CCRI File Report 5

TRANSLOCATION OF BENOMYL IN ELM (ULMUS AMERICANA L.)

X SOME ASPECTS OF THE PREVENTION OF THE DUTCH ELM DISEASE IN MATURE TREES BY FOLIAR APPLICATIONS

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INTRODUCTION

Foliar spray field trials were conducted for three summers (1970-73) to monitor the uptake and translocation of benomyl in large elm trees. The results, on the whole, showed a limited uptake, mostly through the bark (Prasad 1972, Prasad and Travnick 1973). The question arose, how much real protection of healthy trees against attack by the Dutch elm disease (DED) organism can be achieved by foliar and bark spray methods? With this objective in mind, several field experiments, involving extensive foliar and bark spraying of the fungicide and insecticide combinations followed by natural and artificial inoculation with DED pathogen, were carried out at Shirley's Bay during the summers of 1973, 1974 and 1975, and the results from these experiments are described in this report.

MATERIALS & METHODS

A. Trials with Methoxychlor, Benomyl Salt, and Inoculation by the Bark Beetle

To investigate the effects of applications of methoxychlor and MBC-SO_4 (a sulfate salt derived from benomyl) on vector population and disease incidence, a preliminary experiment was carried out during the summer of 1973.

Three groups of ten elm trees each, of 20 cm (8") average d.b.h. and 10.5 m (35 feet) tall, were selected. Trees in the first group were sprayed with methoxychlor only; the second group was treated with a combination of methoxychlor and MBC-SO_4 with Biofilm added; the third group was left as untreated controls.

The methoxychlor treatment was a 50% W.P. applied at a concentration of 3 lbs/100 gallons of water. MBC-SO_4 was of 2500 ppm concentration

(0.25%) with the adjuvant Biofilm added at 5 ml/gal of solution. This formulation of the insecticide and fungicide was compatible and did not cause any phytotoxicity. However, an emulsion of methoxychlor with xylene was extremely toxic to elm foliage and thus only 50% W.P. mixture was used. Trees were sprayed at the rate of 15 litres per tree, soon after the leaf-flush.

Two weeks after the treatment, all trees were inoculated with the bark beetles *(Hylurgopinus rufipes)*, already infected with DED spores. They were released on the sprayed and unsprayed branches in a manner as illustrated in Fig. 1, and allowed to crawl and infect only in the limited area of the cages.

Two branches of each tree were fitted with this cage and each tree was infested with 20 beetles, to a total of 30 trees. As mentioned earlier, these vectors were previously infected with the DED *(Ceratocystis ulmi)* spores, according to procedures of Gardiner (1973). After 2 weeks, the cages were examined for the distribution and mortality of beetles; and the progression of the disease symptoms (wilting) was then followed up in all trees at weekly intervals. Observations indicated that there was a high mortality among the caged beetles (Table I) and that the disease development was also erratic among the inoculated and control trees. Therefore, in another series of experiments, sprayed and unsprayed trees were artificially inoculated by hand, according to the method of Gregory (1970) with a suspension of conidia (10^6/ml). The progression of the fungal growth in the xylem was then monitored about one foot away from the point of infection. This is documented in Table II; no disease development was found in the branches treated with the fungicide.

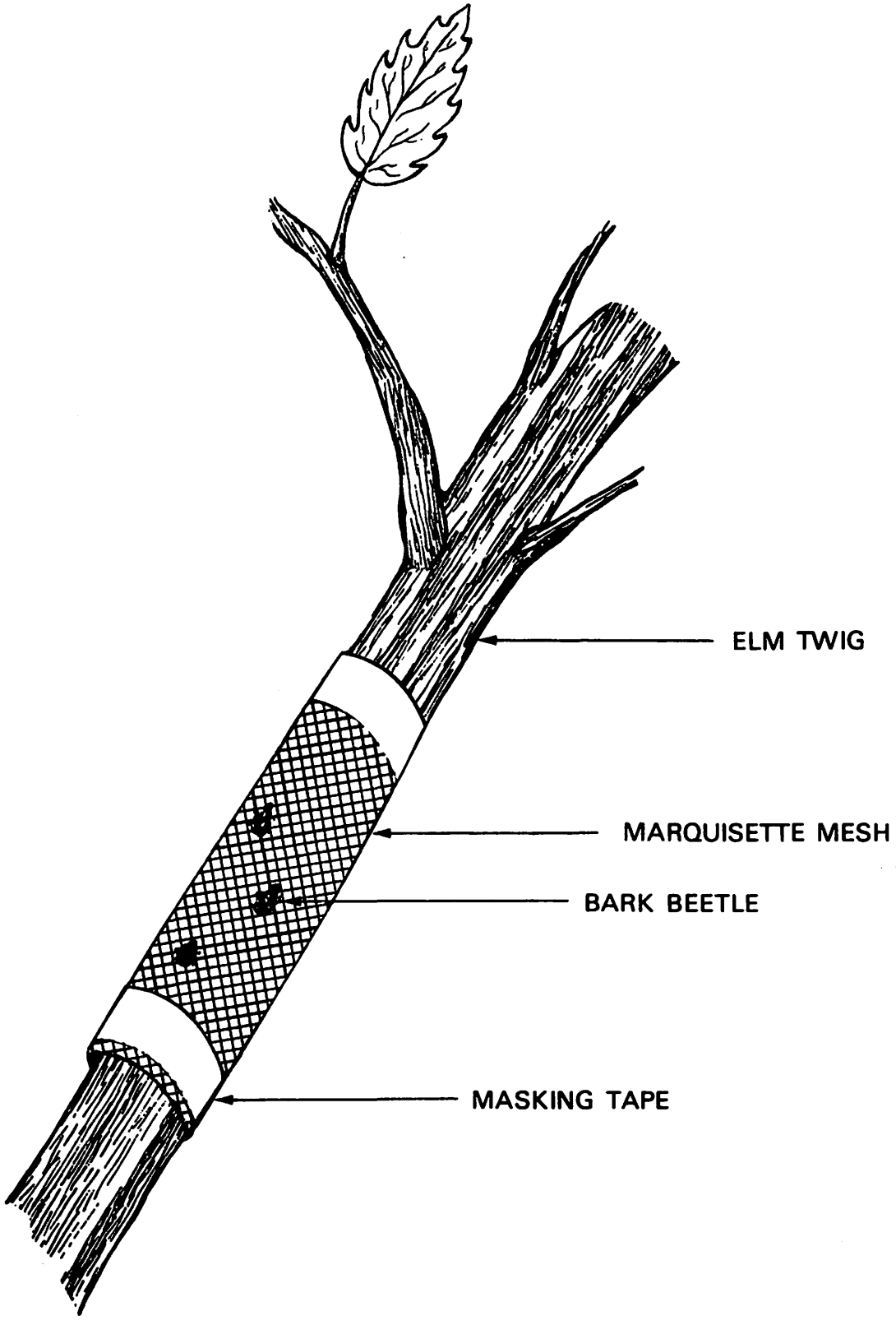
ELM TWIG
MARQUISETTE MESH
BARK BEETLE
MASKING TAPE

Fig. 1 Illustration of the method for caging of beetles infected with DED on elm branches sprayed with and without methoxychlor plus benomyl.

TABLE I

Average Mortality per Tree of Bark Beetles Caged on Elm Twigs For Inoculation of Trees with the DED[1]

Treatment	Number of Beetles			
	Live	Dead	Total	% Mortality
Unsprayed (Control) Trees	9	11	20	55
Trees sprayed with methoxychlor	4	16	20	80
Trees sprayed with methoxychlor Plus MBC-SO_4	1	19	20	95

[1] Counts taken 2 weeks after release of beetles on the branches.

TABLE II

Effects of Foliar Sprays of MBC-SO_4 and Methoxychlor on the Progression of Disease Development in Branches Infected Artificially With the DED[1,2,3]

Treatment	Percentage Infection Produced	
	Above point of inoculation	Below point of inoculation
Control	44.4	44.4
Methoxychlor	11.2	11.1
MBC-SO_4	0	0
MBC-SO_4 plus Methoxychlor	0	0

[1] Twigs inoculated with 1 ml of 10^6 spores 15 days after spray.

[2] Recovery of DED spores 3 months after inoculation into the twig: Samples of infected twigs were incubated on PDA for the development of DED colony.

[3] Samples for recovery of DED taken 1 foot above and below the point of inoculation.

B. Trials with Fungicide and Artificial Inoculation

(i) Tree Material

In mid-May, 1974, 50 mature, healthy trees of 20 cm (8 inch) average d.b.h. and 10 m (35 feet) tall, were selected and tagged at the NCC site at Shirley's Bay. From all trees sampled, twigs were taken before treatment and tested in the laboratory for presence of *Ceratocystis ulmi* by bioassays on potato-dextrose agar media. Only those trees free from the Dutch elm disease infection were selected.

(ii) Spray Application

For the spraying operation, a John Bean hydraulic sprayer with a tank of 20-gallon capacity was used (Prasad and Travnick (1973). Benomyl 50% W.P. (product of Dupont Co. Canada Ltd.) of 5,000 ppm (0.5%) concentration (calculated as 100% active ingredients) was mixed in water and applied by thoroughly drenching the leaves, twigs, and bark with 10 litres of suspension per tree.

From the selected 50 trees, 10 were used as controls and are referred to as untreated controls herein. During the first week in June, 40 trees were sprayed, and 20 trees from this group were treated again two weeks later with a second spray of 5,000 ppm of benomyl at 10 litres per tree. In this way, there were 10 control (untreated) trees, 20 trees treated with a single spray and 20 trees with a double spray. Ten trees in each treated group also had Biofilm (0.1%) adjuvant added to the spray.

(iii) Inoculation of Treated and Control Trees

At the end of June, i.e. 2 weeks after the final spray, all trees were inoculated with virulent strains of *Ceratocystis ulmi* (Buism) Moreau. The virulence was tested on small trees in the same site before-

hand. An extension ladder was used to climb to branches about 45 m (15 feet) above the ground. Two branches on each tree were selected and tagged with plastic ribbons. At a distance of one foot over the trunk of tree, a one square inch (1 x 1") of bark on each branch was removed and the sapwood exposed. One ml of *C. ulmi* spore solution (Hock et al 1969) of 0.2 optical density was then dispensed on a folded filter paper, which was applied to the exposed area cut on the branch and covered over with a masking tape.

(iv) Tests on Disease Protection

After inoculation of trees with *Ceratocystis ulmi* all trees were visually checked at weekly intervals for detection of disease symptoms. Unfortunately the soil in this location was very shallow, and a prolonged drought-spell caused dessication of leaves and this interfered with final evaluation of disease symptoms in this particular area. As a second method of monitoring the disease progression, the inoculated branches were removed in the fall and samples from a section 3 feet above the inoculated spot were bioassayed in petri dishes containing potato-dextrose agar, for presence of *C. ulmi*. The results of these tests are presented in Tables III and IV.

RESULTS AND DISCUSSION

Table III summarizes the visual observations in field of treated trees, two and four weeks after the onoculation with *C. ulmi*, expressed as percentage of protection afforded by foliar sprays. Values are averages of 20 treated trees and 10 control trees.

TABLE III

Visual Observations of the Effect of Benomyl Foliar Spray On Mature Trees Inoculated with DED spores (1974)

Treatment	Disease Symptom Development After:	
	2 weeks	4 weeks
Controls	0%	80%
Single Spray	5%	80%
Double Spray	5%	95%

Because of drought conditions that prevailed during the investigation, the results were rather onconclusive. The experiments were repeated the following year, 1975; again very little or no protection was obtained from foliar applications.

TABLE IV

Progression of Disease Development in Inoculated Control and Benomyl Treated Branches After 6 Weeks

Treatment	Number of Samples	DED Detected	% "Infection" by DED
Controls	33	4	12.12
Single Spray	60	4	6.66
Double Spray	60	2	3.33

Even though from the above table it is evident that the progression of Dutch elm disease is curtailed in treated as opposed to untreated branches, there is not much gain by foliar and bark applications on a large scale. Perhaps the uptake and translocation patterns of benomyl in large trees is not great enough to warrant large scale treatment. Thus, some of the observations are in accord with findings of Hart (1972) and Smalley et al (1973) that the disease can be arrested inside the elm trees, and some of the results are contradictory. Probably drought conditions at the site repressed the degree of protection offered by foliar sprays.

SUMMARY

Using a hydraulic sprayer, field trials on foliar spraying of methoxychlor, MBC-SO_4 and benomyl to two groups of large elm trees as a single and double treatment, was carried out. Trees were then inoculated naturally by bark beetles, and artificially with spores of the Dutch elm disease pathogen *(Ceratocystis ulmi)*. Results indicated that limited protection could be achieved by the foliar sprays of benomyl and MBC-SO_4 probably by uptake through the bark, but on large scale treatment, protection is not significant.

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REFERENCES

1. Gardiner, L.M. (1973) Personal Communication.

2. Gregory, G.F. (1970) A technique for inoculating plants with vascular pathogens. Phytopathology, Vol. 59 p. 1014.

3. Hart, J.H. (1972) Control of Dutch Elm Disease with Foliar Applications of Benomyl. Pl. Dis. Reptr.56(8): 685-88.

4. Hock, W.K., B.R. Roberts and D.E. Wuertz (1969) A Technique for Rapid Standardization of Conidial Suspensions of *Ceratocystis ulmi*. Plant Disease Reporter 53(8): 640-41.

5. Prasad, R. (1972) Translocation of Benomyl in Elm (Ulmus americana L.) III. Retention on and Penetration Through the Bark. Dept. Env. Inf. Repts. CC-X-47.

6. _____ and D. Travnick (1973) Translocation of Benomyl in Elm *(Ulmus americana L.)*. V. Distribution Patterns in Mature Trees Following Trunk Injection Under High Pressures. Dept. Env. Inf. Rept. CC-X-53.

7. _____ and D. Travnick (1973) Translocation of Benomyl in Elm *(Ulmus americana L.)*. IV. Uptake and Movement Through the Foliage Under Laboratory and Field Conditions. Dept. Env. Inf. Rept. CC-X-52.

8. Smalley, E.B., C.J. Meyers, R.N. Johnson, B.C. Fluke, and R. Vieau (1973) Benomyl for Practical Control of Dutch Elm Disease. Phytopathology 63: 1239-1252.