conditions during spraying were steady and did not exceed 5 miles per hour. Spraying time was early morning between 5 and 7 a.m. and in the evening between 6 and 8 p.m. Each tree received on the average 2 quarts of 12.5% spray emulsion.

Treatment VI: The smaller elms in the University married housing area with a height of 10-12 m were treated from the ground with a John Bean 301-G roto mist sprayer (Figures 10, 11). The same 12.5% spray emulsion was employed as for treatment V of about two gallons per tree.

(B) Spray Applications 1970

In 1970 most of the elms on campus were treated with the Michlin Chemical Co. 'MX-2' Elm Spray diluted to give a 12.5% emulsion. Dacagin was added for spray drift control at a rate of 2 pounds per 100 gallons emulsion. Amoco Elm Spray which is similar to Michlin Chemical Co. 'MX-2' in its composition was also used as 12.5% emulsion and with 2 pounds of Dacagin per 100 gallons of spray. The applications were made at the end of April in early morning by helicopter as described for 1969, but the average amount of spray per elm was somewhat less than for the previous year (less than 2 quarts). Wind conditions were favorable and not exceeding 6 miles per hour.

Several 15-18 m elms close to dormitories and other buildings, where helicopter application did not seem feasible, were treated with a John Bean 301-G roto mist. Again, Michlin Chemical Co. 'MX-2' was used as a 12.5% emulsion but without Dacagin.

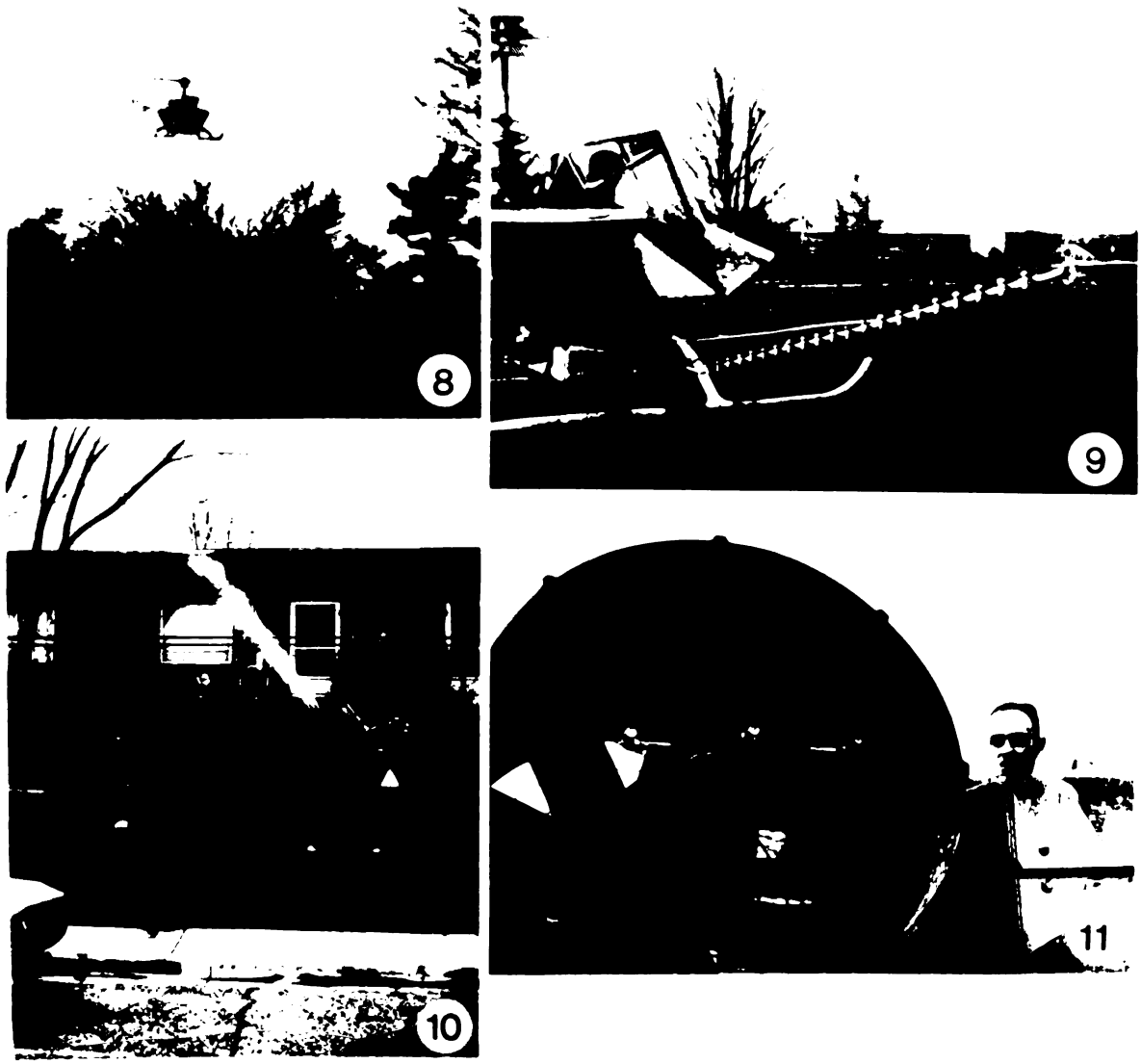


Figure 8. Aerial spraying of elm trees with Bell G-2 helicopter.
Figure 9. Bell G-2 helicopter: part of boom and nozzle assembly.
Figure 10. Ground spraying of elm trees with John Bean Rotomist (model 301-G).
Figure 11. John Bean Rotomist: blower and nozzle assembly.

(C) Twig Sampling

1969: From each of the 6 spray plots 2 elms were chosen at random and twig samples were taken 6 and 10 weeks after application. The sampling of each tree was designed to take into account the vertical and horizontal variation of spray deposits within the crown. Each tree was subdivided into 3 horizontal sample strata (Figure 12).

HEIGHT LEVEL 1: TOP (15 m and above)

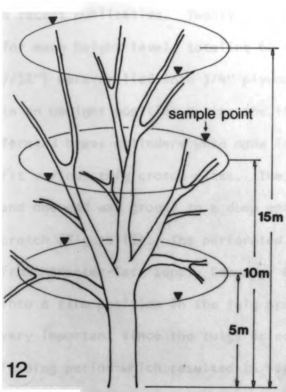
HEIGHT LEVEL 2: CENTER (10 m)

HEIGHT LEVEL 3: BOTTOM (5 m)

0.6 - 0.9 m long branches were taken at 2 points within each height level which gave a total of 6 sample points per tree. About 100 1-5 year old twig crotches were cut from both branches and bulked to one sample. The fresh twig samples were transferred into polyethylene bags, labeled and transported in styrofoam coolers to the freezer for storage (temperature -10^{o} C).

Samples were stored up to 2 months while awaiting analysis depending on availability of beetle material or manpower. The trees were usually sampled with the MSU Grounds Department Stratotower which made the sampling relatively easy (Figure 13). When this equipment was not available, an extendable aluminum pole pruner with a light-weight head was used for sampling trees up to 16 m (Figure 14).

1970: 10 helicopter-sprayed trees were chosen at random from the elm population on the MSU campus and were sampled 2 and 8 weeks after application. Twig sampling followed the same scheme as in 1969. 2 mist-blower-sprayed trees, both close to dormitories, were sampled as well at the same time. The tree heights of the sampled trees ranged between 15 and 19 m.



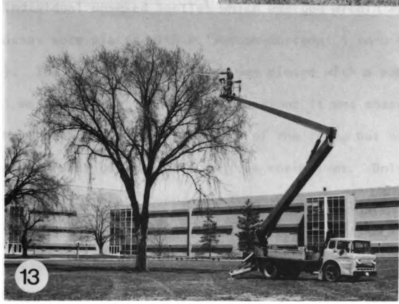


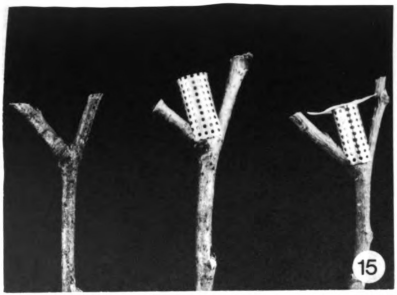
Figure 12. Sampling scheme for treated campus elms: location of sample points at three height levels.
Figure 13. Twig sampling with Stratotower.
Figure 14. Twig sampling with aluminum pole pruner.

(D) Bioassay Technique

The technique used here for the feeding assays was suggested by Lincoln (1968) and described and illustrated by Barger et al. (1971) in a recent publication. Twenty twig crotches, 5-7.5 cm long, were bioassayed for each height level, totaling 60 twig crotches per tree. Holes (diameter 7/32") were drilled into 3/4" plywood boards to hold the twig crotches in an upright position during the feeding trials (Figure 17). Small perforated brass cylinders were made in 3 diameters, 4.0, 4.5 and 5 mm, to fit various twig crotch sizes. The length of all brass cages was 13 mm and one end was ground to a deep notch to give a tight fit in the twig crotch (Figure 15). The perforated brass was obtained as sheet metal from McMaster-Carr Supply Company, Chicago. The brass cage was pressed into a firm position in the twig crotch to assure a tight fit. This was very important since the twigs dried up and shrank during the 24-hour feeding period which resulted in several cases in loose cages and escaping beetles. Individual unsexed beetles which emerged within 24 hours previous to the bioassay were placed with a 'vacuum-forceps'[1] into each cage (Figure 16). The open end of the cage was closed with a small piece of $\frac{1}{4}$" Permacel masking tape. On several occasions it was observed that beetles got trapped on the sticky surface of the tape, but healthy, vigorous beetles were usually able to free themselves. Only 'healthy' beetles were used for the bioassays. The beetles were considered 'healthy' if they were able to climb the side wall of a 1 pint 'SEALRIGHT' ice cream carton. The duration of each bioassay was set at 24 hours at 25° C and 80% ($\pm$ 10%) relative humidity and the light conditions were set at a 12-hour day. After 24 hours, the bioassay was terminated and the beetles

1) Chemical Rubber, Cleveland, Ohio

Figure 15. Fitting of brass cage in twig crotch for bioassay.
Figure 16. Placement of S. multistriatus in brass cage with vacuum forceps.
Figure 17. Assembled twig crotches on plywood tray.
Figure 18. Classification of feeding response: F3 (weak: xylem not visible), F2 (medium: xylem visible), F1 (strong: xylem deeply penetrated).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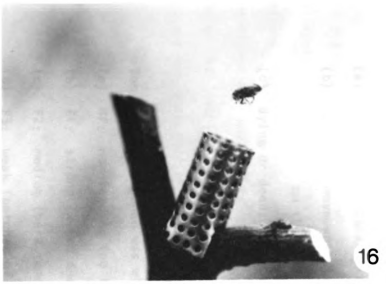
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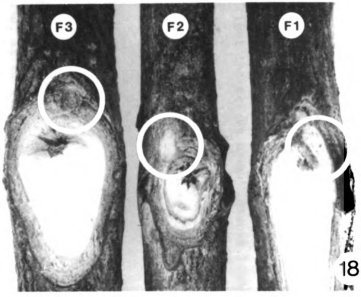
F3
F2
F1
18

were removed from their cages. The beetles were then sexed and classified according to their physiological stage as:

(a) alive: full capability of movement, no jerky movements

(b) (alive): movements jerky, fall sometimes to the side or on their backs

(c) dying or dead: cannot right themselves under intense light, slight spastic movements of antennae or legs, wings often times spread out with rhythmic motions of abdomen or complete immobility

The feeding response was either indexed as (Figure 18):

(a) NF: non-feeding (no feeding activity noticeable)

(b) F1: strong (xylem deeply penetrated)

(c) F2: medium (bark removed, xylem visible)

(d) F3: weak (patches of bark eaten, xylem not visible)

or the length of the scar was measured directly with a 20 mm disc micrometer reticle in a LEITZ stereo microscope.

(E) Scolytus Rearing

The bioassays required a large number of Scolytus beetles with preferably a high degree of physiological uniformity, same age and equally good health. Therefore, a rearing technique was required which provided beetle material with the desired characteristics. Several rearing containers for bark beetles are described in the literature, ranging from 5-gallon metal pails (Fox, 1958), fiber drums (Valek, 1967), vented 20-gallon metal trash cans (Clark and Osgood, 1964), to collection containers made of 35-gallon lever-lock steel drums (Truchan, 1970). Mold and fungus growth on the logs is a common problem in rearing containers due to insufficient ventilation. Even if a high percentage of bark

beetles emerges and is recovered, their physiological condition might render them unsuitable for bioassays. A further requirement was the immediate extraction of newly emerged beetles from the rearing containers to guarantee fresh beetle material of uniform age. S. multistriatus is strongly phototrophic like many other insects. This behavioral feature allows efficient collection shortly after emergence. Many of the aforementioned emergence drums have collection devices which allow the insect to go back into the emergence drum, or the light conditions in the container are such that the emerging insects are not drawn immediately into a collection jar. Both of these conditions result in beetle material of different age which is not desirable for a standardized bioassay. Therefore, emergence drums were designed with a ventilation system to prevent fungus and mold growth and with an efficient extraction system which did not allow the insect to move back into the rearing container.

Emergence drums (Figure 19: A, B)

Three 35-gallon steel drums served as rearing units securely positioned horizontally on a slotted angle steel rack (Figure 20). The drums were covered in front with circular black 'Poly Cover' plastic sheeting which was conveniently tied down with 5/16" rubber cord. A circular air screen was inserted in the lower half of the plastic cover to aid air circulation. A piece of black plastic sheeting loosely covered the screen vent to prevent light from penetrating into the inside of the drum. The actual extraction device consisted of a 5 cm long piece of PVC pipe (diameter 3") glued into a circular hole close to the lower rim of the drum after which a 500 ml transparent plastic bottle from which the bottom had been cut out was fitted snugly over the PVC pipe. One end of a short piece of $\frac{1}{2}$" tygon tubing was inserted into the mouth

Figure 19: Design of emergence drum and ventilation system

Emergence drum plus extraction device

A Front View

B Side View

Ventilation system

C Front view: blower housing and air pipe plus support

D View from above: blower housing

E Side view: support of air pipe with valve half closed

1. 35 gal. steel drum
2. Black plastic sheeting
3. Tie-down rubber cord
4. Air-screen
5. Black plastic sheet covering air screen
6. PVC pipe (diameter 3")
7. 500 ml transparent plastic bottle
8. Tygon tubing
9. 1 gal. ice-cream carton
10. 20 WATT cool white neon light
11. Blower housing
12. 2.5 m long PVC pipe (diameter 3")
13. Wooden support with adjustable valve
14. Dayton blower (model 2C781)
15. 3-way switch
16. 5 cm copper tubing (diameter 5/8")

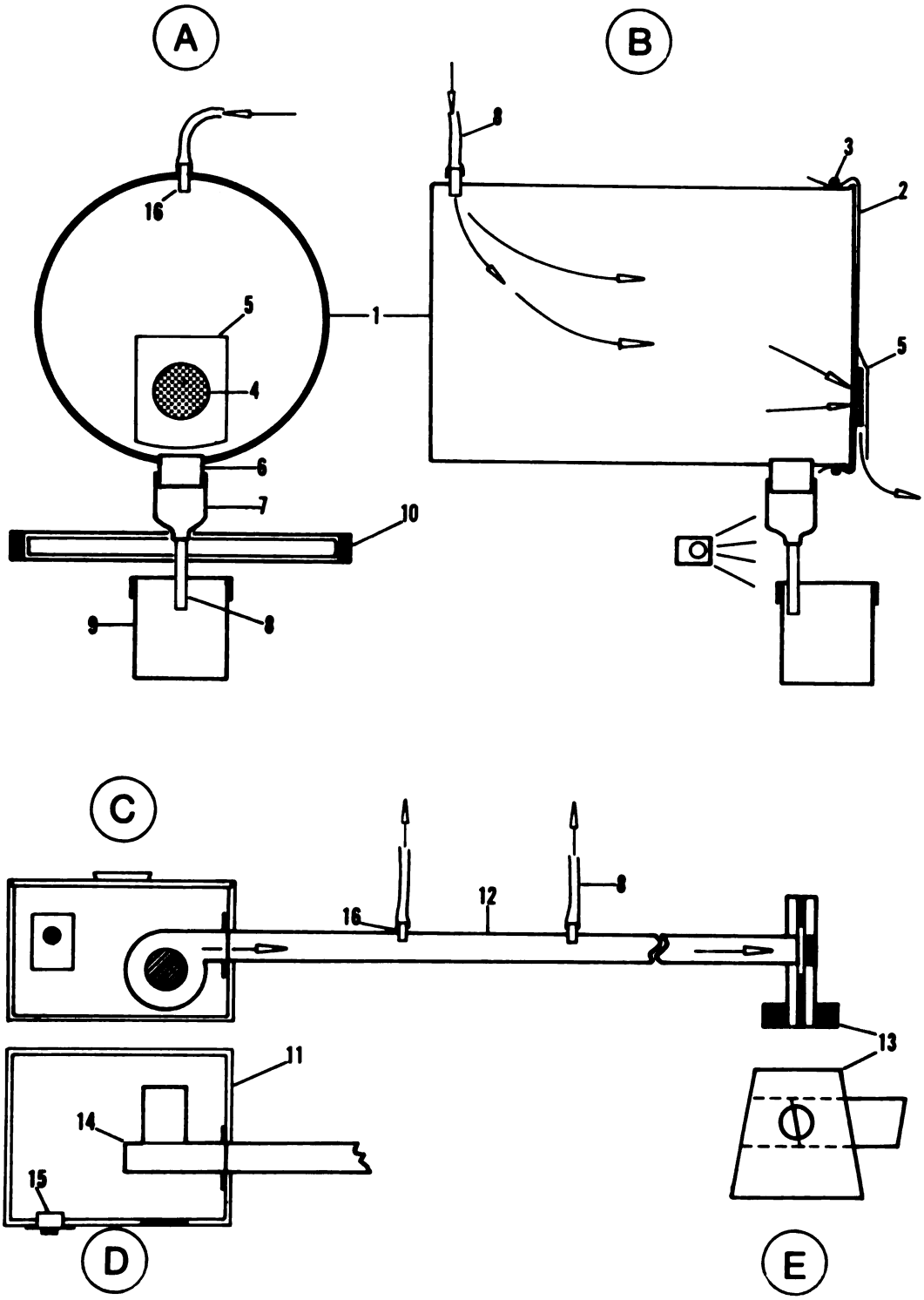

Figure 19. Design of emergence drum and ventilation system.

of the bottle and the other end placed straight down into a 1-gallon ice cream carton. The lower end of the tubing reached several centimeters into the carton, and prevented the beetles from crawling back up into the drum.

Small collection jars with very little space for free movement have the disadvantage that the emerging beetles inflict injuries on each other, rendering them unsuitable for bioassay work. The 1-gallon cartons were a definite improvement in this regard and with frequent emptying of these collection cartons, beetle-inflicted injuries were very rare. A 20-watt cool white neon bulb provided the necessary light stimulus for drawing the emerging beetles into collection containers.

Ventilation system (Figure 19: C, D, E)

The ventilation system consisted of the blower-housing with blower, a long PVC pipe (diameter 3") with several air outlets and an adjustable valve to regulate the airflow (Figure 21). The necessary airflow was generated by a blower (Dayton Model 2C781) housed in a $\frac{1}{2}$" plywood box. It could be set at 3 speeds with a 3-way switch. A 2.5 m long PVC pipe (diameter 2") was connected to the blower with air outlets every 30 cm. Five cm long pieces of 5/8" copper tubing were glued into the PVC pipe to serve as air outlets. A $\frac{1}{2}$" diameter Tygon tubing guided airflow into the emergence drums. Inlet into the drum was provided by a 5 cm long piece of 5/8" copper tubing with a fine-mesh screen soldered to one end to prevent insects from entering the air tubing. The first 20 cm of the Tygon tubing next to the air inlet was wrapped with black plastic tape to keep light from entering the drum. The other end of the PVC pipe rested firmly in a wooden support which consisted of $\frac{1}{4}$" masonite board sandwiched between 2 pieces of 3/4" plywood. A sliding

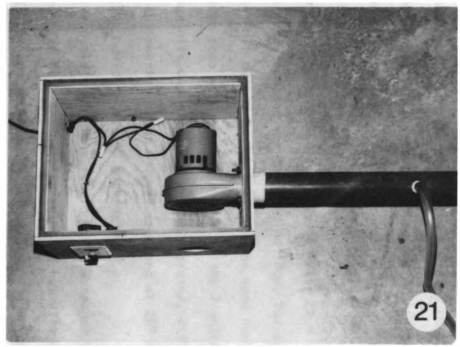

Figure 20. Emergence drum with elm logs on steel rack.
Figure 21. Ventilation system for emergence drums.

piece of masonite board allowed continuous regulation of airflow into the rearing container. With the end of the PVC pipe completely open and the slide pulled out, airflow into the drums was at a minimum. This setting was used for the whole rearing operation and guaranteed sufficient air exchange.

For comparison purposes, relative humidity (R.H.%) and temperature (^{o}C) data were recorded for the rearing room, for an unvented and a vented rearing unit over a 2-week period. The same number of fresh logs from the same tree were placed in each of the 2 units. Temperature in the 2 rearing units did not, as might be expected, differ from the temperature in the rearing room, and was constant at 26^{o} C (± 1.5). However, the relative humidity varied considerably.

Relative humidity for the rearing room averaged 54.8%, whereas in the vented rearing drum the average relative humidity was constantly higher at 67.8%, the mean difference being 13%. The unvented rearing unit had constantly maximum humidity and never went below 100% R.H. during the 2-week period. The logs in the vented drum were free of mold with beetle emergence high, and mortality low. The high humidity in the unvented drum caused extensive fungus growth and an overall unsatisfactory environment for beetle development. Emergence was very poor due to high mortality inside the drum. No quantitative comparisons of mortality data in the vented and unvented rearing units were made.

The beetles used for the bioassays were reared from field-infested elm logs. A lab culture of S. multistriatus was also maintained. Fresh elm logs were artificially infested with S. multistriatus in completely closed cardboard boxes and kept there for 2 weeks before they were placed in one of the emergence drums. Parasites or predators (clerids and ostomids) were never a problem in these cultures.

(F) Quantification of Methoxychlor Residues on Elm Bark

1. Bark sampling

For accurate quantification of methoxychlor residues in twig crotches, it was necessary to obtain uniform bark samples. Most commonly, residues on elm bark were expressed in weight per unit of bark surface (micrograms/cm^2) (Miller, 1951; Matthysse et al., 1954; Nielsen, 1962; Parker and Nielsen, 1967). None of the previously suggested methods allowed easy sampling and exact measurement of the twig crotch area. Since feeding scars along the sides of the twig crotch are apparently more conducive to true infection (Ouellette, 1962), bark samples had to include this area. With a No. 1 cork borer (inside diameter 4 mm) one bark circle with an area of 12.56 mm^2 was taken from each twig crotch (Figures 22,23). Fifty twig crotches from each height level were sampled in this manner, which gave a total of 6.28 cm^2 bark surface analyzed per height level.

2. Chemical analysis (Figures 24,25)

The analytic procedure basically followed the one described by Wallner, Leeling and Zabik (1969).

Extraction Procedure

The bark was extracted with a hexane-acetone solvent pair (1:1, 50 ml) in a 500 ml Erlmeyer flask. The sample was allowed to sit overnight and the next day it was shaken on a Burrell-Wrist-Action shaker for 20 minutes at a setting of five. The extract was decanted into a 500 ml separatory funnel. Following this, another 50 ml of the solvent-pair was added to the sample and again shaken. The same step was repeated a third time so that the extract amount was finally equal to 150 ml. The acetone fraction of the solvent was then removed

Figure 22. Bark sampling in twig crotch with cork borer No. 1.
Figure 23. Bark samples from twig crotches.
Figure 24. Extraction and clean-up of bark samples.
Figure 25. Analysis of samples with gas chromatograph (Beckman GC-4).


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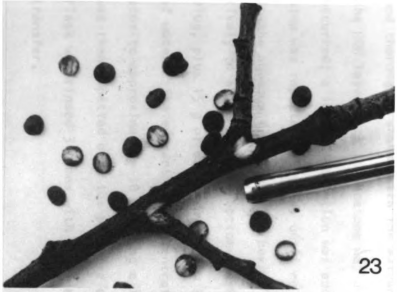
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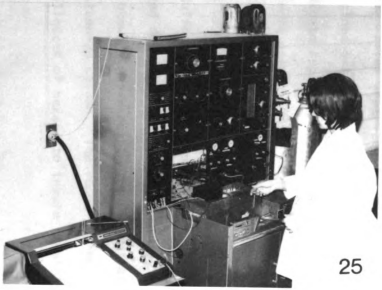
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with distilled, deionized water. 300 ml of distilled HOH were added and thoroughly shaken with the extract. After separation of the 2 layers the HOH layer with the acetone was drained off. If excessive emulsion occurred, a 10% NaCl solution was added to separate the 2 phases. This step was repeated 4-5 times until no more acetone smell could be detected in the aqueous fraction. The hexane extract was transferred quantitatively to a 500 ml round bottom flask using a funnel with a glass wool plug, plus 15 g of anhydrous Sodium Sulfate to remove remaining traces of water. The extract was concentrated down on a rotary vacuum evaporator to approximately 10 ml using a lukewarm water bath. The concentrate was then pipetted onto the prepared clean-up column. The round bottom flask was rinsed 3 times with small aliquots of hexane to insure complete transfer.

Preparation of Clean-up Column

A 22 x 500 mm clean-up column was used with thin Teflon tubing as the outlet. The column was prepared with 10 g of Florisil: Celite (5:1) between 1 cm layers of Sodium Sulfate. The Florisil was 12% deactivated with distilled water and then tested with a known sample of methoxychlor. The total amount of methoxychlor was eluted between 350 and 420 ml, which was considered as adequate. The Florisil was saturated with hexane before the sample was pipetted onto the column. Methoxychlor was eluted with 500 ml redistilled hexane into a 1000 ml round bottom flask. The sample was then concentrated down to about 15 ml on a vacuum-evaporator as mentioned before, quantitatively transferred to a screw-top vial with a Teflon-lined cap and filled up to exactly 25 ml. The samples were stored at -18° C while awaiting analysis on the gas chromatograph.

Quantitative Analysis

The methoxychlor samples were quantified on a Beckman GC-4 gas chromatograph (electron capture detector- ECD) fitted with a 6 ft. long, 1/8" diameter stainless steel column (Figure 25). Column packing for the 1969 analysis consisted of Gas Chrom Q 60/80 with 3% SE-30. Temperature for the column was 200° C, for the injection port, 240° C, and for the detector, 300° C. The retention time for this column was 4.5 minutes at 200° C. Gas Chrom Q 60/80 with 4% DC-11 in a stainless steel column of the same dimensions was used for the 1970/71 and some 1972 samples. The retention time for this column was 4 minutes at 210° C. Most of the analysis in 1972 was done on a Beckman GC-65 (electron capture detector) which was equipped with a 6 ft. glass column (diameter 1/16") packed with Gas Chrom Q 60/80 with 3% SE-30. Figure 26 is a sample run of 3 standards: methoxychlor, pp'DDT and pp'DDE. The peaks of DDT and DDE were superimposed to show retention time of these 3 compounds and the relative sensitivity of the electron capture detector. Amounts of 2 microliters per sample were injected with a Hamilton microliter syringe. Standards were injected at the beginning of a run, after each 10-12 samples and at the end of the run. Peak height was measured and converted to PPM with the standard graph to give the amount of methoxychlor in the sample. The conversion to micrograms/cm^2 bark surface was calculated with the following formula:

$$\frac{\text{(PPM) (DILUTION FACTOR) (SAMPLE VOLUME, ml)}}{\text{BARK AREA analyzed } (cm^2)} = \text{micrograms MeCl}/cm^2 \text{ bark}$$

The methoxychlor in several samples was confirmed with thin-layer chromatography (silica gel-H plates).

3. Efficiency of extraction procedure

The methoxychlor residues in the bark samples were undoubtedly

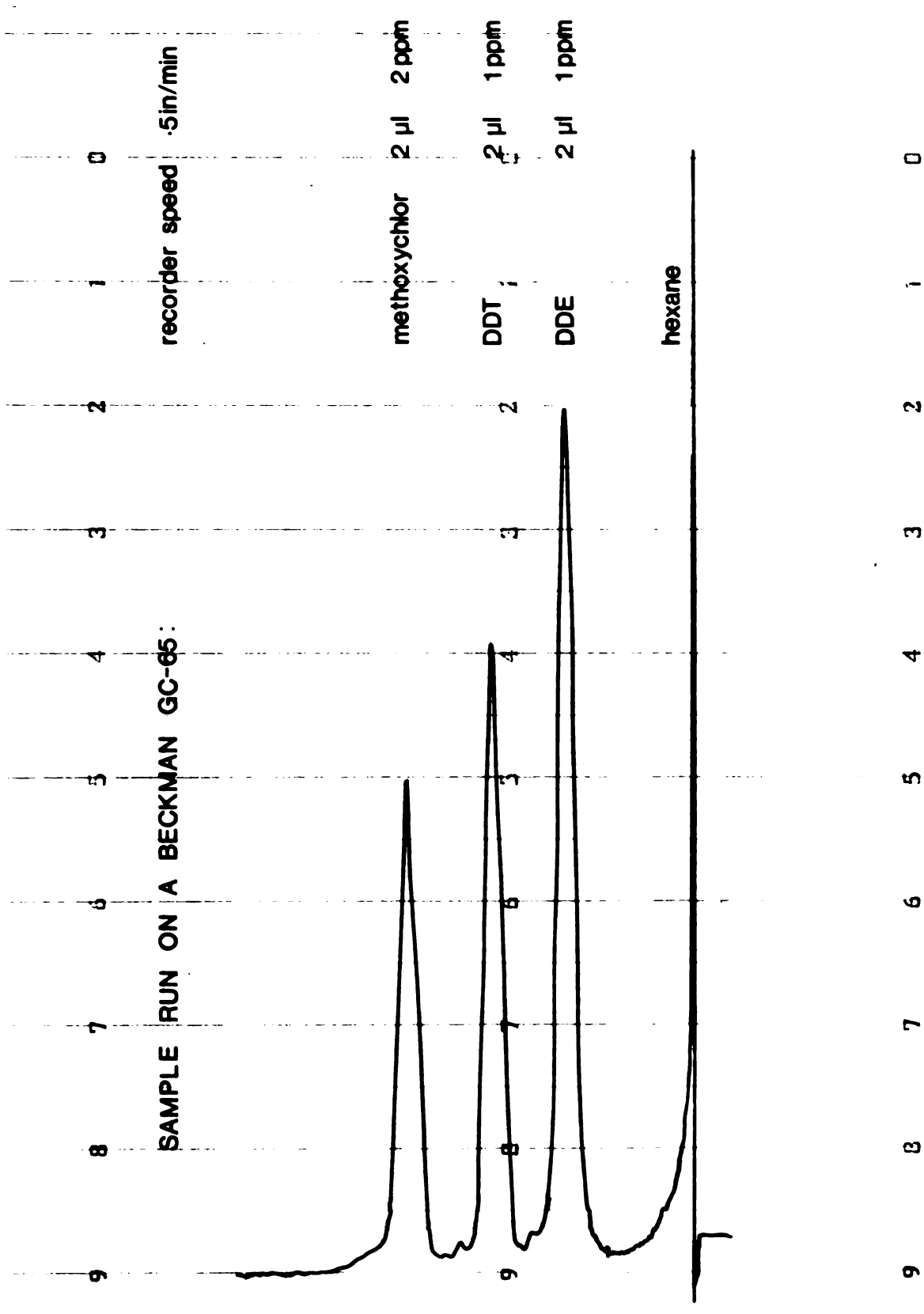

Figure 26. Sample run on a Beckman GC-65: DDE, DDT and methoxychlor.

underestimated because of either incomplete extraction or some loss during the extraction procedure. Since it was desirable to work with the corrected actual residue quantities the degree of efficiency had to be determined experimentally for the standardized extraction procedure. Known amounts of methoxychlor dissolved in acetone were applied with a microsyringe to elm bark. The bark was stripped from young untreated elm branches and cut into 5 cm^2 pieces. After a drying period of several hours the samples were subjected to the standardized extraction procedure. Table 5 summarizes percent recovery for the 20 microgram and 200 microgram samples.

Table 5. Rate of recovery for methoxychlor from elm bark samples.

Amount MeCl Applied (µg)	Amount in micrograms/cm^2	% Recovery	% Av. Recovery
20	4.0	85.00	
20	4.0	86.30	85.03
20	4.0	83.80	
200	40.0	81.63	82.82
200	40.0	84.00	

The average rate of recovery was somewhat less for the 200 microgram samples. All bark residue values were corrected with above figures. If the amount of methoxychlor in a sample was below 20 micrograms/cm^2, 85.03% was used as corrective figure; otherwise 82.82% was employed. Variation in the rate of recovery appeared to be small.

4. Quantitative changes of methoxychlor residues during extended freezer storage

Some of the twig samples taken in the summer of 1970 had to be stored over a longer period of time until the bioassay or the chemical analysis was completed. It was therefore important (a) to prevent desiccation and preserve freshness of the samples (with respect to feeding trials) and (b) to stop further decomposition of the insecticidal residues on the bark. The possibility that the freshly cut twig samples could be subjected to a pre-cold storage treatment such as quick-freezing in liquid nitrogen in order to instantly stop further break-down of residues (Lincoln, 1968) was considered. However, since methoxychlor is a relatively stable compound, a pre-cold storage treatment was omitted. Barger et al. (1971) demonstrated that pre-cold storage treatments had no influence on feeding response or decomposition of the unstable systemic dicrotophos. Nevertheless, an experiment was designed to ascertain any quantitative changes of bark residues over longer periods of time at the given storage conditions (-10° C, no light). 120 twig crotches from an untreated elm tree were dipped in a 1.5% MeCl xylene solution (diluted from 25.1% Michlin 'MX-2' elm spray). After a drying period of several hours, the twigs were randomly grouped in batches of 10, placed in plastic bags and stored in the freezer. At each sampling date, 25 bark circles were taken with a No.1 cork borer from 2 batches labeled A and B respectively, and subjected to the standard chemical extraction procedure. The analysis proceeded as shown in Table 6.

It is noteworthy that over an entire year the residues on the treated twigs remained practically unchanged. The differences between sample dates of that year probably represent inherent variation attributable to distribution of residues, sampling errors, and variation in

Table 6. Stability of methoxychlor residues during freezer storage.

Date of Analysis	Weeks After Application	Sample A	Sample B	$\frac{A + B}{2}$ [1]
6/22/70	APPL.	52.6	57.4	55.0
7/22/70	4	50.4	50.7	50.6
8/23/70	8	50.7	49.2	50.0
8/15/71	60	55.5	53.3	54.4
4/16/72	95	44.2	2)	44.2
10/3/72	118	39.0	47.9	43.4

1) expressed in micrograms per cm^2 bark surface
2) sample was lost

the efficiency of the chemical analysis. It is difficult to speculate upon possible causes of the 20% drop in recovery in the last two samples. After long storage in the freezer desiccation of the twigs was found to be extensive. It is possible that decomposition or translocation of bark residues had something to do with the desiccation process. The storage period for the field-collected twig crotches never exceeded 8 weeks. As was proven here, this lies well within the time limit where no quantitative changes occur.

Results and Discussion

(A) Bioassays 1969:

Table 7 summarizes the mortality and feeding response data of the feeding trials. Both figures, mortality and feeding response, associated with each treatment represent the mean of 2 trees, A and B. These figures are percentage values (proportions) for which a confidence limit

can be calculated according to the formula for the confidence interval for the binomial case (Snedecor and Cochran, 1967, p. 211). Figures 27 and 28 are a graphic representation of these data with the corresponding confidence limits.

The mortality figures are corrected for natural mortality in the controls using Abbott's formula. This was applied throughout the assays.

$$\text{Corrected Mortality (\%)} = \frac{x-y}{x} 100 \ (\%)$$

x= % alive in control assay

y= % alive in treatment assay

The first sample date (6 weeks after application) fell within the peak emergence of the 1st generation in central Michigan. The second series of twig samples was taken 10 weeks after application (end of July) which approximates the end of the susceptible period to the disease. Elm protection against feeding has to last at least until the end of July or the beginning of August, since susceptibility of elms to infection is greater during spring and early summer.

Mortality

Helicopter treatments I to III were very ineffective. The mortality figures ranged between 9 and 20%. The natural mortality in the controls of the May bioassays was unusually high (11.8%) which resulted in lower corrected mortality figures. In the July assays the mortality in the controls was 4.5%, considerably less than for May. Therefore, the slight increase in mean mortality for treatments I, II, III and V from May to July expressed more the variability in natural mortality than a real treatment difference between these 2 sample dates. The only helicopter applications which gave somewhat higher protection were treatment IV and V. In particular, application IV with 60% and 45% mortality at the 1st

Table 7. Responses of S. multistriatus in bioassays of treatments I - VI (1969).

Treatment [1]	Mortality (%) [2] 6 Weeks	Mortality (%) [2] 15 Weeks	'Failure to Score Xylem' (%) [2] 6 Weeks	'Failure to Score Xylem' (%) [2] 15 Weeks
I Helic.	9.3 ± 6.1	15.2 ± 7.2	16.4 ± 7.4	23.6 ± 8.4
II Helic.	12.4 ± 6.7	20.0 ± 8.0	23.6 ± 8.4	29.3 ± 9.0
III Helic.	10.3 ± 6.2	20.0 ± 8.0	22.7 ± 8.3	26.4 ± 8.7
IV Helic.	60.8 ± 9.6	45.7 ± 9.8	56.4 ± 9.8	53.6 ± 9.8
V Helic.	26.8 ± 8.8	34.3 ± 9.4	40.9 ± 9.7	40.9 ± 9.7
VI Mist. Bl.	93.8 ± 5.0	96.2 ± 4.1	88.2 ± 6.5	95.5 ± 4.4

1) described on p. 64
2) mean of 2 trees plus confidence limit ($\alpha = .05$)

and 2nd sample dates respectively stood out from all the other helicopter treatments. The formulation used in treatment IV was a 25.1% Michlin 'MX-2' elm spray diluted to 12.5% and applied with the anti-drift material Dacagin at the rate of 2 pounds per 100 gallons of spray. The formulation for treatment IV differed from the other formulations. Besides the addition of Dacagin, the carrier was different. However, xylene made up the main portion of the carrier in all these formulations. Therefore, the effectiveness of formulation IV, and its apparent longer persistence, was attributed in part to the addition of Dacagin. In decomposition studies of several methoxychlor formulations Dacagin definitely had a retarding effect, and it increased persistence significantly (see section III of this thesis).

Since elm trees in the treatment areas I to VI were mist-blower sprayed either with DDT or later with methoxychlor before 1968, the suspicion remained that the bioassay results did not reflect the 1969

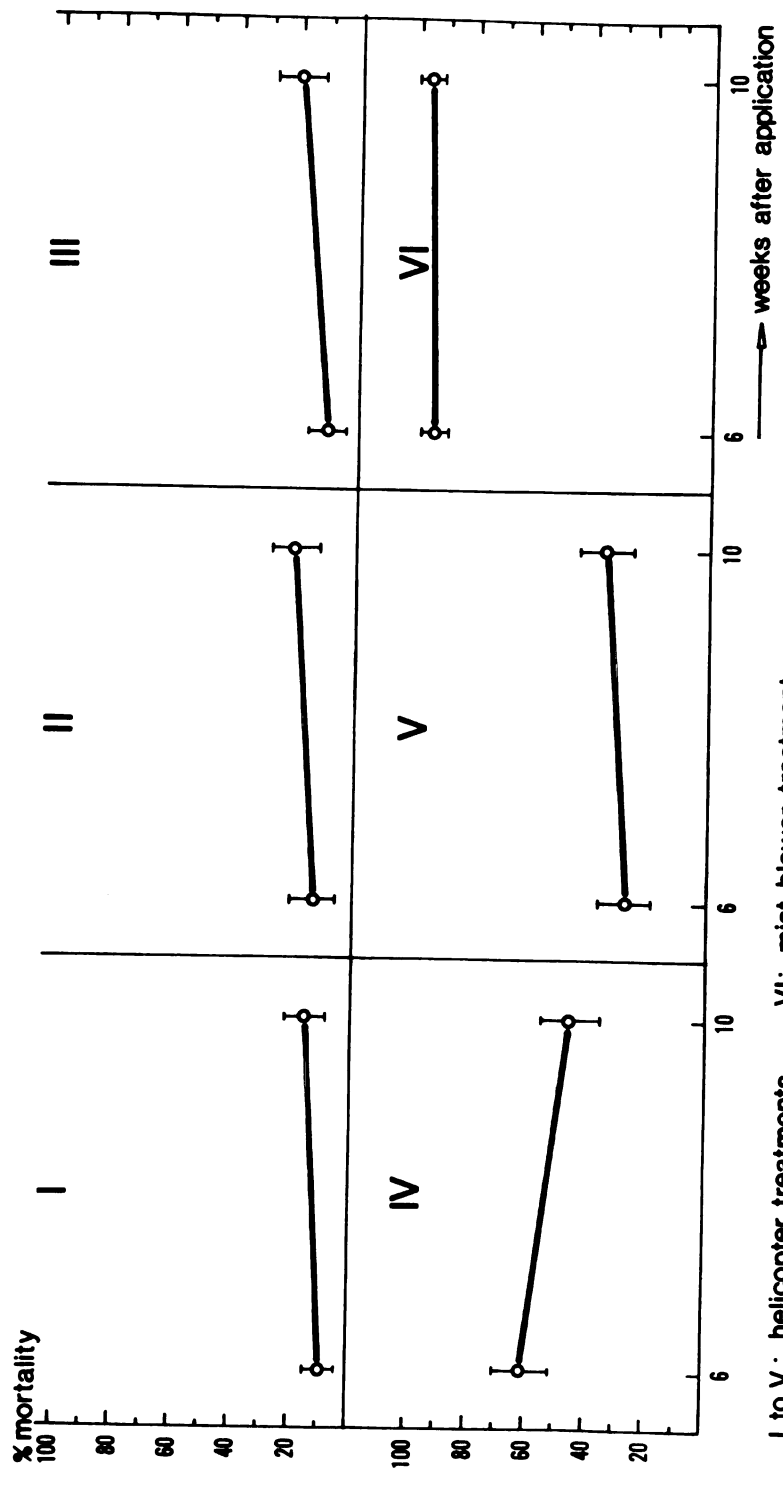

Figure 27. Bioassays 1969: mortality in treatments I to VI.

treatments alone, rather the response in these assays expressed both carry-over and 1969 treatment effects. This caution in the interpretation of the bioassay data was particularly valid for the helicopter treatments I-V. In the mist blower treatment VI the carry-over effect was probably insignificant in light of the high residues accumulated after each spring application. Since this study was initiated after the treatments were applied, it was not possible to differentiate between carry-over and treatment effects.

Treatment VI, a mist blower application, resulted in high mortality at both sampling dates, 93.8% and 96.2% respectively.. The trees in this treatment were smaller than the average helicopter-treated elms (10-12 m). Therefore, a direct comparison of treatment effects between helicopter and mist blower-sprayed trees at different height levels could not be carried out. The mist blower applied 4-6 times as much spray (2 - $2\frac{1}{2}$ gallons) as compared to roughly 2 quarts with the helicopter. This is one reason for increased mortality in the mist blower treatment. Further, greater effectiveness of the mist blower spraying is given by the fact that considerably more of the total twig crotch area is reached than is the case with helicopter spraying. This point was conclusively proven during a later phase of this investigation (see section III).

Feeding Response (Table 7, Figure 28)

The degree of protection of an elm tree against vector feeding is perhaps more truly given by the feeding response itself,or rather, the 'failure to score the xylem', than by the percentage mortality.

F1 and F2, both strong feeding responses with the xylem visible, were pooled to one value which was termed 'successful feeding'. Responses F3 and NF were pooled as well and termed 'failure to score xylem'. It

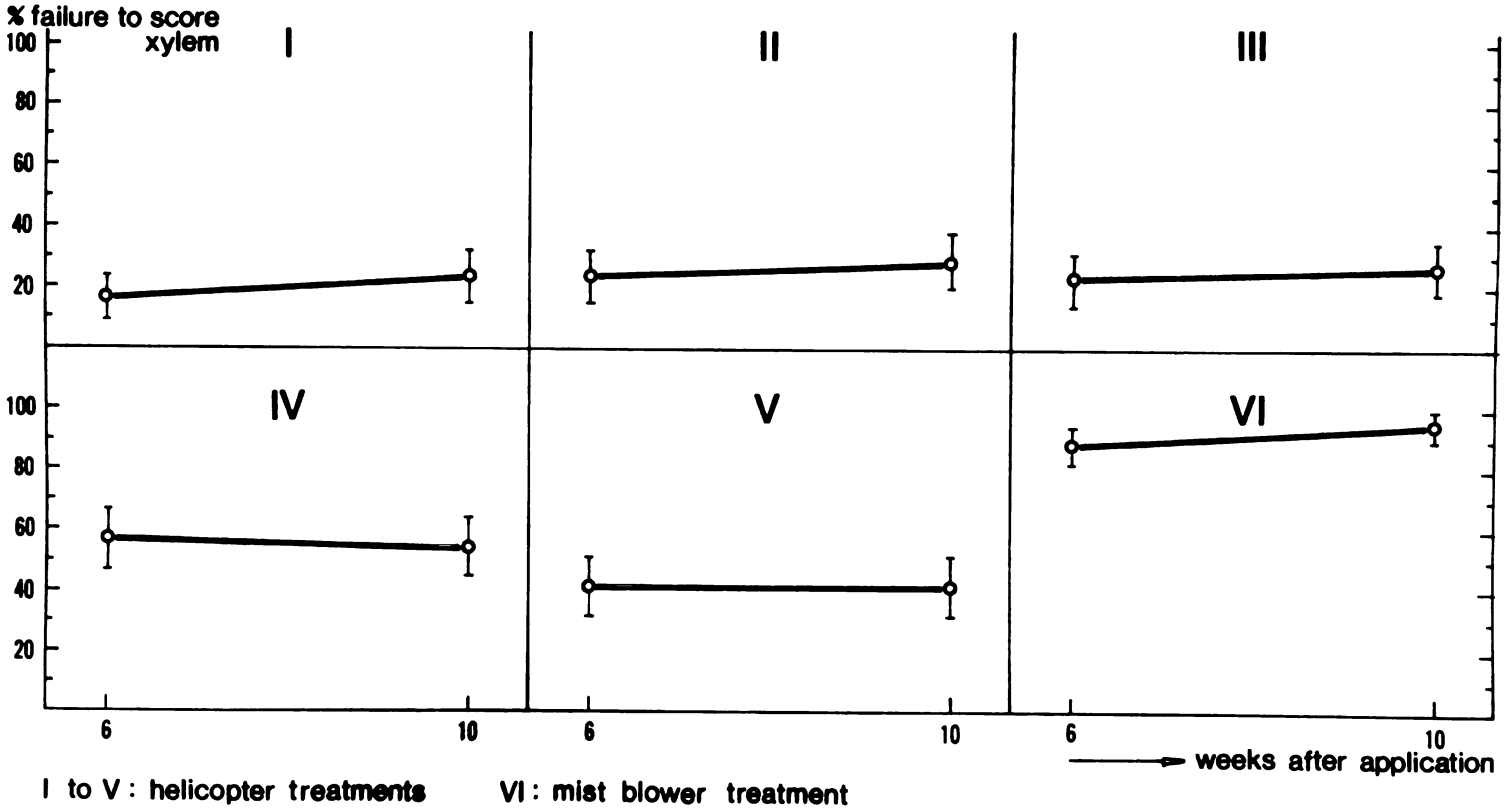

Figure 28. Bioassays 1969: feeding response in treatments I to VI.

was believed that indexing the feeding response was more meaningful than would be an actual measurement of the scar length, since the beetle in close confinement within the brass cylinder could not exhibit 'natural' feeding behavior. Further analysis of feeding and mortality data proved that indexing the feeding response (although only a relative measure of feeding behavior) was very reliable.

In general, the figures of percentage 'failure to score xylem' for treatments I to VI corresponded well to the respective mortality figures. The correlation of these 2 responses received further attention in a separate analysis. Again, the mist blower treatment VI had the highest degree of protection with 88% to 95% in May and July, followed by treatment IV, then V, and with very little protection, the rest of the treatments, I, II, and III.

(B) Bioassays 1970 (Figure 29)

1. Helicopter

Mortality

In general, the helicopter treatment was surprisingly ineffective considering that the first series of bioassays was conducted 2 weeks after application. Mortality figures ranged from 62% to 3.5% with the mean at 23.2% ± 12.5 (C.L.)[1] for the 10 trees, C to M. After 8 weeks (2nd sample period) the mortality decreased to 11.3% ± 10.9 (C.L.) calculated from the 5 sample trees, C, E, G, H, and K.

Feeding Response

Failure to score the xylem in the first series of assays was considerably higher than the corresponding mortality figure. The mean value was 41.0% ± 8.5 (C.L.), which was almost twice as high as the mean

1) $\alpha = 0.05$

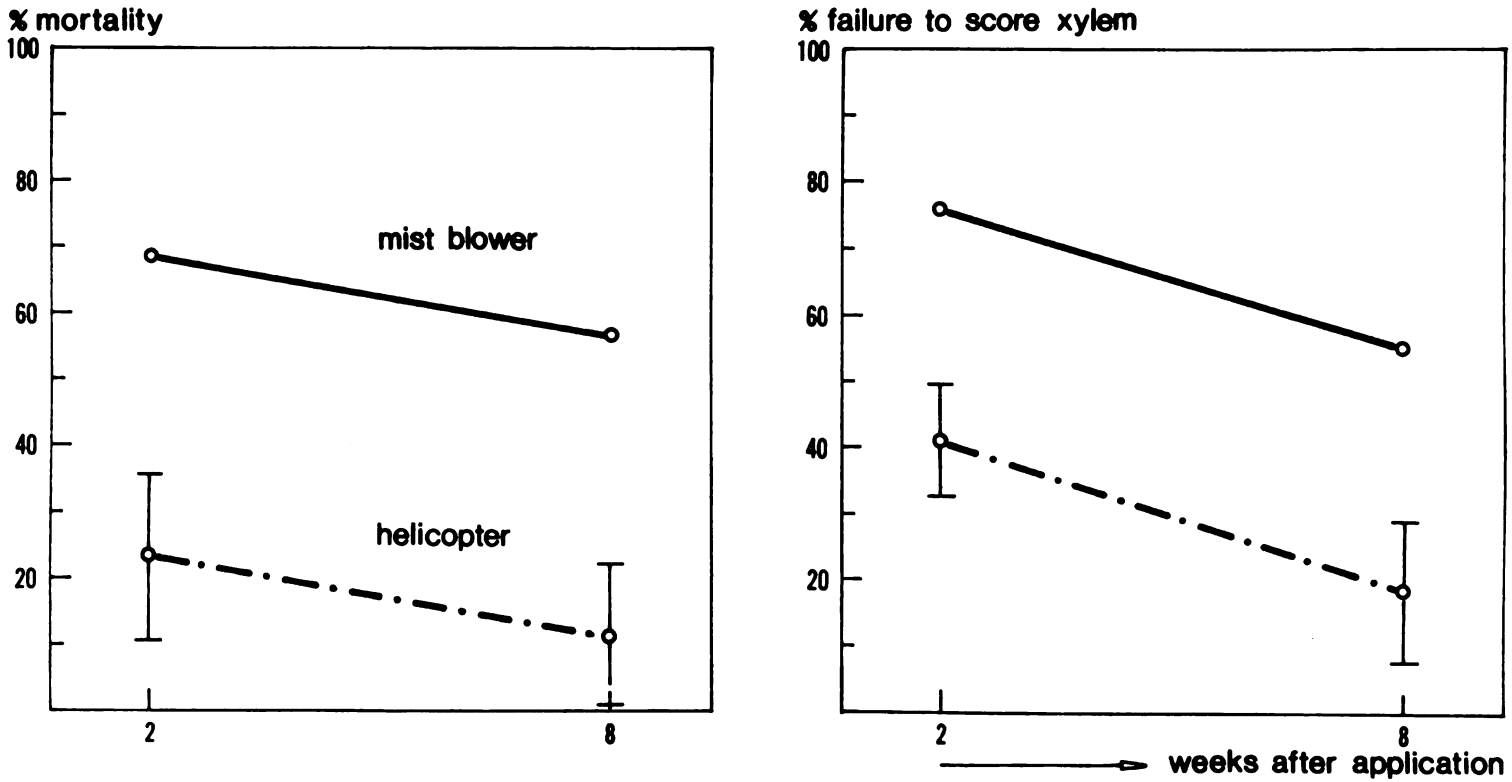

Figure 29. Bioassays 1970: mortality and feeding response on helicopter and mist blower treated elms.

mortality. However, the mean feeding response for the July assays of 18.3% ± 10.64 (C.L.) corresponded well with the average mortality figure of that sample period.

2. Mist blower

Mortality

By comparison, average mortality in the 2 mist-blower-sprayed trees was lying considerably above the mean of the helicopter-treated elms. Tree A, sprayed before bloom, had 82.7% mortality while tree B had only 53.5% due to the fact that the blossoms prevented good coverage of the branches. For the second sample period the mortalities dropped to 66.4% and 47.0% for tree A and B, respectively.

Feeding Response

The percent failure to score xylem and the respective percentage mortality corresponded better in the July assays than in the May feeding trials, as was also noted for the helicopter treatment.

(C) Correlation Between Mortality and Feeding Response (Failure to Score Xylem) in the Bioassays 1969 and 1970 (Figure 30, 31)

These correlations include the figures for all sample trees, both helicopter and mist-blower-treated, of a given sample period. The mortality figures in these correlations were not corrected for natural mortality. The feeding response or failure to score the xylem includes all apparently non-feeding individuals plus the cases when only small bark patches were removed (xylem not visible). Both responses are highly correlated in the 1969 bioassays with the correlation coefficients r= .98 for the May samples and r= .97 for July. The slope for the regression lines was in both cases 1.09. The intercept was somewhat larger for the July assays, however it was negative in both cases.

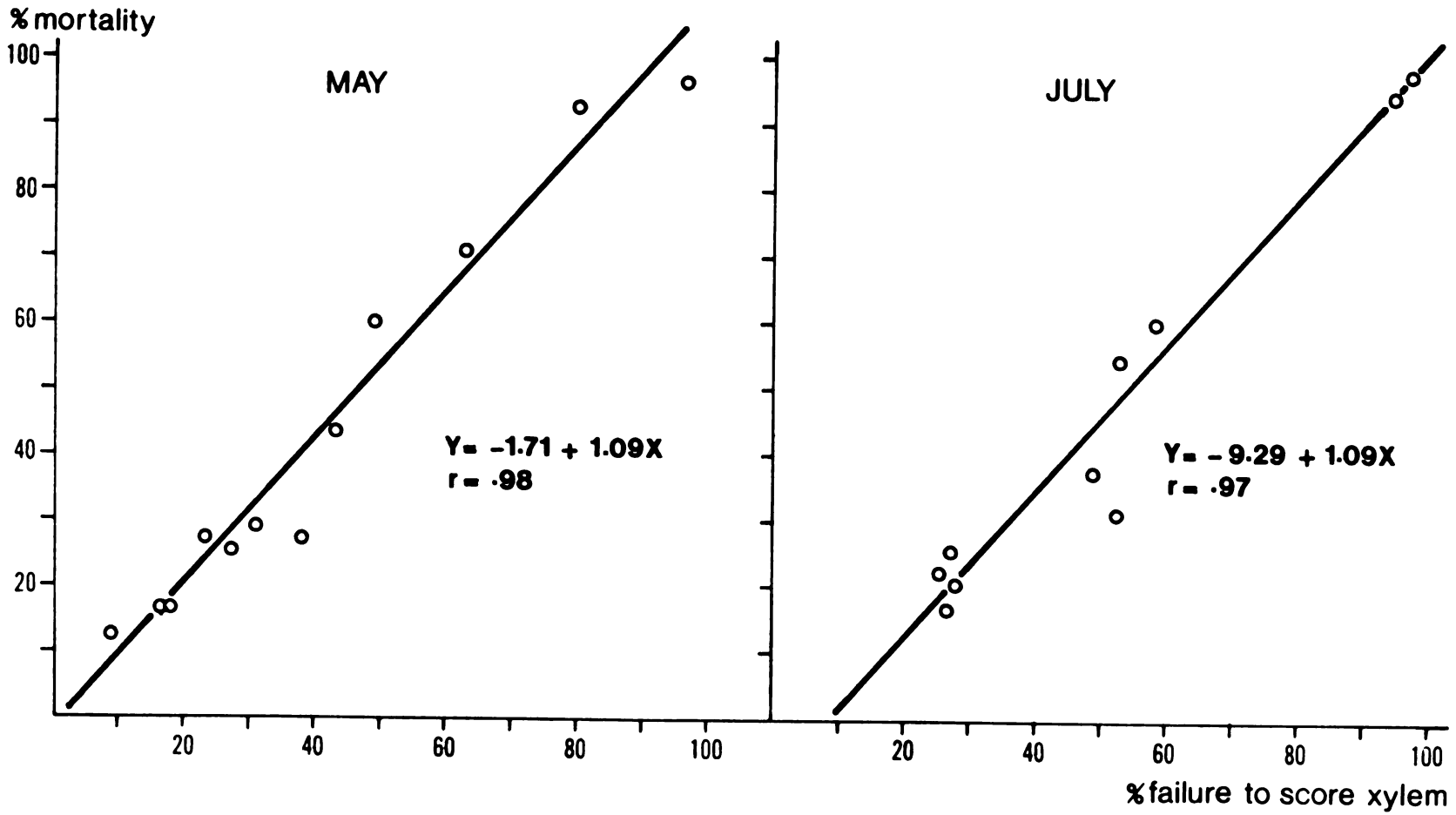

Figure 30. Bioassays 1969: correlation of 'failure to score xylem' and mortality for two sample periods.

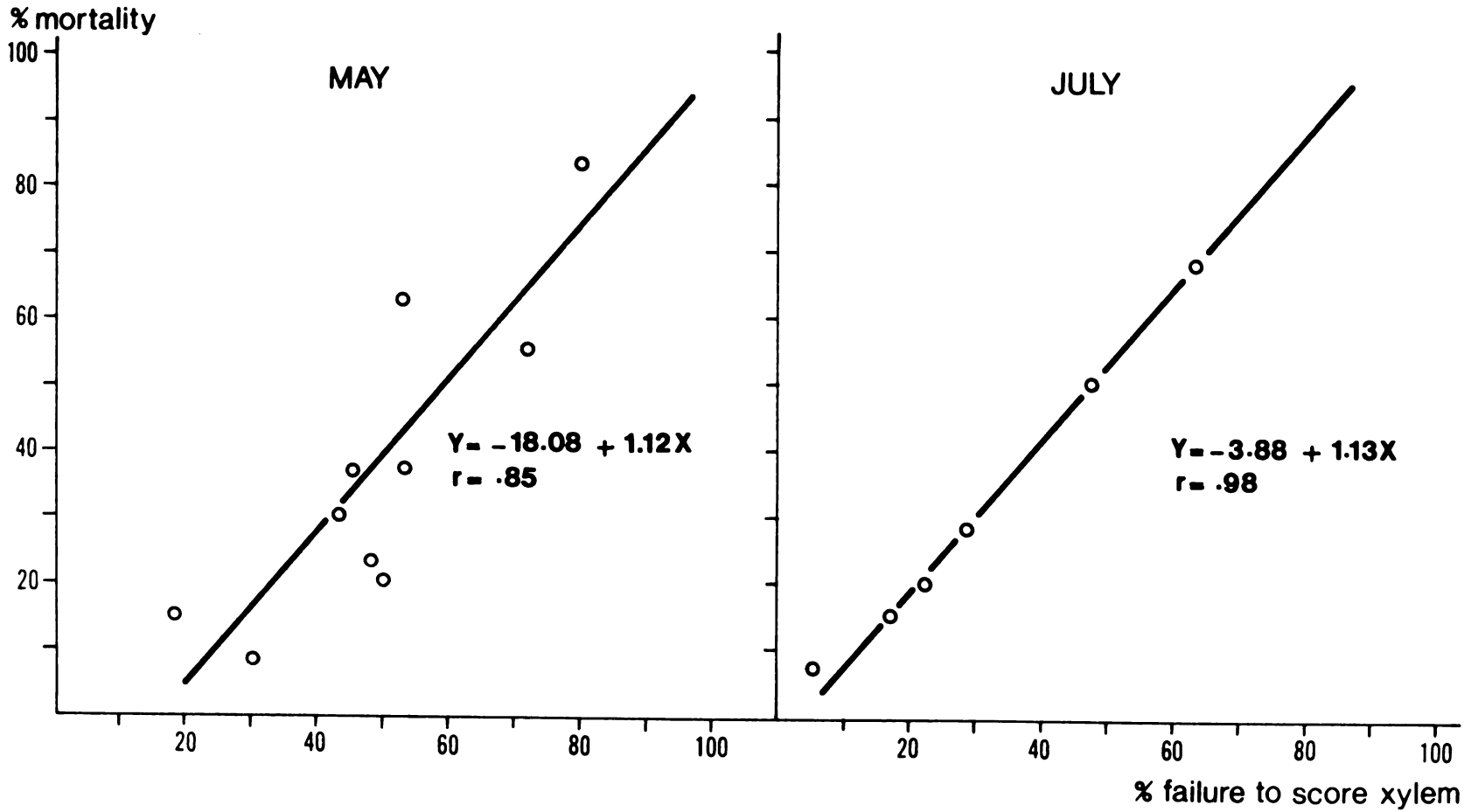

Figure 31. Bioassays 1970: correlation of 'failure to score xylem' and mortality for two sample periods.

The correlation of the 2 responses, mortality and failure to score xylem, in the May bioassays of 1970 is weaker with r= .85; the slope of the regression line was 1.12. In 8 out of 10 data pairs in this correlation, the feeding response (failure to score) was higher than the corresponding mortality figure. A possible reason for this discrepancy between failure to score xylem and mortality could be a repellent action of surface deposits. Cuthbert et al. (1972) suggested that methoxychlor deposits on bark and in the twig crotch might also have some repellent action which keep the beetle from feeding. The May samples were taken 2 weeks after application; that is, enough surface deposits could still have been present in these samples, which prevented a stronger feeding response. It is conceivable that it takes some time (longer than 2 weeks) for the surface deposit to penetrate into the bark (or decompose on the surface) and a particular twig crotch might become attractive again after this penetration (or decomposition) process is completed.

The data for July, 1970, were again well correlated with r= .98 and the slope for the regression line was 1.13.

Under the conditions of the bioassay, feeding response indexing was very reliable, which was proven by the good correlations between failure to score xylem and mortality. These correlations suggest that beetle mortality could be predicted from observed feeding responses.

(D) Mortality at 3 Height Levels

The sampling scheme was designed to take into account not only the horizontal but principally the vertical variation in spray coverage (Figure 12). With a mist blower operating from underneath the tree and a helicopter from above the crown region, definite differences in residue deposits at the 3 height levels were expected. It is a well-known fact

that with increasing tree height the mist blower loses its effectiveness, that is, less spray is carried into the tree top region. The preferential feeding pattern, as elaborated in section I suggests that the greatest protection is needed in the tree top region with no particular preference as to a compass direction. The helicopter application seemed to offer a valid alternative to the mist blower application since it presumably deposits more spray in the top region. Theoretically the deposits should decrease from the top to the bottom due to the interception of spray in the upper tree region. In other words, it was expected that the helicopter would lose efficiency in coverage from the top to the bottom while the mist blower would lose efficiency from bottom to top. If trends of this sort existed, the bioassay data should have reflected differences between height levels with regard to mortality and feeding response. Since mortality was well correlated with the feeding response, only mortality data from the helicopter applications in 1969 and 1970 and from the mist blower treatment in 1970 were examined. Because of the nature of the data, a non-parametric test was employed to demonstrate possible differences in mortality between height levels.

1. Bioassays 1969/70: Helicopter

The mortalities at the bottom, center and top level of all trees of a sample period were compared in Friedman's two-way ANOVA by ranks. A separate analysis was performed for each sample period in 1969 and 1970. All data were corrected for natural mortality in the controls. In two instances the corrected data had to be coded with +10 because of some negative values after correction with Abbott's formula. The results of the ANOVA are listed in Table 8 and show a contrasting pattern for 1969 and 1970. It was expected that mortality would be highest in the upper

Table 8. Friedman's ANOVA by rank of mortalities at 3 height levels of helicopter and mist blower-treated elms.

Sample Date	X_r^2	No. Trees	p [1]	Highest mortality at height level
A. Helicopter Treatments				
1969 May	1.25	10	0.569	----
1969 July	5.55	10	0.069	3
1970 May [2]	0.66	9	0.814	----
1970 June	6.30*	5	0.039	1
B. Mist Blower Treatments				
1970: data pooled for May and June	7.13*	4	0.042	3

1) Table N in Siegel, "Non-parametric Statistics" (1956)
2) One tree omitted from analysis because of incomplete data

tree region in all five helicopter treatments. The opposite was the case for July, 1969, where the mortality was highest at the lowest height level. In 1970, 2 weeks after helicopter spraying, mortality was in general higher at the top level; however, the differences were not significant. The mortality in the second series of bioassays, 8 weeks after application, was significantly greater in height level 1. This change of the mortality pattern in the helicopter treatment from 1969 to 1970 probably reflected the slow, but continuous disappearance of mist blower carry-over in the lower tree regions. The cube sample experiments (section III of this dissertation) proved clearly that the helicopter delivers significantly more spray into the top region of the tree than into the lower portions. One must conclude, therefore, that the carry-over from previous mist blower

applications (prior to 1968) reversed the expected vertical mortality pattern in the 1969 helicopter treatments. With further decomposition of the mist blower carry-over the actual distribution pattern characteristic for helicopter-applied residues will become more and more apparent. The vertical mortality pattern of the 1970 helicopter treatment indicates this. The residue analysis of 1969 and 1970 bark samples gave further support for this conclusion.

2. Bioassays 1970: Mist blower

Tree A and B were treated by mist blower. Figure 32 shows the average mortality pattern at the 3 height levels for the 2 sample dates. The mortality pattern of the 1970 helicopter treatment (9 tree average at 2 weeks, 5 tree average at 8 weeks after application) is shown for comparison. Typical for the mist blower application was the continuous decrease in mortality as tree height increased. Since only 2 mist blower sprayed trees were sampled, the mortality data from the May and July bioassays were pooled for the ANOVA. The results are shown in Table 8 and were significant at $p = 0.042$. The high mortality at the lower height levels is a strong contrast to the helicopter treatment.

Since S. multistriatus obviously prefers feeding sites in the top region of the tree (see section I of this thesis), particular attention must be given to good tree-top coverage. A direct comparison of mortality figures at the upper height level on mist blower and helicopter sprayed trees is therefore of great interest. The average mortality in height level 1 of the 9 helicopter-sprayed trees was 27.0% 2 weeks after application (May 1970) and 17.3% 8 weeks (July 1970) after application. There was a decrease in the mean mortality from height levels 1 to 3 in the May samples, 1970; however, the ANOVA did not indicate significant

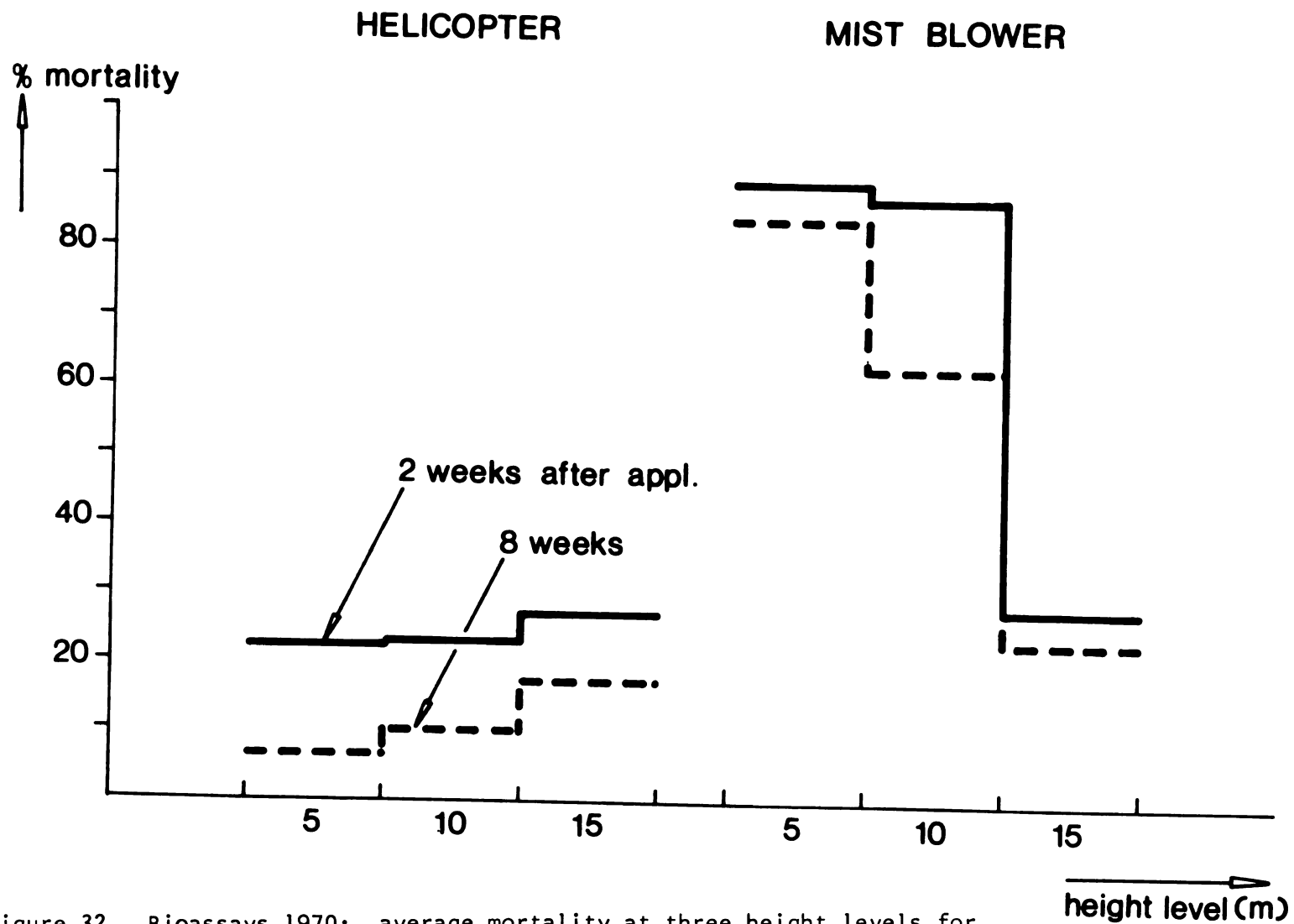

Figure 32. Bioassays 1970: average mortality at three height levels for helicopter and mist blower-treated elms (2 and 8 weeks after application).

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differences between height levels. The mean mortality in the July samples went down from 17.3% in the top to 9.9% in the center and to 6.7% at the bottom. The differences in mortality at the 3 height levels were significant as shown by Friedman's ANOVA.

On the other hand, the mist blower treatment caused the least mortality in the top height level. In the 1969 treatments this did not show up because the trees were smaller (10-12 m) and tree-top coverage was sufficient. The mortality in the top region of these smaller trees ranged between 80% and 100% at both sampling dates (6 and 10 weeks after application). Of the mist blower treated trees in 1970, tree B was somewhat smaller than Tree A and was in bloom when it was sprayed, which resulted in poorer coverage. Consequently mortality was lower than in tree A, particularly in the top height level. Mean mortality was 27.7% at the top but fell to 23.2% 8 weeks after spraying. The cube sample experiments indicated that a well-exercised mist blower application will create sufficient deposits on the tops of 15 m high trees. The mist blower application for tree A and B was probably not thorough enough and was definitely ill-timed because of tree bloom.

(E) Methoxychlor Residues on Treated Elm Trees

One aim of the residue analysis of bark samples was to give support to the findings of the biological assays. The amount of residue present on or in the bark is certainly an accurate parameter for the comparison of spray coverage on elm trees and was suggested by several workers (Thompson, 1965; Wallner et al., 1969). Although useful for comparison of treatment effects, the amount of spray deposit can only be a relative measure of performance and in itself lacks biological meaning. Therefore, for proper interpretation, the amount of residue

needs to be correlated with the response of the insect in the bioassays. This was done in section IV of this dissertation.

1. Carry-over (Table 9)

Prior to 1968, the elm population on the campus of MSU was sprayed yearly in the spring with a mist blower. Section V gives a detailed account of DED spray practices on the campus since 1958 (see also Figure 6 for treatment areas). In 1968, the spray application was performed by helicopter on the majority of the campus elms. Five methoxychlor formulations were applied and evaluated by gas chromatographic analysis (Wallner et al., 1969). Twig samples from elms in treatment areas IV and V taken before the helicopter application had very high methoxychlor residues. The above authors attributed the significant carry-over in these treatment areas to heavy mist blower spraying over the previous years, which frequently overloads the lower tree portions. Methoxychlor deposits on bark were believed to have low persistence. Wallner and Leeling (1968) reported originally that methoxychlor deposits had a short life span, not exceeding one year. This could be true if the deposit is primarily laid down on the bark surfaces where it will decompose much faster. However, if a formulation and/or application technique favors bark tissue deposits over surface deposits, carry-over will be higher since the residue is imbedded in the tissue and is protected against weathering. This point was proven in decomposition experiments of several methoxychlor formulations (section IV). Because of the heavy carry-over from the earlier mist blower applications (both methoxychlor and DDT were used), it is very difficult to make a valid judgement about the superiority of one or the other 1969 helicopter treatment. Carry-over levels were determined each year from 1969 until 1971, shortly before the prefoliar spray

applications. Wallner et al. (1969) found the highest carry-over from 1967 to 1968 on trees in the treatment areas IV and V (Table 9). The difference in carry-over was significant on these trees which were mist blower-sprayed during the previous year with the same methoxychlor formulation. Obviously elm trees in certain locations received greater attention because of their shade tree value. The following year the pre-spray residues were again highest in treatment areas IV and V. Carry-over from 1969 to 1970 was substantial in treatment IV with the highest residues in the lower height levels. Trees in the only mist blower treatment had high carry-over residues in the upper height level. It was intended to use these carry-over data as the base values for the evaluation by calculating the difference between post-spray residue and carry-over. However, variation in spray deposits within and between trees of the same treatment was great. Only extensive bark sampling and residue analysis would have removed this variability. For these 2 reasons, carry-over and variation of spray deposits, the post-spray residue values from the helicopter treated elms must be interpreted with considerable caution. The same caution applies for the interpretation of the bioassay data since the response at the feeding site was a composite of carry-over and treatment effect.

The high amount of DDT in some of the 1970 samples was surprising. DDT was also present in samples from 1969, but it was not quantified. DDT was verified by gas chromatographic analysis with 2 different columns and by thin-layer chromatography on silica-gel H plates. Only trace amounts of metabolites, DDE and DDD, were present. If these residues were indeed carry-over, it was astonishing to find DDT still in its unmetabolized state. A possible explanation may be that DDT had survived

Table 9. Methoxychlor residues in twig crotches of helicopter- and mist blower-treated campus elms: 1968, 1969, 1970.

		1968 [2]			1969 [3]			1970	
		PPM			micrograms/cm^2			micro./cm^2	
Treatment	Height Level	Pre	3 Days Post	130 Days Post	Pre	8 Weeks Post	15 Weeks Post	Pre MeCl	Pre DDT
I	1	84.0	260.2	72.6	6.1	6.2		4.3[3]	
	2					18.8		9.4	
	3					19.4		3.7	
II	1	281.4	328.0	124.4	1.2	39.3			
	2					17.2			
	3					54.4			
III	1	20.5	195.2	32.0	4.0	3.9	2.3		
	2					4.4	2.4		
	3					10.0	2.4		
IV	1	308.8	316.0	257.0	9.5	35.6	16.3	8.7[4]	1.5
	2					83.5	17.8	17.5	2.5
	3					14.7	27.7	28.4	3.5
V	1	404.8	432.0	207.6	15.0	23.8	7.5		
	2					17.3	11.7		
	3					15.8	18.3		
VI	1					172.1	39.9	25.6[4]	1.6
	2					183.5	71.3		
	3					208.3	80.2		

1) I - V: helicopter; VI: mist blower
2) Data from Wallner et al. (1969)
3) Average of 2 trees per treatment
4) Average of 3 trees per treatment

as tissue deposit in the outer bark where neither biological nor environmental factors could induce metabolism. The bark samples were taken from 1-5 year old twig crotches. Therefore, part of the total sample consisted of bark from older twig crotches which apparently carried the DDT residues. On some trees the amount of DDT carry-over was suspiciously high considering that the last mist blower applications with DDT were performed 5 years prior to sampling.

2. Methoxychlor residues 1968/69 (Table 9)

The results of the residue analysis of twig crotches from treatments I to V had a similar pattern in both 1968 and 1969. In 1968, for both sample dates, coverage appeared to be best in treatments IV, V and also II, followed by I and III. The sequence from highest to lowest residues for the first sample date in 1969 was: treatment IV, then II, V, I, and III. The last two treatments, I and III, again had the poorest coverage, as was the case in 1968. Results from the second sample date in 1969 established once again that treatments IV and V had the highest residues, while treatment III had very poor coverage.

The 1969 samples were collected from 3 height levels to demonstrate vertical distribution of residues within the crown region. In general, the highest residues were found at one of the 2 lower height levels while the top level had the lowest deposits. The reverse was actually expected in these helicopter treatments since the upper tree portion presumably intercepts most of the spray. It is possible that the vertical distribution of carry-over from previous mist blower applications still overrode the distribution pattern created by helicopter spraying. If this assumption was correct, one could expect a continuous transition from a 'mist blower residue pattern' to a 'helicopter residue

pattern'. The mortality patterns at the 3 height levels in 1969 and 1970 reflected this trend and supported the above hypothesis. Also, methoxychlor deposits could decompose faster at the top region where twigs are more directly exposed to environmental influences. This would create a similar effect whereby residues in the lower tree region would appear to be higher after a longer weathering period. However, this is only speculation.

Treatment VI, the mist blower spray plot, had considerably higher residues than did the best helicopter treatments. The typical mist blower deposit pattern, with the lower tree portion having the highest residues, was not very distinct in this treatment. The reason probably was that the low height of the trees in the spray block permitted the entire tree crown, even the tops, to receive equally good coverage.

3. Methoxychlor residues 1970 (Table 10)

There were only 2 treatments in 1970, a helicopter and a mist blower treatment. Samples were taken shortly (2 weeks) after application, but residues were considerably lower at all height levels from the year before. Again, the variation in spray deposits between helicopter-treated trees was great. The average deposits at the 3 height levels showed minimal differences 2 weeks after helicopter spraying. Eight weeks after application the deposits were greatest in the top height level. As for 1969, the vertical distribution of residues corresponded well with the mortality pattern at the 3 height levels. The strong presence of DDT in many samples from both sample periods was mentioned already. Significant amounts of DDT were detected in the first sample of 4 helicopter and 2 mist blower sprayed trees at all height levels. Two helicopter and 2 mist blower sprayed trees had DDT present in the second samples, but

Table 10. Methoxychlor residues in twig crotches of helicopter- and mist blower-treated campus elms: 1970.

Treatment[1]	Height Level	2 Weeks Post MeCl	2 Weeks Post DDT	8 Weeks Post MeCl	8 Weeks Post DDT
Mist Blower[2]	1	5.7	4.9	3.7	3.7
	2	17.7	15.1	7.8	5.4
	3	27.9	22.7	19.7	15.9
Helicopter	1	12.8[3]		8.4[4]	
	2	12.8		5.8	
	3	12.5		6.3	

1) For description of formulations see page
2) Average of 2 trees, heavy DDT deposits
3) Average of 9 trees, DDT present on 4 trees
4) Average of 7 trees, DDT present on 2 trees

in smaller amounts.

Considerably more DDT was found on the mist blower-treated trees than on the helicopter-sprayed trees. The vertical distribution of deposits, both methoxychlor and DDT, was typical for the mist blower. The deposit figures for the mist blower in Table 10 represent the mean of 2 trees and are almost 10 times lower than the figures for treatment VI in 1969. Data from the cube sample experiments in 1972 suggest that initial mist blower deposits can be extremely high (several hundred micrograms/cm^2 bark) in the lower tree portions. Either the extraction and the quantitative analysis was unusually unreliable or the application itself was poor. One of the 2 mist blower trees was already in bloom when the spray was applied. Consequently, on that tree the coverage was particularly poor.

Basically, the bioassays and the residue analysis yielded the same information. Both methods established the mist blower as the most effective application technique, at least for trees with limited height. Also, the same sequence of most effective to least effective helicopter treatment was indicated by the two methods with the exception of treatment II which had high residues but little mortality. Treatment IV, the Dacagin formulation, appeared to be the best choice for the helicopter; however, the high carry-over of DDT and methoxychlor in this treatment block confounded the results to a large extent. This applied to the other treatments as well. This circumstance made it difficult to draw valid conclusions from this field experiment and to recommend a superior methoxychlor formulation for the helicopter.

(F) Evaluation of Treatment Effects by Comparison of Disease Incidence in Treatment Areas I-VI: 1968-1969

The effectiveness of pre-attack sprays or other control strategies has frequently been evaluated on the basis of disease occurrence (Plumb, 1950; Matthysse et al., 1954; Barger, 1971). One has to agree that in the case of DED the true test for insecticidal formulations and application techniques is overall disease prevention rather than the performance in a particular bioassay. If the total number of trees lost to DED over a given period of time is used as the sole criterion to compare treatment effectiveness in sometimes widely separated areas, several assumptions have to be made. The most important one is expressed in the following question: Do all elm trees in the treatment areas have the same chance of becoming infested with the disease? Naturally, the probability of disease incidence is directly related to vector density (vector pressure), assuming that the amount of inoculum carried by a

population is proportional to its density and rather stable for a given population size. Equal chance of tree infection presupposes, therefore, a uniform distribution of the vector population over all treatment areas. This is, in all probability, an incorrect assumption. Fransen (Heybroek, 1969) attempted to select in a field experiment elm trees which were resistant to vector feeding. While S. scolytus was feeding rather uniformly on the experimental elm trees, the feeding pattern of S. multistriatus was very spotty and erratic. Wolfenbarger and Jones (1943) made similar observations and found that the feeding pattern among a population of elm trees was influenced by the distribution of beetle-productive trees. Even if the post-emergent flight to a feeding site was random and not triggered by olfactory stimuli (Meyer and Norris, 1964) the feeding and, subsequently, also breeding will always be higher around beetle-productive trees. Furthermore,wind currents can offset the vector distribution (Felt, 1937) and so contribute to an erratic feeding pattern. The design of field experiments where tree losses in sprayed blocks were compared to the losses in the untreated check blocks frequently had inherent flaws. Untended wood lots where the shade tree value of elms approached zero served often as check plots. Naturally, the vector population was higher there than in the sprayed treatment blocks, which were usually roadside plantings or park trees. Because of this vast difference in vector pressure, disease incidence was understandably much higher in the untreated check blocks. In other words, the treatment effect was undoubtedly exaggerated in these comparisons.

The differences in elm die-back in the six treatment areas of the MSU campus over the two-year period (Table 11) can only partially be attributed to the relative effectiveness of one or the other methoxychlor

Table II . Tree losses 1968/69 in treatment areas I - VI[1)]

Treatment[2)] Area	No. of Trees Sprayed	No. of Diseased Trees				% of Diseased Trees	
		1968	1969	Total	Root Graft[3)]	Total	Excl. Root Graft
I	286	10	10	20	7	7.0	4.6
II	90	2	3	5	1	5.6	4.4
III	140	20	13	33	-	23.6	23.6
IV	105	-	4	4	-	3.8	3.8
V	936	44	55	99	24	10.6	8.0
VI	89	6	8	14	8	15.7	6.7

1) Figures taken from records of the MSU grounds department
2) Treatments are described in detail on pages 64 to 66
3) Not confirmed

formulation or application technique. Differential vector pressure in the treatment areas was definitely present. In particular, the western section of the campus is close to extensive untended wood lots which were, at the time, still vector productive. In addition, an increasing influx of vectors must have started in 1968 when the city of Lansing, also to the west of the campus, began to reduce DED control practices (see section V). Other confounding factors which made an objective evaluation of these treatments I to VI difficult were root graft transmissions and possible carry-over from previous DDT treatments (the latter applied also to the feeding trials). Figure 7 shows the campus areas which were continuously mist blower-sprayed with DDT from 1958 to 1967. Disease occurrence in 1968 in the treatment areas I to VI did not differ very much from the figures in 1969.

The highest percentage of die-back occurred in area III in the northwestern part of the campus, followed by area VI, the only mist blower-treated area, then came areas V, I, II, and IV (see Figure 6 for exact location of treatment areas). Root graft transmission was suspected to be responsible for the substantial tree kill in area VI since most of the infestations were in immediate proximity of each other. Also, it was believed that vector pressure in this area was higher than in any other part of the campus. Treatment IV had the smallest percentage of trees killed overall by the disease. With the exception of treatments III and IV, the results of the 1969 bioassays were a strong contrast to the above picture. Treatment VI (mist blower) emerged as the most effective in these feeding trials, giving 90% protection up to 10 weeks after application. From the five helicopter treatments, only IV was satisfactory, followed by V and, giving minimal protection, I, II, and III. The

sequence here was almost the reverse of the disease picture.

Again, probably several of the factors mentioned above contributed to confound the results (die-back) in the respective treatments. In order to correct for the varying vector density in the treatment areas, one would have to know the relationship between vector density and disease infection. Therefore, a different experimental design is necessary to safeguard against confounding variables, such as vector density, which can confuse the whole disease picture and can make comparisons of treatments to control DED meaningless. A completely randomized design or a randomized block design would certainly be more appropriate, but from a practical standpoint, such a design might not be useful since the trees in each treatment would be randomly distributed over a large area. This could make the actual applications complicated and time consuming.

Summary and Conclusions

In 1969, five helicopter treatments and one mist blower treatment were evaluated by feeding trials with S. multistriatus and by analysis of bark residues. A 12.5% MeCl formulation containing Dacagin, a viscous thickening agent, emerged as the most persistent and effective helicopter treatment. Mortality ranged between 50 and 60%. Consequently, this formulation was recommended for the 1970 helicopter application on the majority of the campus elms. The performance of this treatment was very poor. The mean mortality in the bioassays reached only 23.2% 2 weeks after application and fell to 11.3% after 8 weeks. Twig samples taken before the spring applications in 1969 and 1970 suggested that carry-over from mist blower applications prior to 1968 with DDT or methoxychlor was substantial. It was concluded, therefore, that the good protection achieved in 1969 in some helicopter treatment areas was the combined result

of carry-over and the particular helicopter treatment. Carry-over invalidated the results of the comparison study of several helicopter-applied methoxychlor formulations to a great extent since it affected the outcome of the bioassays and residue analysis.

In 1969, the carry-over in the lower tree regions still exceeded the new helicopter deposits in the tree tops. The feeding trials and residue analysis in 1970 indicated that the carry-over in the lower height levels had diminished to the point where tree top residues began to be higher. The slow transition from a mist blower residue pattern, with higher deposits in the bottom region, to a helicopter residue pattern, with higher deposits in the tree tops, was well expressed by the results of the bioassays and residue analysis in 1969 and 1970.

The comparison study of ground application with the mist blower and aerial application with the helicopter was based on bioassay data and bark analysis and definitely favored the mist blower. Tree height seems to be the only factor which would alter the effectiveness of the mist blower. The investigation concerning the preferred regions for feeding attack within the tree crown established the whole upper tree portion as the primary hazard region. The helicopter application in 1970 gave insufficient protection in tree tops, causing only 27.0% mortality, and decreasingly less in the lower height levels (2 weeks after application). The mist blower protected the lower height levels almost completely, with mortality figures ranging between 80 and 100% for the whole season. Mist blower protection was poorer in the tree tops but still better than the helicopter application.

Placing most of the attention during a spraying operation on tree top coverage is inadequate. One has to take into account as well

the hazard regions for effective inoculations with the DED pathogen. Inoculations in the lower tree region, particularly close to the main trunk, have been shown to be more effective and lead to faster disease development in the whole tree. Considering both the vector and the pathogen, the whole tree would need equal protection, because of the higher feeding rate in the tree top and the higher successful infection rate in the bottom region. Neither mist blower nor helicopter gave equally good protection throughout the tree. Their obvious draw-backs were pointed out already.

Two responses, mortality and 'failure to score xylem' (feeding response), were recorded in the bioassays. Both were highly correlated except in one case where twigs with 2-week old deposits were bioassayed. Surface deposits of methoxychlor were still present and they apparently induced a behavioristic type of resistance in S. multistriatus which resulted in low mortality due to non-feeding. This researcher believes that the possible repellent action of MeCl surface deposits is of little significance in terms of tree protection since a searching vector will eventually start to feed in a suitable twig crotch.

Disease occurrence as the sole criterion for an evaluation of spray treatments against DED was critically discussed, using the helicopter and mist blower treatment areas in 1968 and 1969 as examples. Tree kill in each treatment area appeared to be independent of the degree of tree protection (as indicated by the bioassay results). It is suspected that the tree kill reflected more the vector pressure in each respective area. Therefore, a different experimental design was suggested for field experiments where tree kill is used as the criterion of treatment performance.

III. Monitoring of Spray Coverage on Elm Trees

Introduction

During the course of the field evaluations of application techniques and methoxychlor formulations in the spring and summer of 1969 and 1970, it became desirable to determine the degree of coverage of a tree using a more objective method. Bark analysis alone was extremely variable and only extensive sampling would have given the desired accuracy. Besides, carry-over from previous mist blower applications interfered heavily with the evaluation and distorted treatment effects. The objectives were to demonstrate how applications by helicopter and mist blower differed with respect to : (a) variation in spray patterns, droplet size and density along a vertical gradient, (b) horizontal and vertical distribution of actual spray deposits within the crown region, and (c) coverage in a 3-dimensional sense.

A close look at point (c) seemed to have particular relevance in this case because of the 3-dimensional nature of the target sites, the twig crotches. A sampling device was needed, therefore, which could serve the above objectives. Maksymiuk (1971a) successfully used fluorescent dye-tracers for the assessment of spray deposits and spray patterns on cards or aluminum plates. Oil-sensitive cards were found to be useful for the immediate appraisal of oil-based spray deposits. Ideally, a sphere would best show a 3-dimensional coverage. However, since actual deposit determinations had to be made, a cube-like sampling device was selected with filter disks fastened to all 6 sides. The filter paper

trapped the spray in such a way that it could be conveniently extracted and quantified.

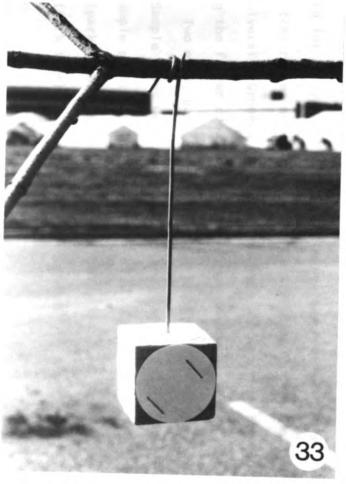
The total analysis consisted first of a visual inspection of coverage and measurement of spray patterns, and, second, of an accurate quantification of deposits on these filter disks.

Materials and Methods

(A) 3-Dimensional Sampling Device

Wooden cubes with the dimensions of 4.25 x 4.25 x 4.25 cm were covered with Permacel masking tape to prevent secondary absorption of spray from the filter paper into the wood. Whatman No. 2 filter disks were stapled to all six sides of the cube (Figure 33). The cubes were suspended on wire from branches at the respective sample points. The top and bottom sides of the cube were horizontal but side areas did not necessarily face certain compass directions. Five cubes were placed in each height level at compass points somewhat within the tree region and at the center (Figure 2).

For mist blower experiments in 1972, attachment of the sample cubes to the branches had to be redesigned because of the strong air current generated by the blower. For an objective experiment, it was necessary to have the sample cubes securely positioned. One end of a 20 cm long piece of steel wire (0.08" diameter) was inserted into the center of the wooden cube; the other end was wound around a branch and tightly squeezed on with a pair of pliers. Also, the filter disks had to be stapled to the cube with at least two staples, again because of the strong air current. For the helicopter experiment, one staple in the center sufficed.



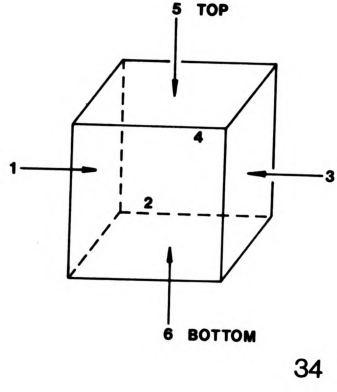


Figure 33. Sample cube fastened to elm branch.
Figure 34. Designation of filter cube sides (1 to 6).

(B) Spray Applications

1. Helicopter application 1971/72

Two elms, one a park tree among a group of shade trees and the other a road-side tree, were randomly selected from the campus elms which were sprayed by helicopter in April, 1971. One day before application, the sample cubes were positioned in the two trees.

Tree A (park tree): 20 m high

Cubes were placed at 3 height levels (5, 10, and 15+ meters) and about 1-2 m within the dripline, $\frac{1}{2}$ meter above ground.

Tree B (road-side elm): 15 m high

The same setup was used as for tree A, however only two height levels (5 and 15 m) were sampled due to the smaller size of the tree. The helicopter used the same type of spray equipment as was employed during the previous years: 30 ft. boom, 59 D-6 nozzles. The weather conditions were ideal with winds less than 5 miles per hour. A Michlin 'MX-2' 12.5% methoxychlor emulsion was employed throughout the campus. As for the 1970 application, Dacagin at the rate of 2 pounds/100 gallons was added for spray drift control. The average amount of spray received by each tree ranged above 2 quarts. The Stratotower from the Michigan State University Grounds Department was made available for placing and sampling the filter cubes in the tree crown region.

Two elms, both 15 m in height, served as experimental trees in 1972. Sample cubes were positioned at two height levels (5 and 15 m) at the 5 sample points as in 1971.

Tree C (park tree): sprayed with same formulation as in 1971

Tree D (park tree): sprayed with same formulation as in 1971 but without Dacagin

The helicopter was equipped with the same spray gear as the year before. Approximately 2 quarts of spray were applied to each tree in the early morning hours. The wind conditions were calm, below 6 miles per hour.

2. Mist blower application 1972

Two days after the helicopter spraying, sample cubes were again placed in the same trees (C and D) at two height levels. These elms were then sprayed from the ground with a John Bean Rotomist 301 G. Approximately $2\frac{1}{2}$ gallons of Michlin 'MX-2' 12.5% emulsion was employed per tree; tree C was sprayed with the Dacagin formulation.

(C) Analysis of Spray Deposits

The first part of the analysis was concerned with a visual inspection of the sample cubes and the spray pattern under long-wave UV. The second portion involved chemical analysis and quantification of the actual deposits. The filter cubes were removed from the trees several hours after spray application and they were stored in a cooler (10^{o} C) up to 14 days until the analysis was completed. Each filter disk was carefully taken from the cube by cutting with a sharp dissecting knife along the staple or by pulling out the staples with needle-nose pliers.

1. Analysis of spray patterns on filter disks

The xylene-based spray droplets which soaked into the filter paper became strongly fluorescent with a light-greenish color under long-wave UV. A Blak Ray (UVL-22) lamp was employed as the stimulating light source. First, the overall spray deposition on the six sides of the sample cubes was examined visually, then the top disks were analyzed with respect to number and size of spray droplets per unit area. Since it

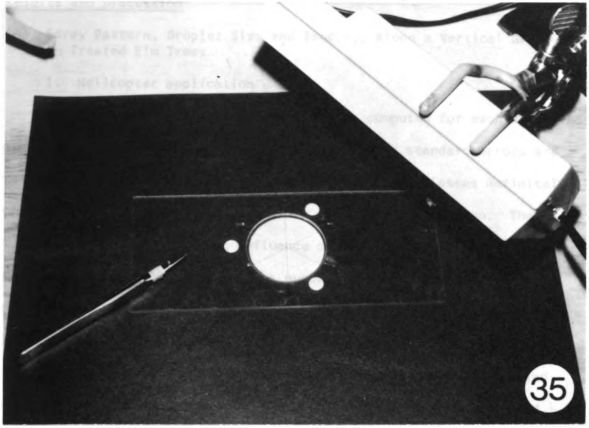
was difficult to count and measure the droplets over the total area of the filter disk at once, the disk was subdivided into smaller sections with a stencil. This was accomplished by placing an acrylite plate with a circular opening above the filter disk. Three thin acetate strings glued across the center subdivided the disk into six equal sections. Only three opposing sections (half the area) were analyzed on each disk (Figure 35). Droplets were classified according to their size into three groups: (1) 2-3 mm, (2) 1-2 mm, and (3) smaller than 1 mm.

2. Quantification of spray deposits on filter disks

After the visual analysis of the spray patterns was completed, the methoxychlor residue on each individual filter disk was extracted with 100 ml of redistilled hexane in a 300 ml Erlmeyer flask. Five grams of sodium sulfate (anhydrous) was added to each extraction to remove traces of water. Each sample was shaken lightly and stirred with a glass rod. After sitting overnight, each sample was analyzed in a Beckman GC-4 gas chromatograph or a 10 ml aliquot was stored in a freezer. The total amount of methoxychlor residue was calculated as micrograms per square centimeter (micrograms/cm^2).

The extraction of residues trapped on filter disks involved only a single step and was, therefore, very efficient. The recovery rate was determined in an experiment where 25 micrograms of methoxychlor in acetone were pipetted onto 3 filter disks. The disks were air-dried for 2-3 hours and then extracted with 100 ml hexane. Percentage recovery was 90.0%, 94.0%, and 95.0%, the average being 93.0%. The residue determinations from the filter disks were not corrected.

Figure 35. Analysis of spray pattern on filter disk under long-wave UV.
Figure 36. Fresh methoxychlor deposits on mist blower-treated elm branch.



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Results and Discussion

(A) Spray Pattern, Droplet Size and Density, Along a Vertical Gradient in Treated Elm Trees

1. Helicopter application

The mean number of droplets/cm^2 was computed for each size class and height level. These means and the associated standard errors are tabulated in Table 12. Droplet density in all size classes definitely changed from the upper height level to the lower tree region. The addition of Dacagin had a strong influence on droplet size even at the reduced rate of 2 pounds/100 gallons. The numbers and equivalent proportions of droplets in each size class per unit area (cm^2) are graphed in Figure 37 at the levels 1 and 3 for the two formulations. It is apparent that the Dacagin formulation produced a higher proportion of larger sized droplets at both height levels. About 20% of all droplets exceeded 1 mm in diameter. The straight emulsion resulted in a pattern with only 6-10% of the total number of droplets being larger than 1 mm in diameter. The proportion of larger sized droplets increased somewhat in the lower height level which could have been the result of spray drift. The proportions of the 3 size classes remained the same at the 2 height levels in the application with the Dacagin. When the mean density per cm^2 in each size class was multiplied by the respective mean diameter (0.5, 1.5, 2.5 mm) the total area covered by residue was obtained. However, since the droplets were measured on filter paper, it is doubtful if these figures of the actual area covered apply also to elm bark with different surface and absorption characteristics. For comparison purposes, they might suffice. It is interesting to note that for both formulations 18% of 1 cm^2 was covered with spray deposit at level 1, while only 6% of

Number of spray droplets per unit area and actual area

Table 12. Helicopter application: number of spray droplets per unit area and actual area covered on horizontal filter disks at 2 height levels of treated elm trees.

Year	Application	MeCl Formulations	Height Level (m)		Mean # of Droplets ± S. E. in Size Classes (mm) 2-3	1-2	1	Total Area Covered per cm^2 (%)
1971/72	Helicopter[1)]	12.5% emulsion + Dacagin (2 lbs/ 100 gallons)	15	MEAN	0.71	5.40	21.93	
				S.E.	0.16	0.62	2.45	
				AREA covered (mm^2)	3.49	9.54	4.30	17.33
			5	MEAN	0.18	2.13	9.18	
				S.E.	0.07	0.30	0.91	
				AREA covered (mm^2)	0.88	3.76	1.80	6.44
1972	Helicopter[2)]	12.5% emulsion	15	MEAN	0.39	3.84	53.40	
				S.E.	0.22	1.57	14.57	
				AREA covered (mm^2)	1.91	6.76	10.47	19.17
			5	MEAN	0.07	1.58	13.64	
				S.E.	0.07	0.60	3.59	
				AREA covered (mm^2)	0.34	2.79	2.67	5.80

1) calculated from 15 filter disks (3 trees)
2) calculated from 4 filter disks (1 tree)

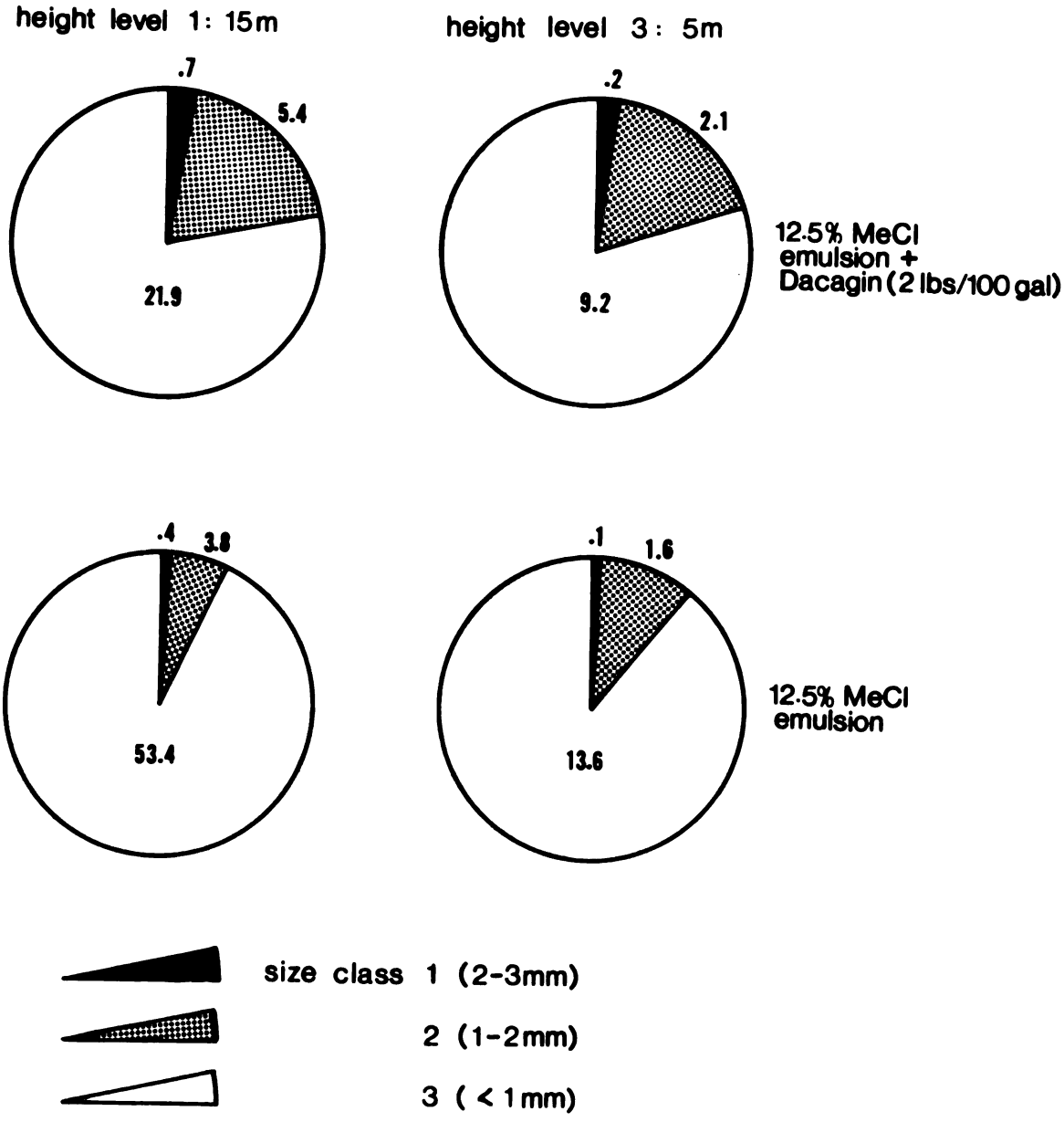
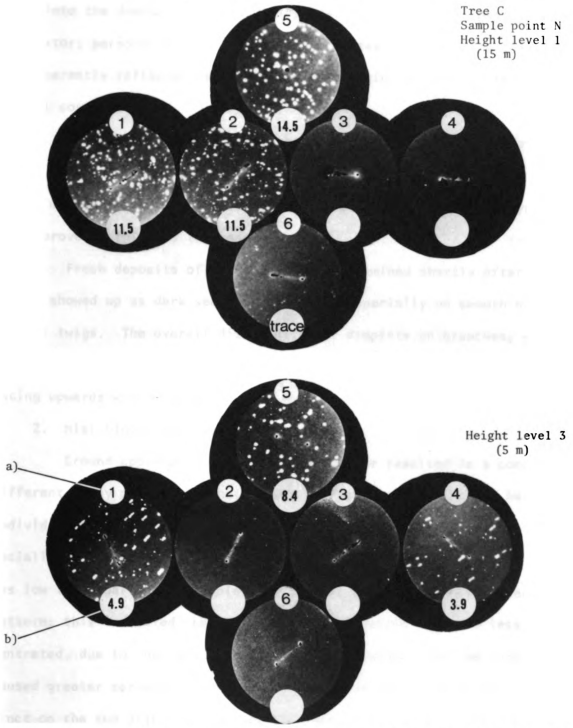


Figure 37. Helicopter application: proportion of droplet sizes per unit area (cm^2) on horizontal filter disks at two height levels of treated elm trees.

1 cm^2 was covered at level 3. That is, although the spectrum of droplet sizes was quite different for the 2 formulations, there appeared to be little difference in the actual area covered. From the standpoint of better and more even distribution of bark residues, the formulation without Dacagin is more desirable with a considerably larger number of droplets per unit area. However, the advantages of Dacagin as anti-drift material and its possible retarding effect on weathering might outweigh the above negative feature.

The overall distribution of spray deposits on the sample cubes was very poor in all helicopter experiments. Figure 38 is an example for the poor coverage of sample cubes at 2 height levels. In all sample cubes, the top disk had the highest droplet density while only one or two side disks had significant deposits. In no case did the bottom disk have any appreciable amount of spray drops. The visual examination of deposit distribution on the sample cubes showed little difference between formulations and height levels. At the lower height levels, however, most of the deposits were concentrated on the top disk, with minimal deposits on side disks. The poor overall coverage of the sample cubes (at best three disks) demonstrated that helicopter application did not create enough air swirl, which is necessary for good coverage of three-dimensional objects (like branches). Also, it was observed that the spray, after being ejected through the nozzles, settled down very calmly with little or no apparent turbulences in the air. The revolving blades of the helicopter create a strong vortex moving downwards only when the aircraft is hovering. However, down-draft and air turbulences are minimal as the speed of the helicopter increases. Elm spraying is usually done at speeds around 20 miles per hour, which is too fast for spray delivery into the downward

Figure 38: Helicopter spray deposits on filter cubes (fluorescence photographs)



a) Numbers 1-6 refer to position of filter disk on sample cube (see Figure 34)

b) Actual spray deposit in micrograms/cm^2.

vortex. Best results with aerial spraying of apple trees were obtained when the helicopter hovered above an individual tree and delivered the spray into the downward vortex (J. Topliss, Montrose, Col.; pesticide applicator; personal communication). The vortex after hitting the ground was apparently reflected back into the tree region which resulted in excellent coverage.

It was speculated that filter disks with no apparent spray deposits, according to the visual examination under UV light, could still harbor micro-droplets. Therefore, only an accurate chemical analysis could prove if this was the case.

Fresh deposits of helicopter spray examined shortly after application showed up as dark spots on the bark, especially on smooth bark of younger twigs. The overall distribution of droplets on branches, even in the top region of the tree, was poor; only the bark areas which were facing upwards were covered.

2. Mist blower application

Ground application with the mist blower resulted in a completely different spray pattern in that it was difficult to distinguish between individual spray droplets. The density of droplets was very high, especially of the smaller size class below 1 mm in diameter. Fluorescence was low in comparison to droplets of similar size of the helicopter pattern; this indicated that the individual droplets might be less concentrated, due to the fact that the strong air blast from the turbine caused greater spreading upon impact. Droplets were usually more distinct on the top disk, and the pattern resembled the helicopter treatment. This was understandable since the pattern on the top disk was created by spray droplets with little velocity settling down from above. Tree C

was mist blower sprayed with a formulation containing Dacagin (same rate as for the helicopter) while tree D received the same formulation but without Dacagin.

Visual inspection of the filter disks on each cube revealed that the overall coverage was very uniform and similar for both formulations. At height level 3, all six disks, even the top disk, had uniform coverage. The filter cubes in the upper tree region had consistently fewer droplets on the top disk, the other side disks and the bottom disk again having uniform coverage.

The superior coverage of the sample cubes (and, therefore, of the branches) with the mist blower application can be explained on the following grounds:

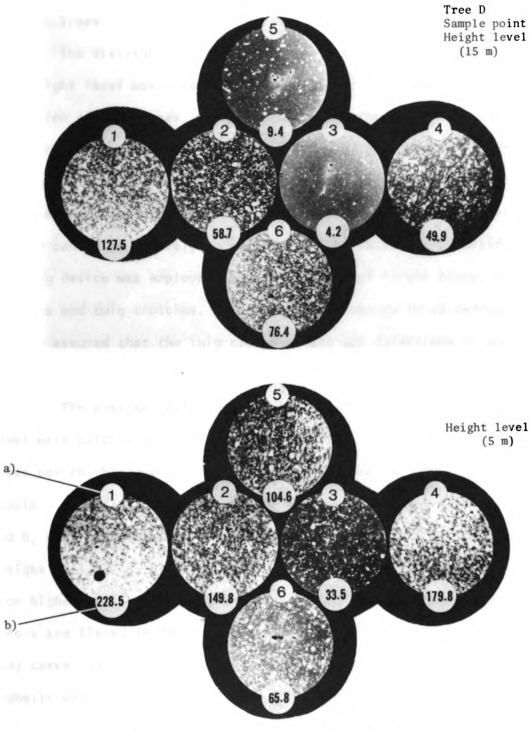
(a) During the spraying operation the blower is moved around the tree, thus covering the tree from all sides.

(b) The strong air stream generated by the blower turbine causes great air movement ('swirl') which is necessary for good coverage in a three-dimensional sense.

(c) The bark areas facing upwards away from the spray direction are eventually covered by spray droplets which settle down from above after being carried into the crown region.

It was not possible to analyze the mist blower sample cubes visually with UV-light in the same manner as the helicopter cubes because of the indistinct droplets. Fluroescence photographs (Kodak Plus-X, 2 Blak Ray UV-22 lamps, Kodak Wratten filter No. 2A) with the spray patterns at the two height levels for mist blower are shown in Figure 39. Observations on the coverage of branches were made shortly after application. The actual deposits on the bark made the twigs look wet. Later only a whitish residue remained (Figure 36). Overall coverage of branches was

Figure 39: Mist blower spray deposits on filter cubes (fluorescence photographs)



a) Numbers 1-6 refer to position of filter disk on sample cube (see Figure 34).

b) Actual spray deposit in micrograms/cm^2.

complete, particularly at the lower height levels.

(B) Horizontal and Vertical Distribution of Spray Deposits on Treated Elm Trees

The distribution of spray on the 6 sides of the sample cubes in each height level was interpreted with respect to actual coverage and protection of bark areas. The hypothesis was that best coverage of target sites will be achieved when all 6 sides of the cube have sufficient spray deposits. 'Sufficient' is defined here with respect to the LC_{50} values as determined in section IV. The researcher is aware that this analogy cannot be entirely correct due to the fact that an artificial sampling device was employed, rather than actual target sites, i.e. the branches and twig crotches. Also, for this analogy to be correct, it must be assumed that the twig crotches face all directions in equal proportion, corresponding to the 6 sides of the cube.

The average methoxychlor deposits on a sample cube per height level were calculated in the following manner. Each tree had 5 sample cubes per height level. The means were computed for each side of the sample cube. First, the average for the two horizontal filter disks, 5 and 6, were calculated. The vertical filter disks on the cube were designated 1, 2, 3, and 4 (Figure 34) according to amount of MeCl deposit, from highest (1) to lowest (4). The means with their associated standard errors are listed in Table 13. The size of the standard error was, in many cases, extremely large, expressing the great variation in spray deposits within each height level. Both spray applications, mist blower and helicopter, gave highly variable deposits at each respective height level.

1. Helicopter application

On the average, about 2-4 times as much methoxychlor was deposited in the upper height level than in the lower tree region (Table 13). Of the 6 filter disks on a cube, only 3 had appreciable deposits. This was consistent in both height levels. The top disk (No. 5) always had the greatest amount of methoxychlor. Only 2 vertical side disks harbored spray deposits, while only small amounts of methoxychlor were detected on the remaining sides of the cube. Deposits were insignificant on the bottom side (No. 6) of the sample cube (Figure 40a,b).

The experimental results suggest that approximately only 50% of the total bark area and, predominantly, those areas exposed to the top would be sprayed. Half on the vertical bark area would receive spray, and to a lesser extent. Residue amounts in the lower tree region were much smaller (Figure 40a,b). The LC_{50} determination on helicopter-sprayed twigs yielded a value of 41.8 micrograms/cm^2 (p. 150) which is represented as a dotted line in Figure 40a,b. Only the deposit on one horizontal top disk approached this line.

Since these figures signify fresh deposits, one can imagine that the degree of protection after several weeks of weathering is even less. By putting the deposit figures from the sample cubes in relation to the LC_{50} one can explain the low beetle mortality in the 1970 helicopter treatment (27.0% in the top tree region 2 weeks after application, and 17.3% after 8 weeks). If all twig crotches in the upper region projected towards the top, an average residue of roughly 40 micrograms/cm^2 could be expected in a twig crotch according to the deposit on disk No. 5 in Figure 40a. A deposit of this magnitude would cause 50% mortality in a given population. However, since only part of the twig crotches in the upper tree region are exposed to the top, and the rest project in

Table 13. Average methoxychlor deposits on sample cubes at 2 height levels of elm trees.

Year	Application	MeCl Formulation	Height Level (m)	Methoxychlor deposits on sides 1-6 of sample cube (micrograms/cm^2)[1] 1	2	3	4	5 Top	6 Bottom	
1971/72	Helicopter[2]	12.5% Emulsion + Dacagin (2 lbs/100 gal)	15	26.4	13.3	2.3	0.7	39.1	0.4	MEAN
				6.7	3.5	0.4	0.2	6.9	0.2	S.E.
			5	9.0	3.7	0.7	0.3	19.7	0.2	MEAN
				2.3	1.3	0.2	0.1	3.4	0.1	S.E.
1972	Helicopter[3]	12.5% Emulsion	15	15.1	6.8	0.9	0.4	26.8	0.2	MEAN
				4.1	1.6	0.7	0.4	6.7	0.2	S.E.
			5	9.0	3.7	0.7	0.3	19.7	0.2	MEAN
				2.3	1.3	0.2	0.1	3.4	0.1	S.E.
1972	Mist Blower[3]	12.5% Emulsion + Dacagin (2 lbs/100 gal)	15	69.2	33.5	22.0	8.9	6.6	73.0	MEAN
				14.8	6.6	7.1	1.5	0.9	12.6	S.E.
			5	177.7	99.4	58.0	17.3	32.0	71.6	MEAN
				61.7	36.2	24.6	4.7	19.1	17.5	S.E.
1972	Mist Blower[3]	12.5% Emulsion	15	213.5	105.7	75.6	37.7	14.7	199.0	MEAN
				32.0	88.1	19.6	12.4	4.8	35.1	S.E.
			5	397.8	304.1	218.5	48.2	61.6	112.5	MEAN
				70.3	85.1	91.1	14.9	16.4	42.9	S.E.

1) for position of sides 1-6 see Figure 34
2) calculated from 15 sample cubes (three trees)
3) calculated from 5 sample cubes (one tree)

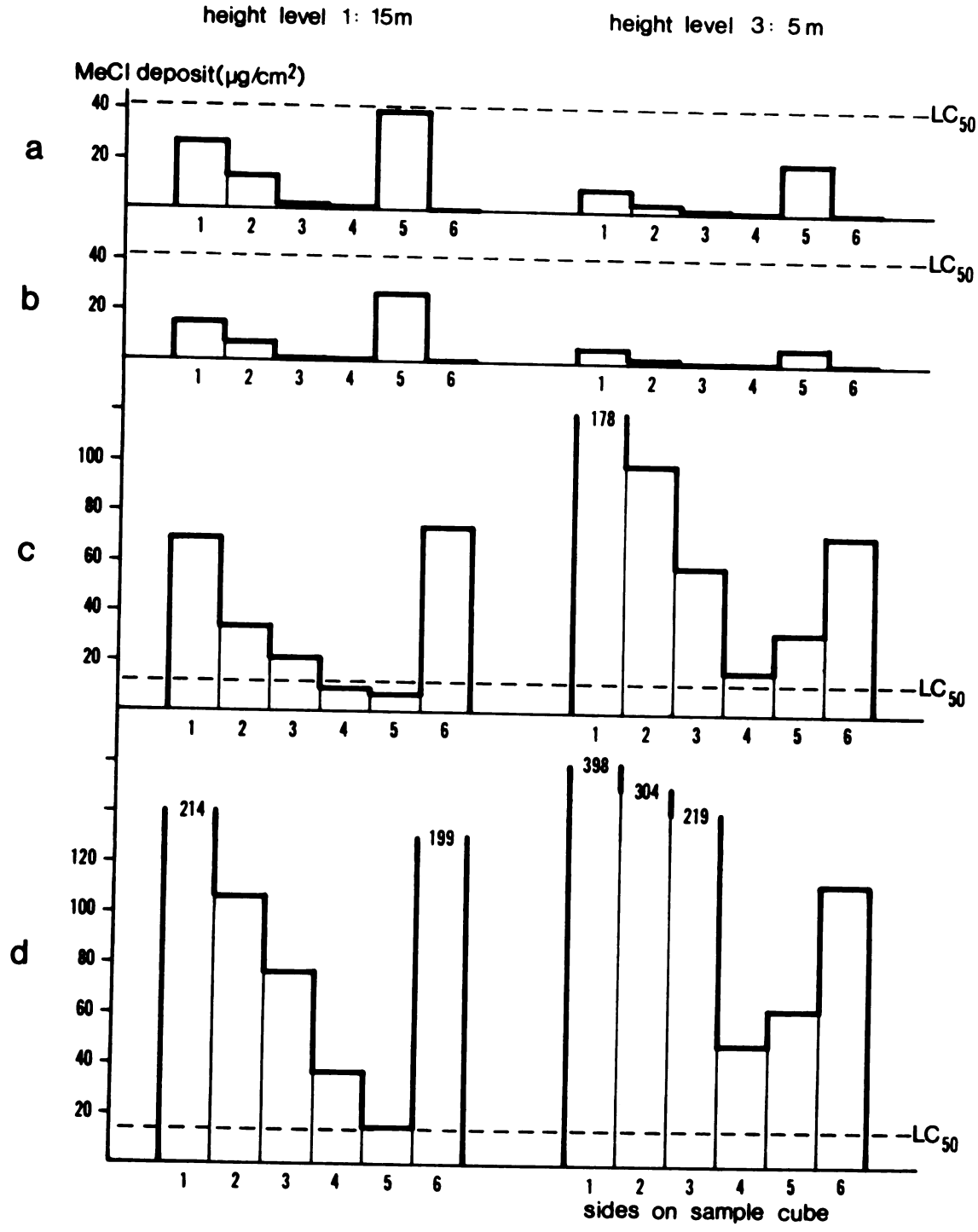


Figure 40. Average methoxychlor deposits on the six sides of the sample cube at two height levels of treated elm trees: helicopter, (a) 12.5% emulsion + Dacagin, (b) 12.5% emulsion; mist blower, (c) 12.5% emulsion + Dacagin, (d) 12.5% emulsion.

various directions, coverage, and, therefore, mortality, will be less (as indicated by the distribution of deposits on the other sides of the sample cube). This points out that the actual effectiveness of a spray application can not be judged by the amount of spray which is trapped on a horizontal filter disk.

Two 12.5% methoxychlor emulsions, one with Dacagin, were tested. Three-dimensional coverage was the same with both formulations (Figure 40 a,b). Deposits on the tree sprayed with the straight 12.5% emulsion were smaller. Because of insufficient replication, no valid conclusions can be drawn concerning the superiority in coverage of either formulation.

2. Mist blower application

The horizontal variation in spray deposits at each height level was substantial and comparable to the helicopter application (see the standard error values, Table 13). The actual deposits were up to 10 times greater than for the helicopter application. The vertical distribution pattern of spray deposits was the reverse of the helicopter pattern. The bottom tree region was heavily overloaded, receiving about 3 times as much spray as the top region. Amounts of close to 700 micrograms/cm^2 were found on single disks in the lower part of the tree. Coverage of the average sample cube in the lower height level was complete. Even the top disk had sufficient coverage. Towards the tree top, the deposits became weaker, but coverage remained excellent for all sides except the top disk. Making the analogy from the sample cubes to the target sites, coverage of the twig crotch areas was more than sufficient in the lower tree portion and decreased towards the top. The amounts of the initial deposits suggests that, even after weathering for many weeks, residues would be sufficient to protect the feeding sites. Since the

dosage-response regression equation for the mist blower-treated twigs was distorted because of DDT residues, the LC_{50} figure from the lab-treated twig crotches was drawn as a dotted line in Figure 40 c,d. The mortality figures from the mist blower treatments of 1969 and 1970 at several height levels correspond well with the vertical distribution pattern of spray deposits as demonstrated here. Mortality in the lower height levels throughout the season never fell below 90%. The percentage dropped significantly in the top on some mist blower-sprayed trees.

The researcher intended to compare the performance of a straight 12.5% emulsion to a formulation of the same strength, but with Dacagin. However, after the spraying was completed, it was discovered that the Dacagin formulation was considerably diluted to a spray emulsion of undetermined concentration. The lower deposits in Figure 40c reflect this. Therefore, no conclusions can be drawn as to the effect of Dacagin on tree coverage with the mist blower.

Summary and Conclusions

Because of the confounding effect of carry-over in the evaluation of formulations and application methods, a sampling system was developed to objectively monitor spray coverage of elm trees. Cubes with filter disks on all sides were employed as sampling devices to investigate spray patterns, droplet density and size spectrum, 3-dimensional coverage and actual deposition. The helicopter applications gave discrete spray patterns. Less than 20% of 1 cm^2 was covered when the spray droplet area was summated. The addition of Dacagin to the 12.5% spray emulsion shifted the droplet size spectrum to the larger sizes. Straight emulsions had a more dispersed spray pattern. The poor 3-dimensional coverage of the sample cubes suggested that helicopter spraying will protect less

than half of the total bark area. Besides, the dosages applied to each tree were found to be too small to provide the necessary protection. A comparison of fresh deposit data from the tree top with the LC_{50} value for helicopter-treated twigs indicated that not more than one-third of the potential feeding sites would be protected. After weathering for several weeks, protection would diminish, especially in the lower tree region.

The mist blower application created almost continuous deposits and 3-dimensional coverage was excellent, even in the upper tree region. Actual deposits often exceeded several hundred micrograms/cm^2 and suggested long-lasting protection. One can deduce from the superior coverage of the sampling cubes that the majority of the potential feeding sites will receive spray protection. This monitoring method nicely showed the distribution pattern of helicopter- and mist blower- applied spray within the whole tree region. The mist blower gave higher deposits at the bottom while the helicopter favored tree top deposits. The bioassay results of the helicopter- and mist blower-treated twigs in 1970 were in close agreement with the findings of the cube sample experiment, which demonstrated the reliability of this monitoring method.

IV. Insecticidal and Decomposition Properties of Methoxychlor Formulations

Introduction

The elm bark beetle conference in 1948 (Scanlon, 1949) laid out certain research objectives for Dutch elm disease control, among which were quantification of bark residues and correlation with bioassay data (feeding response or mortality). Pesticide analysis at that time was in its infancy, and analytical tools were not sufficiently refined to give accurate results at the desired levels.

In this study one of the objectives of the residue analysis of field-collected bark samples was to demonstrate differences in coverage which were attributable to certain formulations and application techniques. This was done in section II of this dissertation. Furthermore, an effort was made to determine residue levels which were sufficient to prevent successful feeding to the xylem. Matthysse et al. (1954) evaluated DDT formulations applied by hydraulic spray gun or by mist blower to tall American elms (15-21 m), with chemical analysis of bark residues and with feeding tests. Assays with twigs dipped in 1.0, 0.25 and 0.05% DDT emulsions (113, 23 and 5 micrograms/cm^2 bark surface respectively) revealed that deposits of 40 micrograms/cm^2 and more gave sufficient control, while deposits below 20 micrograms/cm^2 resulted in poor protection (for both native and European elm bark beetle) (see also Miller, 1951). Other investigators made use of chemical analysis of bark residues to measure the performance of application techniques and formulations, but they did not relate actual residue with bioassay data to give it biological meaning (Thompson, 1965; Wallner and Leeling, 1968; Wallner et al., 1969). It is common knowledge that finely dispersed residue is more toxic than residue of the same amount per unit area but with less subdivision.

Subdivision of spray droplets is primarily influenced by the application technique and formulation (Hoskins, 1962). It was therefore expected that the residue-mortality correlations would not be the same for the mist blower and helicopter treatments because of the different residue patterns on the bark. LC_{50} values were calculated from bioassays where twigs were dipped in methoxychlor solutions of known concentration.

For realistic evaluation of the contact toxicity of insecticidal deposits, it is desirable to use solid support surfaces on which the insect dwells in a natural setting (on leaves, on soil, on bark, etc.). For the purpose of uniformity and convenience, researchers have often resorted to supporting surfaces which were easier to handle. Lyon (1965) measured the residual contact toxicity of several insecticides to bark beetles on fiber boards. He argued that natural bark surfaces introduced too much variability in the way deposit patterns developed from the same formulation and this prevented good reproducability.

Cuthbert et al. (1971) used the World Health Organization procedure to determine the contact toxicity of DDT and methoxychlor applied on filter paper to S. multistriatus and to its braconid parasite, Dendrosoter protuberans. In these tests DDT was about 100 times more toxic than methoxychlor.

Although the contact toxicity of methoxychlor was shown to be low, it was of interest to demonstrate the contact toxicity of various formulations. Further, experiments were carried out to measure if, and to what extent, the feeding response was influenced by prior contact with the surface deposits of methoxychlor.

The behavior of methoxychlor deposits on elm bark was also investigated. The type and structure of bark deposits was described and

documented for various formulations and concentrations. The possible relationship of deposit structure to contact toxicity and weathering received considerable attention in these experiments.

The rate of decomposition of insecticidal deposits can vary greatly depending on the formulation employed (Barlow and Hadaway, 1952; Hoskins, 1962). Wallner et al. (1969) reported highest methoxychlor residues on trees which were treated with a formulation containing the anti-drift material, Dacagin. This suggestion led to several experiments where the break-down of methoxychlor formulations applied to elm branches was followed over a whole year.

When an elm tree is sprayed, many bark areas are covered which are not potential feeding sites. It was often speculated that methoxychlor residues might be translocated passively from the original site of deposition to another location and possibly be accumulated in the twig crotches. Also, if this assumption was correct, discrete spray patterns where droplets are far apart would become continuous over time if passive movement of MeCl residues existed. Therefore, it seemed worthwhile to supply information on the possible movement of MeCl deposits and trace their displacement.

Materials and Methods

(A) Bioassay Procedures for Oral LC_{50} Determinations

In 1970, the majority of elms on the campus of Michigan State University received the same helicopter application. Twigs were sampled from 10 helicopter sprayed trees and 2 mist blower treated trees two weeks after application. Only 5 helicopter treated trees and two mist blower sprayed trees were sampled eight weeks after application. Samples were collected from 3 height levels and subsequently subjected to feeding

tests with S. multistriatus and chemical analysis for methoxychlor residues (for details of sampling, bioassay and chemical analysis, see section II). Regression equations were computed from the data pairs, amount of residue (micrograms/cm^2 bark surface) and mortality (%) after conversion to a log-probit scale. Regression lines were fitted (a) to the helicopter data from 2 weeks after application, (b) to the helicopter data from 8 weeks after application, and (c) to the mist blower data from both sample periods (data were pooled because of the small number of sample trees). Twig samples from helicopter sprayed trees with considerable DDT residues were not considered for the analysis. DDT residues were present in all mist blower twig samples, in several cases almost equalling the amount of methoxychlor (Table 14).

For the lab tests, twig crotches were collected from untreated mature elm trees, cut to size and stored in a freezer at -10° C. Preliminary feeding assays with twig crotches dipped in methoxychlor solutions of contrasting strength were performed to get a starting point for the LC_{50} determination. Consequently, the following methoxychlor solutions (xylene) were prepared from a commercial stock solution (Michlin 'MX-2', 25.1%): 0.1%, 0.15%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6% and 0.8%. Twig crotches were dipped for two seconds in each solution respectively and then placed upright in a ply wood tray. After a drying period of 3-4 hours, the treated twig crotches were subjected to the standard bioassay with S. multistriatus. Extended drying periods beyond 4 hours were avoided since they resulted in a poor feeding response due to desiccation of the twig crotches. Bark samples were taken as described in section II from around 50 twig crotches from the same batch for each concentration. They were then chemically analyzed to determine the

Table 14. Twig crotch residues and respective mortalities on sprayed campus elms (1970).

Tree[1)]	Height Level	2 Weeks After Application MeCl$_2$ µg/cm^2	DDT µg/cm^2	Mortality %	8 Weeks After Application MeCl$_2$ µg/cm^2	DDT µg/cm^2	Mortality %
A	1	6.1	5.6	53.5	4.4	3.1	20.5
	2	20.1	14.8	94.8	9.6	8.2	78.8
	3	37.9	30.3	100.0	26.9	18.7	100.0
B	1	5.3	4.2	1.8	2.9	4.2	25.8
	2	15.3	15.4	79.3	5.9	2.5	47.0
	3	17.8	15.0	79.3	12.4	11.5	68.0
C	1	22.2	3.3	32.8	28.1	13.1	36.4
	2	41.8	19.7	53.5	15.2	Trace	36.4
	3	21.1	20.4	17.3	3.3		15.2
D	1	1.0	Trace	6.9	6.2		25.8
	2			1.8	1.1		4.6
	3			1.8	17.8	5.6	25.8
E	1	30.3	0.7	53.5	13.3		25.8
	2	21.8	1.2	63.8	9.8		9.9
	3	26.0	1.4	69.0	11.5		
G	1	5.0		12.1	0.5		4.6
	2	2.2		12.1	0.9		4.6
	3	3.0		27.6	2.5	0.9	
H	1	23.4	Trace	63.8	1.5		4.6
	2	0.5	0.2	1.8	2.1		
	3	1.9	0.5	17.3	2.0		4.6
J	1	9.8		17.3	4.7		15.2
	2	11.2		12.1	3.4		4.6
	3	11.9	2.1	32.8	1.2	Trace	
K	1	4.5		38.0		Not Sampled	
	2	16.9		32.8			
	3	30.3	2.3	32.8			
L	1	15.4		12.1		Not Sampled	
	2	15.9		22.4			
	3			1.8			
M	1	3.7		6.9	4.7		15.2
	2	4.7		6.9	7.9		15.2
	3	5.6		1.8	5.8		15.2

1) trees A and B: mist blower-sprayed
all others: helicopter-sprayed

amount of residue on the bark surface (micrograms/cm^2). The control treatment consisted of 50 twig crotches dipped in xylene. Finney's probit analysis was employed to calculate the residue-mortality equation and the LC_{50}, the median lethal concentration (Finney, 1964).

(B) Fiber Board Units for Contact Toxicity Testing

Several attempts to test the contact toxicity of methoxychlor deposits on elm bark failed because the bioassay insects invariably began to feed in bark fissures. Therefore, a supporting surface was chosen which was by its nature not stimulating to S. multistriatus and would not induce a feeding response. Untempered 1/8" Masonite fiber board had the desired characteristics: easy to handle and absorption qualities similar to bark. The fiber board was cut into smaller pieces measuring 7.5 x 7.5 cm. A special pen-like applicator was constructed to distribute the measured amount of insecticide over a 4 x 4 cm square. The applicator (Figure 41)was made of a 2 cm wide thin aluminum sheet metal strip which was folded to an acute V on one side and then bent over. An equal amount of each respective formulation was released with a pipette into the V-shaped applicator. Four tiny holes at the bottom of the V permitted the liquid to flow out slowly when the applicator was drawn across the fiber board surface. It took 2 timed strokes with the 2 cm wide applicator to distribute the measured amount evenly over the 16 cm^2 area. Another problem was to keep the assay beetles on the treated surface during the duration of the experiment. Lyon (1965) placed a watch glass over the assay insects, but because of possible fumigation effects during the long exposure time (60 minutes), plastic weighing cups (1 5/8" x 1 5/8")[1] were used instead. Large holes were punched in the bottom of the dish

1) Scientific Products

41
3X
42
3%
44
EMULSION
EMULSION + DACAGIN
43

for proper ventilation. Then the cup was placed upside down above the treated surface and taped down (Figure 42). Unsexed beetles were introduced with a vacuum forceps, then a small piece of plastic screen was placed above the hole to prevent beetles from flying out. The inside wall of the cup was rubbed with emulsifier (Emcol 5100) which kept the assay beetles from climbing up the side wall. Five beetles were tested at a time in one unit over a period of one hour. The choice of exposure time had to be arbitrary since it seemed impossible to determine the average time a beetle will spend moving around on the bark surface before feeding activity is initiated.

After exposure each beetle was placed individually in a gelatine capsule No. 3 to guard against cross contamination (Figure 43). The physiological condition of the beetles was checked and recorded every 6 hours. The same criteria were used here as described for the bioassays in section II:

(a) A alive
(b) (A) dying
(c) + dead

For the final analysis (b) and (c) were pooled. The total observation period was 48 hours. The following formulations were tested for contact toxicity:

6.25% MeCl solution (xylene)
6.25% MeCl emulsion (xylene:water = 1:1)
6.25% MeCl emulsion plus Dacagin (2 pounds/100 gallons)

All formulations were diluted from a 25.1% MeCl xylene stock solution (Michlin 'MX-2' elm spray). Exactly 0.1 ml from each formulation was distributed evenly in a 4 x 4 cm square on the fiber board as descibed earlier. The actual deposit per cm^2 amounted to ca. 400 micrograms. Deposits of this magnitude can be found on the heavily overdosed lower

portions of mist blower sprayed elms. Two replicates with 25 beetles each were run for each formulation and the control with untreated fiber boards. Time-mortality curves were established for fresh deposits (12-hour drying period) and for 4-day old deposits.

To determine the influence of contact with surface deposits on their feeding response, beetles were exposed to deposits on fiber boards as described above. Three emulsions were tested: 6.25%, 3.12%, and 1.56%, which corresponded approximately to 400 micrograms/cm^2, 200 micrograms/cm^2, and 100 micrograms/cm^2 actual MeCl deposit respectively. After a 60-minute exposure to the treated fiber boards, the beetles were each caged in a residue-free twig crotch. Following 24 hours, the feeding test was stopped and mortality and feeding response determined. The deposits on the fiber boards were aged for 10 days. Twenty-five beetles were tested per concentration in 2 replications.

(C) Application of Methoxychlor Formulations on Elm Branches to Investigate the Behavior of Bark Deposits

1. Structure of surface deposits

Small sections of elm branches (15 cm long, 2-2.5 cm diameter) were sprayed with three concentrations of three formulations. All formulations were made from a 25.1% MeCl stock solution (Michlin 'MX-2' elm spray):

Formulation		Concentration
(a)	solution (xylene)	12.55% MeCl 6.25% MeCl 3.12% MeCl
(b)	emulsion (xylene:water 1:1)	same as (a)
(c)	emulsion (as (b) but with Dacagin 2 lbs/100 gal)	same as (a)

An equal volume of each formulation was applied to a branch section with a chromatography spray unit. The 12.55% formulations gave roughly an initial deposit of 500 micrograms/cm^2 and the other concentrations correspondingly less. The crystallization patterns of the surface deposits generated by the various formulations and concentrations were then observed and documented as they developed.

2. Decomposition of bark deposits

Bottom branches of mature elms were sprayed with several methoxychlor formulations. The branches had a western exposure, a diameter of 1-2 cm and the sprayed portion measured between 1.5 m to 2.0 m. It was attempted to make the total bark area for each treatment similar in size. Ten ml of each formulation were applied with a chromatography spray gun to the bark area facing south. The spray settled down as a continuous deposit over the whole treated surface. Three days after application, the bark was sampled for the first time to quantify the original deposit. The bark sample was taken with a No. 1 cork borer (diameter 0.4 cm) by punching out bark circles (Figure 44). A total of 50 bark circles was sampled randomly from a branch amounting to 6.28 cm^2 total bark area analyzed per formulation. Table 20 lists the formulations tested the respective application and sampling dates plus the actual residue data in micrograms per cm^2 at the start of each experiment. The break-down of these formulations was studied in 2 experiments during 1970/71 and 1971/72. All formulations in these experiments had a concentration of 12.55% MeCl and were diluted from 25.1% Michlin 'MX-2' elm spray.

3. Movement of bark deposits

Two twig crotches (2-3 years old) on a young elm at the edge of a woodlot were chosen for this experiment. The position of these twig

crotches was close to vertical. The bark area around the twig crotch was divided into 1 cm sections and marked (Figure 51). The application was made in section B with 5 microliter of a 12.55% emulsion which was equivalent to 599.7 micrograms of actual methoxychlor. A Hamilton microsyringe was employed to dispense this small amount in the specified bark area.

After 4 weeks of weathering the two twig crotches were cut and the bark sections A to D were stripped from the branch. Then each bark section was chemically analyzed for amount of residue.

Results and Discussion

(A) Oral Toxicity of Methoxychlor Deposits in Elm Twig Crotches

1. Residue-mortality regressions calculated from field samples

a. Helicopter application

The data pairs from the first sampling period showed great variation around the calculated dosage-response regression line (Figure 45). The bioassay beetles came from the same batch. Therefore, the encountered heterogeneity of the data could not be attributed to an inconsistent response (other than random variation). Non-uniform, discrete distribution of bark deposits, bark sampling and chemical analysis of methoxychlor residues caused this wide spread of points. The researcher believes that, in particular, the non-uniform, discrete distribution of helicopter spray deposits on elm bark contributed significantly to the variation. Continuous carry-over deposits from earlier mist blower treatments (before 1968) and their different toxicity could have added a further component to the total variation. The low coefficient of determination of r^2 = 0.41 expresses the high unexplained variation.

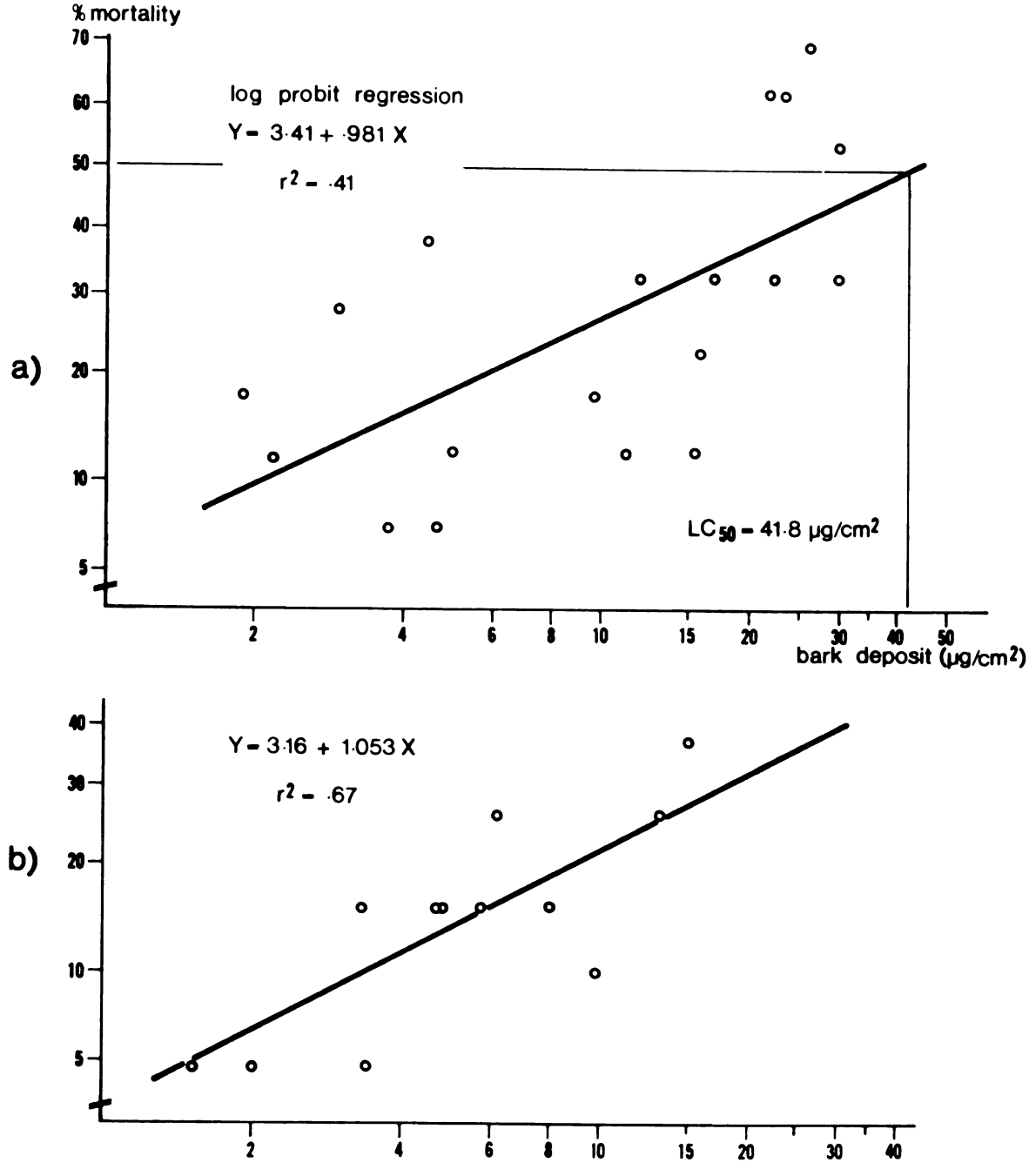


Figure 45. Bark deposit-mortality regression line calculated for helicopter-treated twig crotches
(a) two weeks after application
(b) eight weeks after application.

The LC_{50} was calculated from the log-probit regression equation in Figure 45 which yielded a value of 41.8 micrograms methoxychlor residue per 1 cm^2 bark surface. Since this value is extrapolated (just outside the range of sample points) it must be regarded with caution.

The data from the second sampling period were closer to the computed regression line which resulted in a higher $r^2 = 0.67$. The log-probit equations from the two sampling periods agreed adequately, considering that the second regression line was constructed from data where the highest mortality was 36.4%. The extrapolated value for the LC_{50}, as predicted by the equation in Figure 45, would be 55.9 micrograms/cm^2.

b. Mist blower application

The beetle response in the assays of mist blower-treated twigs had the range necessary for the construction of a valid dosage-mortality equation. The data were in good agreement with the regression line (Figure 46) which was indicated by a high $r^2 = 0.85$. The relationship between dosage and mortality, as shown by the log-probit equation in Figure 46 for the mist blower treatment, was quite different. The slope of the regression line was more than twice as steep as for the helicopter regressions. The higher toxicity of mist blower-treated deposits was attributed partly to better distribution and finer dispersion of residues. The log-probit equation gave a LC_{50} of 6.5 micrograms/cm^2, which was roughly one-sixth of the LC_{50} on helicopter-treated twigs. However, the regression line was computed with residue data which had high amounts of DDT (see Table 14). The beetle response was, therefore, caused by the combined action of both compounds, methoxychlor and DDT. The calculated log-probit regression is, therefore, not indicative of the relationship between methoxychlor bark deposits and mortality on mist blower

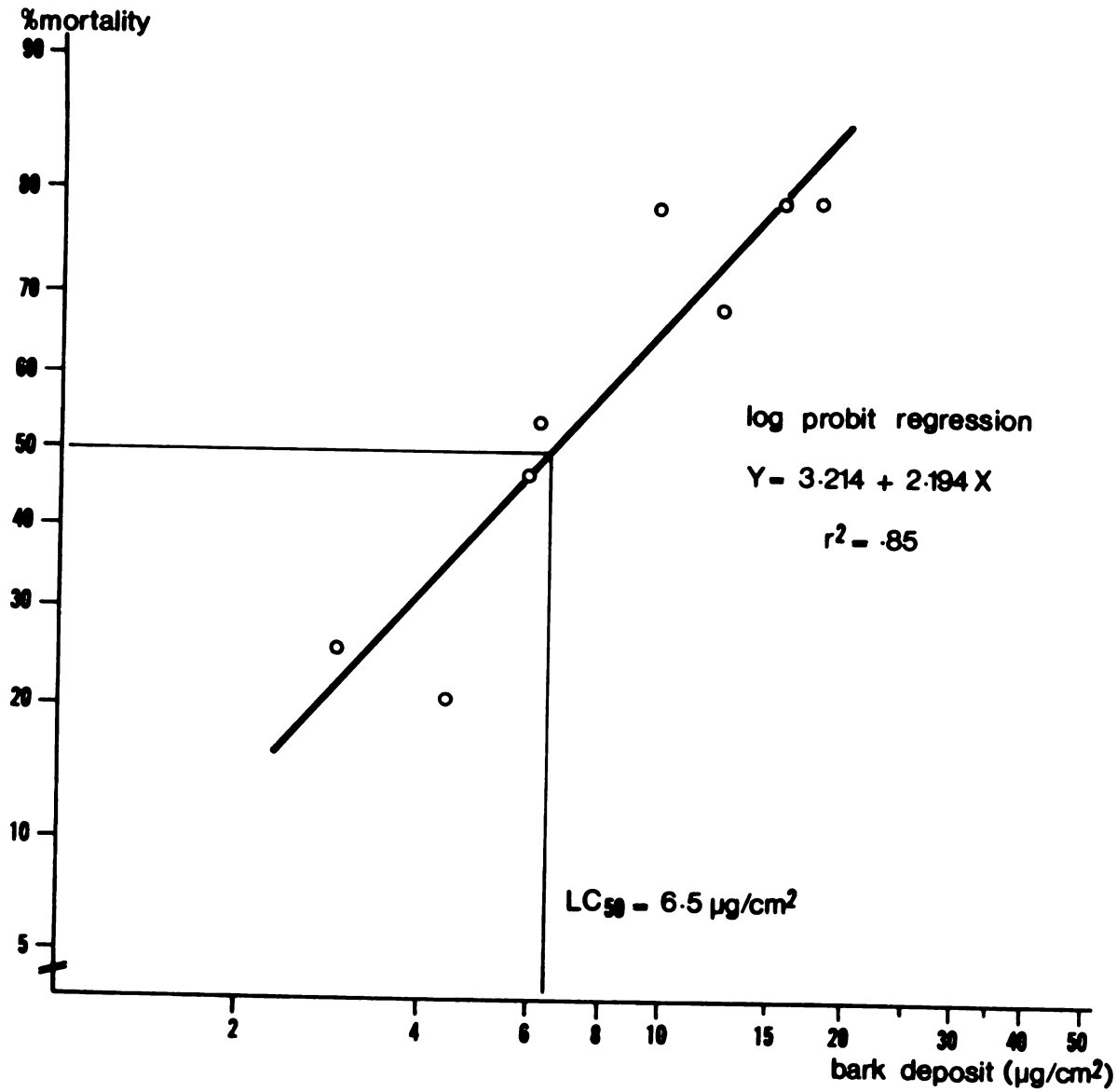


Figure 46. Bark deposit-mortality regression line calculated for mist blower-treated twig crotches.

sprayed twigs and underestimated, consequently, the LC_{50}.

2. Responses of S. multistriatus on lab-treated twig crotches

Both responses, mortality and feeding, were tested over a wide range of methoxychlor concentrations (Table 15). The observed mortality was not corrected since no natural mortality occurred in the control assays. The results of the probit analysis of the deposit-mortality data are listed in Table 16. This regression equation differed considerably from the equation for helicopter treated twigs. The higher beetle response (mortality) on the lab-treated twig crotches was apparently caused by the continuous uniform distribution pattern of bark deposits. The LC_{50} was 12.7 micrograms/cm^2 which was about 1/3 the value calculated for the helicopter treated twigs. A comparison with the results of the mist blower assays is difficult to make because of the interaction with DDT. The substantial difference in response rate on the field-treated and lab-treated twig crotches suggested again that the outcome of lab tests is not necessarily indicative of happenings in the field.

The slight heterogeneity of points around the regression line in Figure 47 is partly the result of a longer time lapse between bioassays. This meant that the physiological response was not completely uniform in all assays since beetle material from different batches had to be used. Further, the inherent variability of the twig crotch material certainly contributed to the heterogeneity. A X^2 test for goodness of fit was almost significant at $\alpha = 0.10$, therefore the variances were corrected with a 'heterogeneity factor' as suggested by Finney (1964). This correction gave a somewhat wider fiducial belt.

The actual feeding response was measured for some bioassays as

Table 15. Response of S. multistriatus in bioassays of lab-treated twig crotches.

Treatment[1]	MeCl Deposit ($\mu g/cm^2$)	No. Beetles Tested	Mortality %	Feeding Response Mean Scar Length(cm)	Conf. Limit(α = 0.05)
1) 0.1%	4.1	100	4	--	--
2) 0.15%	5.2	100	10	--	--
3) 0.2%	7.5	51	20	0.226	0.045
4) 0.3%	11.7	50	38	--	--
5) 0.4%	15.4	55	73	--	--
6) 0.4%	16.7	52	50	0.190	0.033
7) 0.5%	21.1	53	77	0.139	0.026
8) 0.6%	23.6	53	87	0.098	0.026
9) 0.8%	31.2	52	96	0.111	0.019
10) Control 1 (xylene)	--	51	--	--	--
11) Control 2 (xylene)	--	53	--	0.327	0.047

1) bioassays 1, 2, 4, 5, and 10 performed prior to the others; twig crotches were dipped in MeCl xylene solutions

Table 16. Results of Finney's probit analysis of the dosage-mortality data in Table 15.

log-probit regression equation: $y = 0.989 + 3.635x$

slope $\pm$ SE = 3.635 ± 0.326

$\chi^2 = 11.93$ n.s. ($\alpha = 0.05$)

LC_{50} ($\mu g/cm^2$) = 12.7

95% C.L. = 11.1 - 14.5

LC_{90} ($\mu g/cm^2$) = 28.6

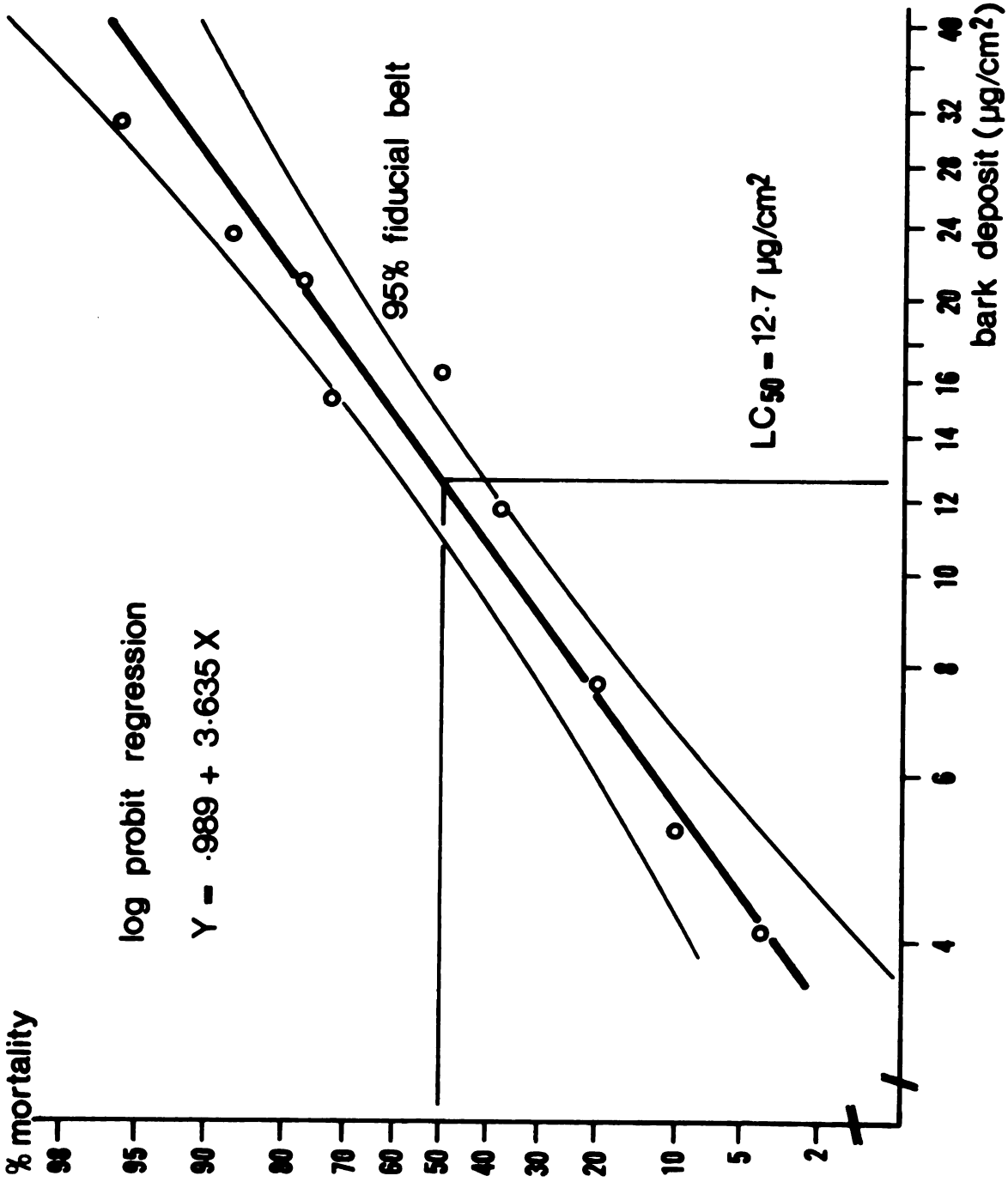


Figure 47. Bark deposit-mortality regression line calculated for lab-treated twig crotches (Finney's probit analysis).

indicated in Table 15. Naturally, the feeding response became weaker as the methoxychlor deposits increased. Accordingly, the lengths of the feeding scars became more uniform for each treatment, which is nicely shown by the narrowing confidence limits associated with the respective means in Table 15.

3. Comparison of male and female feeding response on untreated twigs

For an actual comparison of male and female feeding response it was necessary to use a more accurate way of measuring the feeding. Indexing alone was not refined enough, therefore the feeding scars were measured accurately to 0.1 millimeter (mm) with a disc micrometer reticle in a LEITZ stereo microscope.

The feeding data were taken from the controls for the oral toxicity tests. The total sample amounted to 102 feeding scars, 47 of which were made by females, 55 by males. The mean length of a feeding injury for the males was 3.24 mm, for the females, 3.35 mm. The calculated $t = 0.337$ was smaller than $t = 2.01$ ($\alpha = 0.05$); indicating that male and female feeding responses were not significantly different. For a graphic demonstration (Figure 48) of the distribution of length of feeding scars, the feeding responses were grouped in size classes from .05 - .15 to .55 - .65 cm for both males and females.

4. Relationship between mortality and sex in the bioassays

The helicopter bioassays of July, 1969, and the oral toxicity tests (July, 1971) were examined with respect to the association of beetle sex and mortality. The percent mortality was determined for each sex separately in each assay using the total number of males and females as 100% (Table 17). Differences between male and female mortality were then computed and marked with the proper plus or minus sign. Wilcoxon's

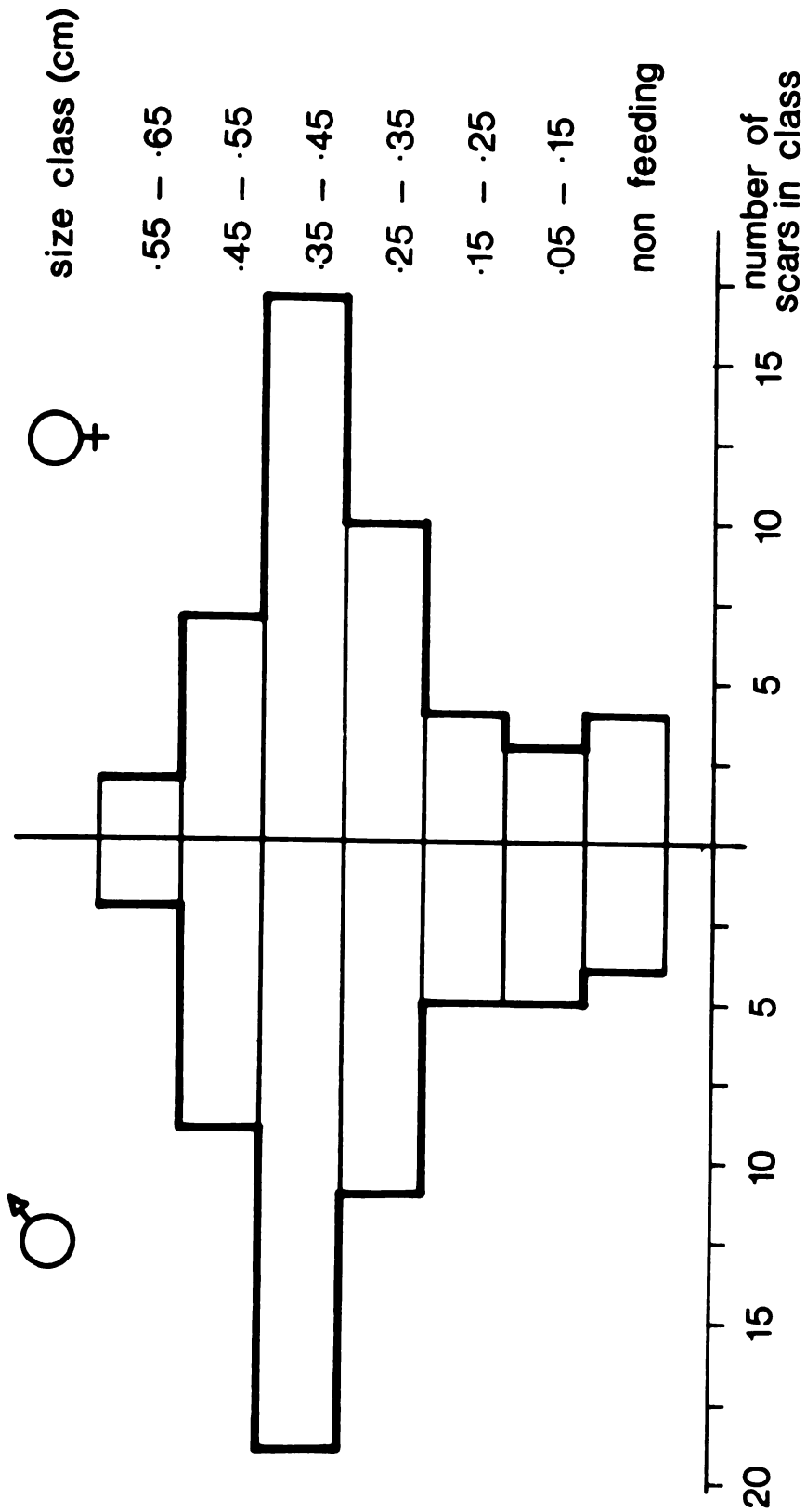


Figure 48. Male and female feeding response on untreated twig crotches.

Table 17. Association of mortality and beetle sex in bioassays of methoxychlor-treated elm twigs.

MeCl Treatment	N (Males + Females)	Males Mort.%	Females Mort.%	Mort.[1] Difference	Sex[2] Ratio
Bioassays, 1969, July: Helicopter treatment					
II A	54	35.5	26.1	+ 9.4	1.35
II B	55	22.2	10.7	+11.5	0.96
IV A	55	55.2	40.0	+15.2	1.16
IV B	55	39.3	33.3	± 6.0	1.04
V A	55	21.4	18.5	+ 2.9	1.04
V B	55	62.5	45.2	+17.3	0.77
Bioassays, 1971, July: Oral Toxicity Tests					
0.2%	51	23.8	6.7	+17.1	0.70
0.3%	51	37.0	41.7	- 4.6	1.13
0.3%	54	82.6	62.5	+22.6	0.72
0.4%	50	52.4	51.7	+ 0.7	0.89
0.4%	50	23.1	16.7	+ 6.4	1.08
0.5%	53	82.6	62.5	+22.6	0.72

1) Mean difference in susceptibility: $\bar{D}$ = 9.7%

2) Sex ratio (males/females): Mean S. R. = 0.96 ± 0.13 (C.L. for α=0.05)

signed rank test for 2 groups, arranged as paired observations, was performed on these data (Sokal, R. R., and F. J. Rohlf, 1969, p. 400).

The difference between males and females with respect to susceptibility to methoxychlor was significant at $\alpha = 0.01$. These data do not suggest to which degree the males are more susceptible than the females. The fact that in 11 out of 12 observed bioassays the males responded with higher mortality suggests that a difference exists. On an average, the females were 9.7% less susceptible than the males. Although this difference in susceptibility might be important for precise toxicological investigations, it can probably be disregarded in practical bioassay work. Cuthbert et al. (1971) did not find a difference in susceptibility between sexes when S. multistriatus was exposed to methoxychlor-treated filter disks.

The mean ratio of males to females in these 12 assays was very close to unity: Sex Ratio = 0.96 ± 0.13 (C. L. at $\alpha = 0.05$). Since the beetles used for each assay were a random sample from a field population (emerged under lab conditions), the above sex ratio is probably a good estimate for the true ratio of males to females in a natural S. multistriatus population.

(B) Contact Toxicity of Methoxychlor Deposits

1. Comparison of 3 methoxychlor formulations

The toxicity pattern which emerged from these experiments corresponded well with previous work (Barlow and Hadaway, 1952; Hoskins, 1962). The contact toxicity of the 12-hour old deposits was somewhat higher than for the 4-day old, weathered deposits (Table 18). Barlow and Hadaway (1952) pointed out that the physical state of a surface deposit determines its toxicity and that a reduction of toxicity usually

goes hand in hand with crystallization. The 12-hour old deposits were probably not completely crystallized and consisted of a mixture of crystals and supersaturated droplets. The reduced toxicity of the weathered deposits can be attributed to complete crystallization of the mixture since it is unlikely that significant weathering occurred during this relatively short period.

Table 18. Contact toxicity of methoxychlor deposits on fiber boards to S. multistriatus.

Time After Exposure (hours)	6.25% Solution Total Mort.%		6.25% Emulsion Total Mort.%		6.25% Emulsion + Dacagin Total Mort.%		Control Total Mort.%	
	A[1]	B[2]	A	B	A	B	A	B
6	9	2	24	19	10	2	2	--
12	17	8	59	43	17	16	2	--
18	27	14	84	61	30	24	8	8
24	48	32	89	67	38	32	24	12
30	69	40	93	73	60	44	38	16
36	81	52	98	77	75	60	48	36
42	92[3]	68	100[3]	83	88[3]	70	60[3]	56
48	92[3]	84	100[3]	88	92[3]	78	78[3]	72

1) deposits aged for 12 hours, mean mortality of 2 replicates (25 beetles each)
2) deposits aged for 4 days, mean mortality of 2 replicates (25 beetles each)
3) mortality of 25 beetles

The solution penetrated well into the absorbent fiber board surface despite its high volatility, and laid down a thin surface deposit. The emulsion, on the other hand, stayed more on the surface and the quantitative relationship between surface and tissue deposit favored the

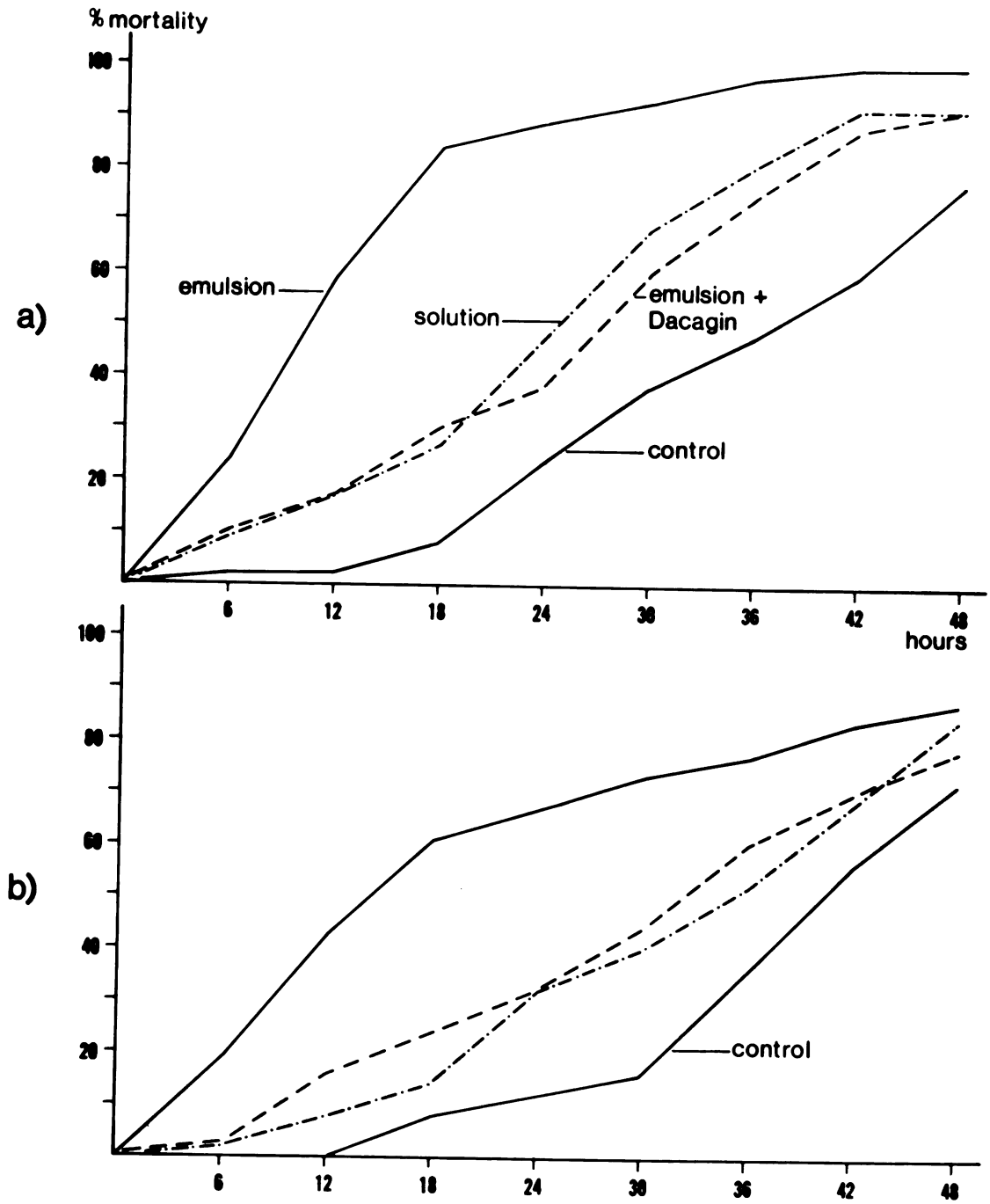


Figure 49. Time-mortality curves for S. multistriatus after exposure to treated fiber boards: (a) deposits aged for 12 hours (b) deposits aged for 4 days.

former. The emulsion containing the Dacagin behaved in the same fashion; it penetrated little and the deposit accumulated on the surface. The degree of contact toxicity was directly related to the type of surface deposit which was formed by each formulation.

The emulsion caused highest mortality because of richer surface deposits and better pick-up of methoxychlor crystals. When the thickening agent, Dacagin, was added to the emulsion, the toxicity dropped markedly and was similar to the solution (Figure 49). Obviously, this gel-like, viscous anti-drift material coated the surface with a thin film which prevented direct contact between insect and residue to some extent and made 'pick-up' more difficult. Since the mortality in the controls was rather high in both series of the experiments, the treatment mortalities were not corrected. The time-mortality curve of the control is graphed for comparison and was a little steeper in the experiments with the 12-hour old deposits. The handling of the bioassay beetles after exposure might have increased mortality overall, since the gelatine capsules offered little room for movement and were a completely artificial environment for the beetle. The bioassay beetles responded in the same fashion to the fresh and the aged deposits. In both cases, the emulsion was the most toxic, the solution and the emulsion plus Dacagin were comparable in their toxic action.

2. Contact toxicity and feeding response

The methoxychlor deposits which the beetle encounters upon emergence have experienced several weeks or months of weathering. As surface deposits they have undergone changes in their chemical and physical make-up. The treated fiber boards in this experiment had been exposed to outside conditions for 10 days in order to approximate the natural

conditions somewhat.

The following Table gives a summary of the beetle response in these assays.

Table 19. Beetle mortality and feeding response after exposure to treated fiber boards.

MeCl emulsion	Mortality %	Failure to Score %	Mean Scar Length(cm)
1.56%	4	32	22.2
3.12%	6	30	21.5
6.25%	6	36	21.1
Control	4	28	22.7
Fumigation Control	6	22	25.2

The cause of poor feeding response in the controls and in the experiment as a whole is not quite clear. The twigs were untreated and were collected from young elm trees with smooth bark in late summer. Meyer and Norris (1964) suggested that feeding is motivated in part by rough bark. Dixon (1964) deduced from his data that feeding in twig crotches is primarily a thigmotactic response. This is one explanation. Elm twigs collected in late summer are not as succulent as during the early season, which could be the cause for the poor feeding response. However, this researcher believes that a comparison to the control is still valid. The mortality and the feeding response in all treatments were not different from the control. The time-mortality curves in Figure 49 show high mortality after an observation time of 24 hours for the fresh and aged 6.25% emulsion deposits. After 10 days weathering, the surface

deposits on the fiber boards had apparently lost their contact toxicity and this resulted in insignificant mortality. The mean values for the measured feeding response decreased a little with increasing concentration. The difference to the control proved to be insignificant. To test for possible fumigation effects in these experiments, fiber board units were used where a screen separated the treated surface from the assay beetles. As the low vapor pressure of methoxychlor suggested, no fumigation effect was noticeable. These experiments bore out that the contact toxicity of weathered methoxychlor deposits is extremely low and that the feeding response does not appear to be influenced by prior contact with aged deposits.

(C) Structure of Surface Deposits of Methoxychlor

Solution

The 6.25% and the 12.55% solution produced a fine network of long crystals (Figure 50a) but the bark had still its dark natural appearance. Very light crystal formation was observable on bark treated with the 3.12% solution. Crystals, thin and long, sometimes radially branching out from a center, adhered tightly to the bark surface.

Emulsion

All 3 concentrations produced surface deposits primarily of large-sized crystals and resulted in continuous coats of whitish appearance (Figure 50b). Surface deposits were heavier on the branch sections treated with the higher concentration. Crystals ranged from very small to large and some were definitely plate-like (Figure 50c). The radial crystal type was also common, but in contrast to the solution, here the crystals frequently projected away from the surface. Crystallization started uniformly several hours after application over the treated surface.

Figure 50. Structure of methoxychlor deposits on elm bark
(a) solution: radial crystal
(b) emulsion: radial and plate-like crystals
(c) emulsion: large plate-like crystals
(d) emulsion plus Dacagin: uneven distribution at lower concentrations (whitish spots)

50a
100 μ
50b
200 μ
50c
200 μ
50d
3%
6%
12%

However, a mixture of crystals and supersaturated droplets persisted for 1-2 days until complete crystallization occurred.

Emulsion plus Dacagin

The surface deposit patterns developed by the 3 concentrations were distinctly different (Figure 50d). The pattern formed by the 3.12% formulation showed several irregular whitish spots where deposits seemed to be more concentrated. These spots became more frequent on the bark which was sprayed with the 6.25% emulsion and they consisted of ray-like radial crystal structures. The thickener itself showed up in some places as thin transparent film. The area between these centers of intense crystallization was covered with a net of ray-like crystals. Their branches were long, sometimes needle-like, and adhered tightly to the bark surface. The spottiness and obvious uneven distribution of deposits disappeared with the 12.55% emulsion. The crystal coat was thick and continuous.

Ray-like crystals branching out from a center were produced by all 3 formulations. The solutions resulted in very delicate radial structures which adhered flat to the bark and did not project away from the surface. The straight emulsion deposits were characterized by many plate-like crystals. The Dacagin emulsions generated both types of crystal structures but plate-like crystals were not as frequent. In general, the solutions produced the thinnest surface deposits since the xylene penetrated well into the bark tissue. The emulsions did not penetrate as well which resulted in rich surface deposits. Although the xylene-based MeCl solution generated better tissue deposits than both types of emulsions, xylene as the sole carrier cannot be recommended because of its high volatility. Heavier oils would be more practical since they favor tissue deposits, but their phytotoxicity makes them

oftentimes not usable for tree applications (Whitten, 1949).

(D) Decomposition of Methoxychlor Formulations

The rate of decomposition on bark depends to a great extent on the quantitative relationship between surface deposits and tissue deposits (Lyon, 1965). The penetration into the bark is determined by the carrier. Solutions usually penetrate better and create higher tissue deposits than emulsions. At higher concentrations, however, solutions and emulsions were found to penetrate equally well (Lyon, 1965). This observation was confirmed in the first break-down study, 1970-71, where the solution and the emulsion deposits weathered equally fast by 61.1% and 66.5% over a one year period (Table 20). The emulsion containing the thickening agent, Dacagin, produced a very persistent deposit which was reduced by 34.3% after one year. During the first 4 weeks break-down was slow, 15% for the straight emulsion and the solution, and 5.5% for the Dacagin formulation. That thickening agents can have a retarding effect on decomposition of insecticidal deposits was mentioned by Barlow and Hadaway (1952). Since higher concentrations of Dacagin caused nozzle clogging during helicopter applications, the 1971-72 experiment had the purpose of determining concentration levels for Dacagin at which a break-down retarding effect was still noticeable. The experiment was started on September 1, 1971, and was carried through the winter to obtain a measure of the residue degradation during the dormant season. Up to the tenth week only little break-down occurred for all formulations (around 10%). Decomposition was slower during the first 5 weeks than during the first 4 weeks of the previous year. Colder temperatures in the fall might have retarded degradation. The residues of all formulations dropped significantly over the dormant months. The

Table 20. Decomposition of methoxychlor formulations on elm bark over a one year period.

Formulation[1]	Orig. Deposit[2] micrograms/cm^2	% Decomposition N Weeks After Application[3] 2	4	5	10	32	52
1970 - 1971							
Solution (xylene)	391.0	6.4	15.0				61.1
Emulsion (xylene:water= 1:1)	569.0	6.6	15.0				66.5
Emulsion + Dacagin (4 lbs/100 gallons)	379.9	2.5	5.5				34.3
1971 - 1972							
Emulsion	457.6[2]			4.5	10.2	61.8	72.0[2]
Emulsion + Dacagin (2 lbs/100 gallons)	527.3[2]			0.7	9.8	45.4	67.7[2]
Emulsion + Dacagin (0.5 lbs/100 gallons)	470.9[2]			5.1	11.7	48.4	65.4[2]

1) formulated from 25.1% Michlin 'MX-2' elm spray and diluted to 12.55%
2) mean of 2 samples
3) based on original deposit after application

Table . Decomposition of methoxychlor formulations on elm bark over a one year period.

Formulation 1)	Orig. Deposit 2) micrograms/cm²	% Decomposition N Weeks After Application 3) 2	4	5	10	32	52
[illegible]							
Solution							
Xylene & [illegible]							

straight emulsion was broken down by 61.8% by the following April. The formulation with the higher Dacagin concentration had decomposed by 45.4%. After more than one year, 28% was left over from the original emulsion deposit, 32% and 35% from the high and low Dacagin deposits. Break-down of the Dacagin formulation in the 1970-71 experiment was much slower than in the second experiment. The higher concentration of Dacagin in the formulation of the 1970 experiment was certainly one reason. The quantitative deposit changes of both Dacagin formulations (2 lbs/100 gal and 1 lb/100 gal) in the 1971/72 experiment were comparable.

These experiments indicated that methoxychlor deposits have a life-span which definitely exceeds one year. The deposits of formulations without Dacagin were reduced to roughly 1/3 of the original amount within one year. Decomposition was twice as strong for the first 32 weeks (September to April) than it was during the next 20 weeks. This suggested that the weathering of surface deposits was completed after 32 weeks. The break-down of the remaining tissue deposits proceeded, then, at a much slower pace. After 10 weeks, a fine net of methoxychlor crystals was still observed on the bark of all treatments. No surface deposits could be detected in April after 32 weeks of weathering. Dacagin, at the lower concentrations (1 lb/100 gal and 2 lbs/100 gal), must be credited with the retarding action on the decomposition of methoxychlor deposits during the dormant season. This protective action, however, disappeared during the rest of the year. It appeared that this thickening agent can increase persistence at higher concentrations significantly, as was shown in the 1970-71 experiment.

The results of these break-down experiments might give an answer to a key question: can methoxychlor be applied in the fall? For the

mist blower, the answer is 'yes' for trees with limited height (up to 15 m) provided the application is done thoroughly. Mist blower deposits frequently reach several hundred micrograms/cm^2 in the lower tree region and up to 200 micrograms/cm^2 in the upper height level. Given a decomposition rate of 70% over one year, the 200 micrograms/cm^2 would have decomposed to 60 micrograms/cm^2 which is still sufficient for good protection. The helicopter achieves deposits of 70 micrograms/cm^2 in the top of elms. Using the same decomposition rate, 25 micrograms/cm^2 would remain after one year. This deposit could cause up to 40% mortality according to the residue-mortality regression. In reality, however, this figure would be lower because of the poor spray distribution on the target sites, the branches. All in all, a helicopter treatment in the fall appears to be ineffective.

(E) Movement of Methoxychlor on Elm Bark

Table 21 summarizes the residue amounts recovered from the various bark sections. Within the 4-week period, very little displacement of residue had taken place. Section A, the bark area above the application point, had zero residue. Almost the entire recovery of residue was made in section B where the insecticide was originally applied. A small amount of residue was translocated downwards to section C, which included the crotch area, and section D. The recoveries for the twig crotches I and II were almost identical. These results clearly indicated that movement of methoxychlor deposits from the original point of application to another location is minimal.

The insignificant displacement which occurred during the 4-week weathering period was probably due to the washing effect of rain water running down the bark. Since the water solubility of methoxychlor is

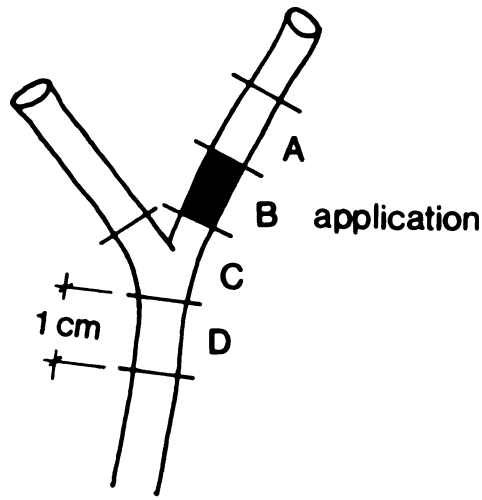


Figure 51. Translocation experiment: designation of bark sections on twig crotch.

Table 21. Residue recovery from bark sections A, B, C, and D.[1)]

Twig Crotch	Bark Section	Application micrograms MeCl	4 Weeks After Application micrograms MeCl		Recovery[2)] %
I	A	---	0.0		0.0
	B	599.7	529.4		88.3
	C	---	5.9		1.0
	D	---	4.2		0.7
				TOTAL	90.0
II	A	---	0.0		0.0
	B	599.7	514.7		85.8
	C	---	6.2		1.0
	D	---	4.6		0.8
				TOTAL	87.6

1) for location of bark sections, see Figure 51 above
2) based on total amount applied originally

extremely low, it is conceivable that translocation was purely mechanical. Therefore, the degree of coverage is definitely determined by the capability of the application machine and the quality of the application itself, since bark residues of methoxychlor were not redistributed after initial deposition.

Summary and Conclusions

The correlation of beetle response (mortality) with the respective amounts of residue on field-treated twig crotches yielded LC_{50} values for the helicopter and the mist blower application. The LC_{50} on helicopter-treated twigs was 41.8 micrograms/cm^2 while the LC_{50} on mist blower-treated twigs was only 6.5 micrograms/cm^2. However, DDT residues on mist blower-sprayed twigs interfered with the LC_{50} determination. Therefore, the LC_{50} underestimated the true deposit-mortality relationship. Feeding tests and residue analysis of lab-treated twig crotches with continuous bark deposits yielded an LC_{50} of 12.5 micrograms/cm^2 which was also much lower than the LC_{50} for the helicopter-sprayed twigs. The difference was explained by the fact that discrete spray patterns are less toxic than spray patterns with great dispersion. These findings pointed out a further advantage of the mist blower application, in addition to better tree coverage. That is, less methoxychlor per unit bark area is required for protection than for the helicopter.

Contact toxicity of several methoxychlor formulations applied to fiber boards was substantial when the deposits were fresh. The emulsion deposit caused greater mortality than a solution deposit. The addition of Dacagin to the emulsion reduced contact toxicity of fresh deposits considerably. Weathered surface deposits had only little contact toxicity. The contact with aged deposits prior to feeding had no impact on

the feeding response. The physical structure of methoxychlor surface deposits was documented and discussed in relation to contact toxicity.

These experiments pointed out that methoxychlor deposits are only effective when they are taken in during the feeding process. Since surface deposits are avoided by the vector, the whole toxic action must come from tissue deposits. In order to obtain long-lasting protection, formulations must be employed and developed which favor tissue deposits.

Methoxychlor deposits proved to be quite persistent. Deposits from 12.5% emulsions on elm bark broke down to about 30% after one year. When Dacagin was added, even at very low rates, decomposition was definitely delayed. The persistence of methoxychlor deposits suggested that fall applications could be effective with the mist blower provided excellent coverage of the whole tree region is obtained.

It was often suspected that methoxychlor residues are partly translocated from the point of application and are accumulated in twig crotches. However, no such displacement was observed in 2 experiments.

V. Dutch Elm Disease Control on the Campus of Michigan State University 1958 - 1971.

In 1958 the first elm tree succumbed to DED on the campus of Michigan State University. Since then, the elm population on the campus has been subjected every year to a rigorous and well-planned control program. This was largely due to the excellent cooperation between the Department of Entomology and the Grounds and Maintenance Department of Michigan State University. Between 1958 and 1963, DDT emulsions were applied by mist blower throughout the campus, but some trees were sprayed by hydraulic equipment. In 1964, the campus was subdivided into 2 treatment blocks (Figure 7). One block with about 900 trees was treated with methoxychlor; in the other, DDT was used on approximately 1500 elms. Two woodlots within the campus area were never sprayed and served as check plots. For 3 consecutive years, these blocks were treated in the same way. At the end of 1966, the methoxychlor plot compared favorably to the DDT treated plot in terms of elm losses over the 3 years. However, as Butcher et al. (1967) pointed out, it was difficult to evaluate the effect of DDT carry-over in the methoxychlor plot. Table 22 compares the losses in the 2 treatment plots and the check plots. In 1967, the majority of the campus elms was sprayed with methoxychlor since it appeared that this insecticide was a satisfactory substitute for DDT. With methoxychlor, all spraying had to be completed during 2 weeks before bud break whereas DDT applications were made partly in the fall as well as in the spring. Treating the more than 1500 campus elms by mist blower within a

short period of 2 weeks posed a particular problem to Grounds Department personnel and very often the timely completion of elm spraying could only be accomplished by over-time work. Increasing cost factors (methoxychlor is about twice as expensive as DDT), problems with manpower, and growing concern about pesticide contamination on the campus stimulated the search for more effective and more economical ways to treat elms.

Table 22. Tree losses in DDT and methoxychlor blocks, 1964/65. (from Butcher et al., 1966)

Year	Insecticide	Number of Trees	Dead Trees No.	Dead Trees %
1964	DDT	1274	38	2.90
	Check [1]	647	238	36.78
	Methoxychlor	1163	41	3.53
	Check [2]	604	398	65.89
1965	DDT	1163	41	3.53
	Check [1]	--	--	--
	Methoxychlor	793	8	1.08
	Check [2]	480	237	49.37

1) Kalamazoo woodlot - never treated for bark beetle control.
2) Sandord woodlot - never treated for bark beetle control. Woodlot disturbed by sewage plant construction in 1965.

Thus, in the spring of 1968, based on previous experience with experimental spraying of city elms in Birmingham, Michigan, all campus elms were treated by helicopter (Wallner and Leeling, 1968; Wallner et al., 1969). Helicopter spraying, treatment areas and methoxychlor formulations employed for 1968 and 1969 are described in detail by Wallner et al. (1969) and on page 64 of this dissertation. In the spring of 1970 and 1971, a 12.5% methoxychlor emulsion with 2 pounds of Dacagin per 100 gallons

spray was applied by helicopter to most of the campus elms. Around 200 elms in 2 University housing areas, University Village and Cherry Lane, and several elms close to dormitories have been sprayed consistently by mist blower (Figure 6). Particularly valuable elm trees were sprayed twice, once by helicopter and again by mist blower. In 1969, Vapam [1] was injected into the soil in several places where root graft transmission was suspected. According to the elm tree count at the end of 1967, there were about 1900 elms left from the original elm population of 2300 trees (estimate). This amounted to a total of roughly 400 trees or 16.6% lost during the first 10 years of DED on the campus from 1958 to 1967. An additional 18.0% was lost between 1968 and 1972. The elm population on the campus is now down to about 1500 elms which represents a loss of 34.6% since 1958. The history of DED control on the MSU campus for the years 1958 to 1971 inclusive is summarized in Table 23 which lists also the yearly tree losses owing to Dutch elm disease. Figure 52 shows a dramatic increase of losses to DED on the MSU campus since 1967.

One might conclude that this increase was due to the switch from DDT to the less effective methoxychlor or due to the less thorough aerial application technique. This conclusion is probably correct to some degree and might explain part of the upward trend in elm losses in recent years. However, the most important variable which is definitely correlated with the number of tree losses is the vector density. Unfortunately, no population estimates are available for the campus or the greater Lansing area for the period following 1958. The production of Scolytus vectors on the campus itself is probably very low due to the stringent and well-exercised sanitation and tree removal program. An indication of a possible 'population explosion' in the Lansing area and the subsequent increase of

1) product of Stauffer Chemicals

Table 23. Summary of DED control and elm losses on the campus of MSU: 1958-1971

Year	No. trees yearly total	No. trees[1] yearly loss	% loss[2]	%loss[3]	No. trees sprayed with DDT	No. trees sprayed with MeCl	% trees sprayed	% trees sprayed by mistbl.	% trees sprayed by helic.
1958	2285	1	0.0	0.0	1157	-	50.6	100	-
1959	2284	8	0.4	0.4	1641	-	71.9	100	-
1960	2276	17	0.7	0.8	1960	-	86.1	100	-
1961	2259	45	2.0	2.0	2007	-	88.8	100	-
1962	2214	34	1.5	1.5	2214	-	100.0	100	-
1963	2180	33	1.4	1.5	2180	-	100.0	100	-
1964	2147	44	1.9	2.1	1515	898	95.3	100	-
1965	2103	49	2.1	2.3	1274	793	89.8	100	-
1966	2054	69	3.0	3.4	1163	616	89.8	100	-
1967	1985	79	3.5	4.0	208	1320	89.8	100	-
1968	1906	112	4.9	5.9	-	only MeCl used	undetermined[5]	5[6]	95
1969	1794	126	5.5	7.0	-			5	95
1970	1650	90	3.9	5.5	-			5	95
1971	1560	84	3.7	5.4	-			5	95
1972	1476								
			34,6[7]						

1) including root graft infestations
2) % based on tree total of 1958
3) % based on yearly tree total
4) 18 trees, removed due to construction, not included
5) probably between 95 to 100%
6) estimate
7) % total loss from 1958 to 1971

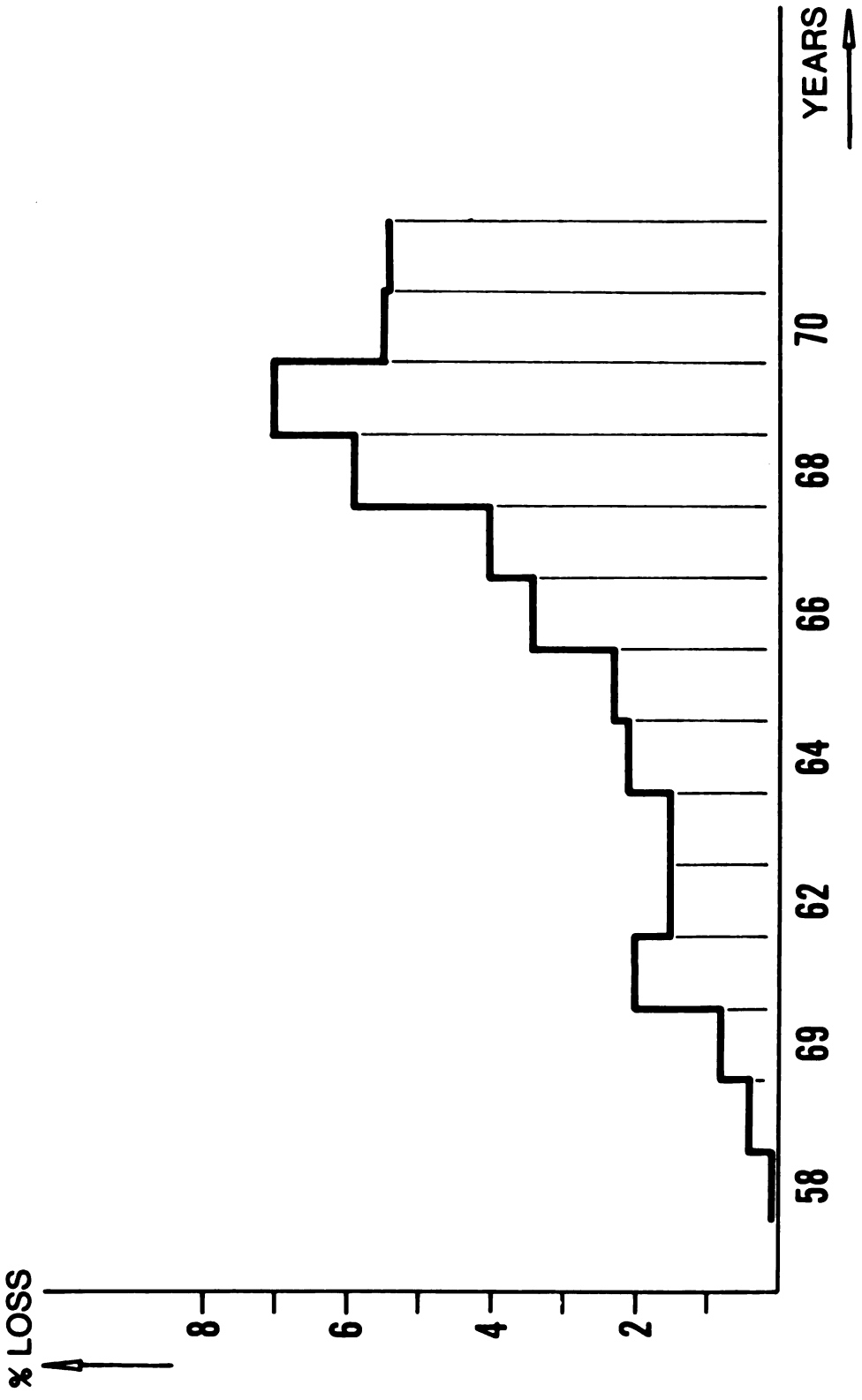


Figure 52. Losses to Dutch elm disease on the campus of Michigan State University: 1958-71.

tree infections in surrounding areas such as the campus is the fact that 1967 was the last year where all Lansing city elms received DDT spray protection. In the following years, methoxychlor was used exclusively by the city of Lansing, however, only some of the more important park and street trees were sprayed (Table 24). City crews were not able to keep up with timely removal of diseased elms and sanitation of elms was not practiced sufficiently. All these factors led to an increase in the vector population in the Lansing area and the rising losses of elms on the MSU campus since 1967 are probably a partial reflection of that.

Table 24. DED Spraying in Lansing, Michigan. [1)]

Year	Number of elms sprayed	Estimated total No. elms
1968	1200	5000
1969	1400	?
1970	700	?
1971	spraying discontinued	2500

1) according to figures supplied by the city forester of Lansing, Michigan

The original elm population in the city of Lansing of 8000 trees suffered a loss of 69% and as of 1971 is down to an estimated 2500 trees. The city of East Lansing to the north of the campus lost a similar percentage of its original number of elms. The loss of elms on private property is not included in these figures. The 34.6% loss to DED since 1958 on the campus compares favorably to the above figures of around 70% in the city of Lansing and East Lansing. Yearly elm losses in some cities in the northeast were as high as 15% (Miller et al., 1969). Most of the bigger elms in the woodlots surrounding the campus have disappeared

because of Dutch elm disease, so that the urban elms in the vicinity of the campus can be considered as the only source of beetle production. The chances that the majority of the well-tended campus elms can escape the disease is enhanced by the fact that the elm density in the surrounding urban communities will eventually reach a level where the disease becomes endemic. With a basically vector-free belt surrounding the campus, a minimal sanitation and spray program will be necessary for adequate protection of the campus elms. By maintaining or even improving the present control measures for Dutch elm disease, a fair chance exists to preserve the elms on the campus of Michigan State University.

SUMMARY AND CONCLUSIONS

Data on the distribution of feeding zones in elm trees were collected because of their obvious importance for elm protection. Extensive sampling of untreated elm trees showed a linear increase in feeding attack by S. multistriatus from the bottom to the top. The upper tree region was designated, therefore, as the preferred feeding zone of S. multistriatus. However, inoculations in the lower half of an elm tree reportedly result in faster disease development and tree kill because of the shorter distance between feeding sites (inoculation points) and main trunk. Consequently, twig crotches in the whole tree region require protection.

The actual target sites during a spraying operation are the twig crotches which make up only a fraction of the total bark area in an elm tree. Only a small portion of the spray settles in the twig crotches, the rest 'contaminates' other bark areas or drifts to non-target sites in the vicinity of the tree. No spraying method will ever be able to direct the protective spray exclusively to the twig crotch areas. In practice, the whole tree, all bark area, has to be covered to assure protection.

What is adequate protection? Theoretically, vector feeding in one unprotected feeding site could result in effective inoculation and disease development. The logical objective of pre-attack spraying is therefore to prevent inoculation via vector feeding in all potential

feeding sites during the whole period of susceptibility. The problem is two-fold. First, to achieve the goal of complete protection, sufficient amounts of insecticidal spray have to be deposited in every twig crotch. This is basically a spray-technological problem. Second, the duration of protection is determined by the persistence of residue on and in the bark, which is dependent on the properties of the chosen insecticide and formulation.

The performance of two spray systems for elm protection, the helicopter and the mist blower, was critically evaluated in this dissertation by bioassays, by chemical analysis of insecticidal residues on the bark, and by a monitoring technique for spray coverage. Neither of the two tested application techniques achieved complete coverage or insured 100% protection. However, the mist blower was, in general, 3 to 4 times more effective than the helicopter. This was conclusively shown by all three evaluation methods. Reduced tree-top coverage at greater tree heights seemed to be one important short-coming of the mist blower application. The monitoring technique, employing sample cubes which were suspended at several height levels in elm trees, provided an objective evaluation of spray coverage. This technique was unique in the sense that it permitted the study of droplet size spectra, actual deposits and 3-dimensional coverage. Although the degree of protection provided by a certain application technique should be the main criterion for its selection for elm spraying, other factors, such as environmental and economic considerations, must be taken into account in the decision-making process. The following criteria appear to be crucial to the evaluation of application techniques for elm spraying:

1) degree of coverage: Coverage depends on the capabilities of the application machine to reach all target sites. The actual amount

of insecticide applied per unit bark area and droplet size spectrum are critical for the degree of protection.

2) insecticidal input to the environment and drift: Dutch elm disease spraying is practiced primarily in urban areas where the addition of insecticide to the environment should be kept at a minimum. Spray drift should be low because of the obvious health hazards which could arise in the city environment.

3) economics of spraying operation: The actual spraying costs per tree are certainly a prime consideration. They are composed of the direct cost of the spraying machine, cost of manpower and insecticidal formulation. However, indirect costs could evolve from environmental damage and spray damage to property and human health.

4) speed and accuracy of application: Timing for Dutch elm disease spraying is critical. It is desirable to complete the spraying operation in the weeks before bud break. In urban areas spraying is usually carried out during early morning and evening hours because of reduced interference with city life. Low temperatures and unfavorable wind conditions can greatly limit the available spraying time before bud break. Speed of the spraying operation is then of great importance. Further, with persistent insecticides such as DDT the spraying operation could be stretched out over a longer time period (fall and spring applications) which is not possible with fast-weathering insecticides. This, too, increases the need for faster application techniques. In terms of accuracy, elm identification is better executed from the ground than from the air, which could mean that, in an aerial spraying operation, not all elm trees receive spray coverage.

Utilizing the criteria established above, the helicopter and the

mist blower application techniques can be compared for a final evaluation. The mist blower was, overall, the more effective application machine. It delivered ca. 4-6 times more spray to each tree (2-3 gallons), reached ca. twice as many feeding sites and gave between 80 and 90% protection during the first 10 weeks after spraying, as compared to 15 to 30% for the helicopter. Also, helicopter-applied bark deposits were found to be comparatively less toxic than mist blower deposits, owing to the higher dispersion of mist blower spray. The helicopter's spray input to the environment is considerably lower (2 quarts per tree) which should be considered when environmentally hazardous insecticides (such as DDT) are used for Dutch elm disease spraying.

The question as to which spraying method causes less drift is still open, however drift control was achieved in helicopter-applied spray by the addition of an anti-drift material.

The economics of both spray methods was not investigated, however, helicopter application is 2 to 3 times cheaper. The helicopter saves insecticide, requires less manpower and finishes a spraying job in a shorter time. For instance, on the campus of Michigan State University helicopter spraying of ca. 1200 elms requires a total of ca. 6 flight hours (one pass over each tree) while the mist blower application needs several weeks for completion.

The mist blower application can be adjusted better to characteristics of the individual tree and involves no tree identification problem. A pre-requisite for a good spraying job from the air is a thorough knowledge of the terrain by the pilot applicator. It is certain, however, that always an unknown number of elm trees escape spray coverage from the air. It was suggested recently (Barger, 1971) that 'saturation'

spraying (treating all elms in a given area) might not be necessary. This might be a reasonable assumption provided the vector pressure is low.

In summary, one can state that the helicopter application, as it is practiced today, cannot be recommended as a substitute for the mist blower application because it fails to give adequate protection. Its positive features of speed of application and reduced cost make it desirable to use if it can be improved as an application machine. Such improvements can be made either in the technical design of the actual spray equipment, adapting it especially for tree spraying, or altering the mode of the spraying operation. Reducing speed during application above each tree will cause a more favorable down-draft and better coverage, and will result in higher spray dosages for each tree. Another possibility is to make two passes over each tree ('double application') which will result in twice as much insecticide per tree and possibly better coverage since the spray settles from two different directions. These suggested modifications will, however, increase the expense of helicopter spraying; one would have to weigh the economics of a 'double application'.

Methoxychlor is now the exclusive chemical in use for Dutch elm disease vector control. It is surprisingly effective and has long persistence, exceeding one year. From an environmental standpoint, it is a safe compound with high biodegradability and low mammalian toxicity. Because of these positive features, no change to another insecticide will be necessary. However, new formulations are needed which favor bark tissue deposits and create persistent deposits so that fall applications will be feasible. Formulations which are self-spreading after application could improve deposit distribution on the branches. Such

a formulation would complement and aid the helicopter application with its poor 3-dimensional coverage and discrete spray pattern.

Over the long run, the expensive yearly spraying operations against DED cannot be carried on indefinitely. Many communities, discouraged with the results of many years of intensive DED control, are giving up all protective spraying operations. No control method to date has been able to halt disease spread. Spraying against vector feeding certainly delayed disease development and gave city foresters a chance to replace the dying elms with other shade tree species. This delaying action is probably the biggest merit of elm spraying, but control of the disease cannot be achieved by it.

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