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TRANSLOCATION OF BENOMYL IN ELM (*Ulmus americana* L.)

III. RETENTION ON AND PENETRATION THROUGH THE BARK

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INTRODUCTION

During a field experiment concerned with the control of Dutch elm disease by foliar and bark spraying of benomyl, it was found that bark and bark tissues retained considerable quantitites of the pesticide applied (Prasad 1971). For instance, samples of bark and bark tissues taken from different parts of the sprayed trees and subsequently bioassayed for the presence of benomyl, produced very large zones of inhibitions on the agar plates seeded with spores of *Penicillium expansum* L. and *Ceratocystis ulmi* (Buism) C. Moreau. Since benomyl persisted in young bark (taken from juvenile branches and crotches) where the bark beetle feeds, as well as in old bark near the main body of the trunk of the trees, the question arose whether these tissues are actively involved in the accumulation of benomyl, and if so, whether they can prevent the initial infection brought about by the vector during its feeding activity. With this in mind, a laboratory experiment was designed to test the retention, penetration and accumulation of radioactive benomyl (methyl-2-benzimidazole carbamic acid, MBC-C^{14}) into bark and associated tissues.

MATERIALS AND METHODS

(i) Culture of Plants

Elm seedlings were raised in a greenhouse according to a standard procedure as described in the first report of this series (Prasad 1972). When the plants were about 4 months old, they were transferred to a growth cabinet set at 3000 ± 200 foot candles (16 hr.

photoperiod), 76 ± 2°F and 70 ± 10% relative humidity. A mixture of soil, perlite and peat moss served as culture medium and the plants were irrigated regularly with half strength Hoagland solution to maintain a vigorous growth.

(ii) Method of Application

To investigate penetration, a known length (12.2 mm) of bark around young and old stems (Fig. 1) was selected and then painted with a concentrated solution of benomyl (MBC-C^{14}) of conc. 1500 ppm, activity 45 x 10^6 dpm and pH 3.5. A spreader-sticker type of adjuvant (Biofilm) was added (1.3 ml/l) to all test solutions. Before treatment, the area of the bark was also measured and examined under a magnifying glass for cracks, crevices and lenticels. The root and soil mixture in each pot was then covered with a polyethylene sheet so as to prevent contamination of the soil-perlite-peat mixture. Because the painted material dried out on the bark surface after 30 minutes, several coatings were applied to ensure that enough activity (3 ml) deposited on the surface. Then a serum cap (vial stopper) was snugly fitted around the bottom of the treated stem in the same manner as suggested by Gregory (1970) so that radioactivity did not wash down to the soil. In some experiments the selected area was punctured with a needle and in others, the bark was partially removed before the application of MBC-C^{14}. The object of these experiments was to provide an answer to the nature of the barrier on the bark surface, involved in the penetration process. Each experiment was replicated fourfold (one tree constituting one replicate) and ran for 6 days in the growth chamber maintained at conditions specified. At

the end of this period, the plants were harvested, sectioned into different parts and assayed for radioactivity as described earlier (Prasad 1972). Fig. 1 illustrates the method of application and sampling of the radioactive parts of each plant. Separate plants were used for treating young and old bark but for the sake of convenience both treatments are shown in the same diagram.

In experiments where persistence on and penetration through bark of mature elms was studied, large trees (30 feet, 40 years) were thoroughly sprayed with a benomyl suspension (conc. 2500 ppm, pH 5.2) from top of the crown to bottom trunk. Care was taken not to contaminate the soil or root under the sprayed tree. Before the onset of spraying, the soil under the tree was completely covered with a plastic sheet (40 x 40 ft.) and was allowed to remain there for four weeks. Periodic samples of juvenile bark from top branches and old bark from the trunk were removed and bioassayed on agar plates seeded with Penicillium expansum for benomyl (MBC) according to a special procedure. (Prasad and Travnick 1972).

(iii) Microscopic Examination of Bark

For the determination of size and distribution of the lenticels, the old and young bark were peeled off the wood and examined under a microscope (magnification- 100X and 400X). Further details concerning the morphology and anatomy of lenticels were obtained from Esau (1960) and Smith *et al.* (1953).

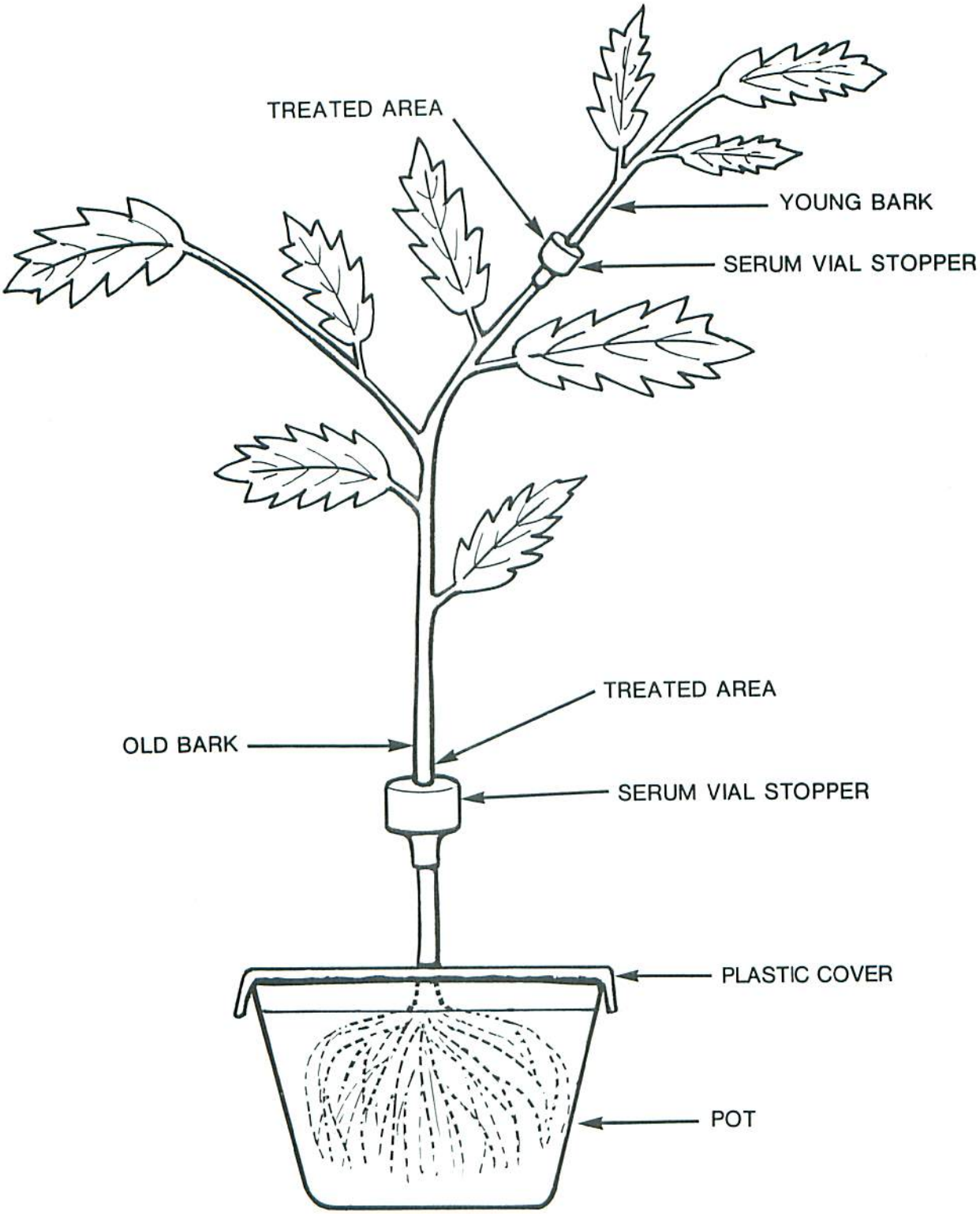

Fig. 1. A diagrammatic illustration of the painted and non-painted areas from which samples were removed for determination of radioactivity. Separate plants were used for treating young and old bark surfaces in the experiments.

RESULTS

The results obtained from laboratory and field experiments are presented in Tables I, II, III and IV. Evidently there are marked differences in the retention capacity and penetrability of young and old bark surfaces.

TABLE I

Distribution of MBC-C^{14} in Various Parts of Plants Following Application Through Bark *

Content of MBC-C^{14}

Plant Part	Bark Painted		Bark Punctured		Bark Excised	
	µg/gm	Percentage	µg/gm	Percentage	µg/gm	Percentage
Bark	934.130	99.43	866.380	98.29	1090.500	96.24
Wood	3.080	0.32	4.230	0.48	32.650	2.88
Stem	0.595	0.06	6.100	0.69	4.899	0.43
Leaves	1.570	0.18	4.596	0.52	4.962	0.44
Roots	0.095	0.01	0.185	0.02	0.128	0.01
Total	934.470	100.00	881.491	100.00	1133.139	100.00

* Concn. 1500 ppm; sampled after 6 days.

Comparing the three treatments it is quite evident that bark does serve as a barrier to penetration and translocation, because as soon as its surface is ruptured with either holes or excisions, MBC surges through the vascular systems. Hence the concentration in wood, stem, leaves and roots rise. There is a about threefold increment in the foliar content and

tenfold rise in the stem after puncturing the bark surface. Another interesting feature to note is the high degree of deposition of residues onto the bark. Even though the same concentration of benomyl was applied in all three treatments there are differences in the residue levels at the bark surface. Perhaps this variability is expected because of the differences in the method of applications and retention capacity of the bark. Therefore the data were transformed to percentages and even on this basis, the original conclusion remains valid and unaltered. On the whole, the contents are higher in wood and stem and this would be expected since the compound has to translocate through the stem first. The amount moving downward into the root is rather insignificant but it seems that some downward movement does take place.

The uptake and translocation from younger bark tissues (branches and twigs) was also investigated. Bark of these parts was painted with the same concentration of MBC (1500 ppm) and allowed to translocate for the same length of time (6 days) as with the old bark tissues. When the contents were calculated on the basis of fresh weight from both old and young bark tissue, the results were rather interesting. There is greater uptake and translocation through the young bark (Table II), with considerable retention and accumulation into the treated areas particularly in the case of young bark. Separation of bark from wood was found to be difficult in young branches and perforce they were sampled together for the determination of radioactivy. Even though these two parts retained most of the MBC-C^{14}, appreciable quantities did translocate to other parts of the tree viz stem, leaves and roots.

TABLE II

Distribution of the MBC-C^{14} into Various Parts of Elm Seedlings Following Penetration Through Young and Old Bark for 6 Days. *

Plant Part	Content of MBC-C^{14}			
	Old Bark		Young Bark	
	µg/gm	Percentage	µg/gm	Percentage
Bark & Wood	938.010	99.77	2776.470	99.60
Stem	0.595	0.06	3.062	0.11
Leaves	1.527	0.16	7.902	0.28
Root	0.095	0.01	0.223	0.01
Total	940.227	100.00	2787.557	100.00

* Concn. 1500 ppm; activity 45×10^6 dpm.

As described earlier, during the course of painting, the radioactive solution dried out quickly from the bark surfaces and this seemed to inhibit uptake. Therefore it appeared logical to investigate penetration from a continuous liquid source. An experiment was designed to test this. A serum vial cap was again snugly fitted around the stem and 5 ml of radioactive solution was directly pipetted into it and allowed to stagnate there for 3 days. The results are presented in Table III.

TABLE III

Retention and Distribution of MBC^{14} in Different Parts of Elm Seedlings Following Penetration Through Old Bark from a Liquid Solution for 3 Days. *

Plant Part	Contents of $MBC\text{-}C^{14}$			
	Application by Painting		Application by Solution	
	µg/gm	Percentage	µg/gm	Percentage
Residue in Bark	1143.70	99.58	721.03	98.14
Wood	2.45	0.21	8.71	1.19
Leaves	2.37	0.21	3.09	0.42
Root	0.05	0.00	1.87	0.25
Total	1148.57	100.00	734.70	100.00

* Concn. 1500 ppm; activity 45×10^6 dpm.

It is apparent from the above data that the extent of penetration and distribution from the liquid solution is greater suggesting prolonged retention and penetration of MBC through the bark. Conversely, these findings also suggest evaporation and drying may have depressed penetration and as a consequence the pesticide content declined in wood, leaves and root. However, when the serum cap was filled with MBC solution, there was greater penetration and transport into wood, leaves and roots. Because the transpiring leaves once again exhibited greater accumulation than roots it seemed much of the transport was apoplastic. The results from the field experiments (Table IV) indicate that the juvenile bark surfaces retain benomyl or products thereof (MBC) far more per unit of weight than those from the main stem of the tree.

Another interesting feature to note is the persistence (6 months) of benomyl on the bark surfaces.

TABLE IV

Retention and Persistence of Benomyl on Young and Old Bark of Mature Elm Trees Under Field Conditions at Shirley's Bay, Ottawa. *

	BARK			PERSISTENCE	
Age	Area Sampled (mm)	Dry Wt (mgms)	Lenticel (Number)	Zone of (Z.I.) Inhibition (mm) (*a*)	Z.I. /Wt. (*b*)
Old	21 x 5	0.212	Invisible	57.50	271.20
Young	21 x 5	0.051	12.1	47.12	923.92

* Time of application - July, 1972.

Samples taken for bioassay - January, 1973.

Dosage applied - 2.1 ozs/tree with Biofilm (1.3 ml/l) added.

Method of application - Hydraulic spraying of large (40 yrs. old, 30 ft.) elm trees.

(a) Bioassayed on Potato Dextrose Agar seeded with Penicillium expansum spores.

(b) All determinations are average values of 15 samples.

The observation that longer persistence of benomyl on young bark seems to be positively related to distribution of lenticels, raised the question whether lenticels are, in any way, responsible for greater penetration and movement through juvenile bark surfaces.

This hypothesis was investigated with seedling trees and radioactive MBC and the data (Table V) demonstrate that there is no direct correlation either with the number or with the width of the lenticular openings and pesticide penetration.

TABLE V

Relationship Between Lenticels and Translocation of MBC-C^{14} Through Bark of Elm Seedlings *

	BARK					Translocation Into Shoot & Root
Age	Area (mm^2)	Thickness (mm)	Fresh Wt (mgms)	Lenticel (number)	Lenticular Opening (mm)	µgm/gm
Old	235.42	0.68	317.75	8.5	0.634	2.217
Young	103.82	0.28	57.60	5.4	0.251	11.187

* Seedlings - 4 months old; Concn. 1500 ppm; Activity 45 x 10^6 dpm. Period of retention and penetration - 6 days.

DISCUSSION

The data demonstrate that very little uptake and translocation takes place through the bark surface of the elm seedlings, and in particular the old bark seems to offer a barrier to penetration because as soon as this restriction is removed partially (See Table I) the pesticide begins to surge through the vascular system and accumulates largely in leaves. It was also seen that the quantity of the fungicide moving through the young bark was greater than

through the old bark. This would be expected because the young bark is morphologically and physiologically distinct from the old one.

Morphologically, the young bark is thin, chlorophyllous and impregnated with fewer lenticels. The translocation of MBC to root, leaves and shoots away from the site of treatment showed a direct relationship with the fresh weight of the bark. Thus there was a five fold difference in the rate of translocation and in the amount of fresh weight of bark tissues treated between young and old bark. This would tend to suggest that translocation is neither related to size of lenticels nor to their number. Rather it seemed to be related to weight and nature of the area of treatment.

Physiologically, young bark being chlorophyllous may be active in photosynthesis. Young and juvenile barks of many woody species contribute significantly to photosynthesis and wood formation (Kramer and Kozlowski 1960) and if this is the case with the elm branches then uptake and transport of MBC may be related to products of photosynthesis. Experiments on foliar penetration of benomyl into elm with sucrose, amino acids and urea indicated that the transport of MBC-C^{14} was somewhat increased. (Prasad 1972)

The fact that a considerable proportion of the fungicide resides in the bark or associated tissues and very little penetrates the vascular systems of the seedling does not necessarily imply that bark treatment would be ineffective for DED control. It could be that spraying of the branches and trunk of the healthy trees with benomyl may provide the same kind and magnitude of prophylactic

protection as normally obtained by the methoxychlor treatments. If this proves to be so, a combination of methoxychlor and benomyl spraying might turn out to be a better control practice than application of one pesticide alone. According to J. White (1972), of the National Capital Commission, Ottawa, who carries out extensive operation of methoxychlor sprayings to prevent the recurrence and new infection for the disease in the capital city, many more elm trees die each year of the DED in spite of the insecticide treatment. Thus methoxychlor alone is not sufficient to eradicate the vectors, (*Hylurgopinus rufipes* Eich. and *Scolytus multistratus* Marsham). Therefore supplements of benomyl formulation with the insecticide mixture are likely to confer additional protection to the trees. This contention, of course, assumes that the fungicide and insecticide formulation are compatible with each other and that there is an additive or synergistic effect. In this connection, Smalley (1972) carried out field trials and found the action of mixtures of methoxychlor and benomyl to be superior to effects of either of the ingredients used alone. How benomyl precisely operates to reduce the incidence of infection by bark treatment was not elucidated but it seems on, *a priori*, ground that germination of the DED spores which are deposited on bark surfaces by the beetles, is somehow arrested by the fungicide. Alternatively, it may be that much of the benomyl residues on the bark was washed down to the soil by rainfall and thereafter absorption and translocation took place through the roots. That benomyl has a high degree of systemicity from the root is well established (Delp and Klopping 1967, Hock and Schreiber 1971, Biehn and Diamond 1971, Smalley 1971, Kondo

1972, and Prasad 1972). According to Erwin *et al.* (1970) benomyl, after being absorbed, is degraded into a more stable and potent molecule, 2-methyl benzimidazole carbamic acid (MBC) and this by-product circulates freely into the tree and finally confers acquired resistance to elm trees against invasion by DED spores.

If MBC enters the root so easily, it could very well arrest the development of further infection through the root-grafts and it may be that the extra protection that Smalley (1972) observed following methoxychlor and benomyl spraying resulted from a suppression of new infection by the root grafts.

The ability of younger bark to retain and possibly accumulate high quantities of benomyl is of considerable relevance to DED control. For, these young and juvenile branches and bark (crotches) are the primary feeding sites of the vectors and thus the ubiquitous nature of MBC in bark tissue is likely to deter the spread of new infection brought about by the beetle. In this connection the report of Thomas (1972) is of some pertinence. After an elaborate experiment he concluded that vectors (H. rufipes) loaded with DED spores emerge from the old bark, crawl downward on the trunk and then fly to young branches for feeding. Clearly bark spraying of benomyl could be very useful in "sterilizing" the crawling surfaces of the beetle which apparently transports infection to other parts of the tree. Or benomyl soaked bark might act as repellent to the beetles but this was recently refuted by Galford and Schreiber (1972). The transport of benomyl through bark could be greatly accelerated by addition of certain adjuvants apart from Biofilm which has been used in this study. Peterson and Edgington

(1970) have found several organic solvents (DMSO, Sunoco folicate, Volck 50 and 66) which appreciably augment the rate of penetration. Thus there is need for further work on the enhancement of penetration with the aid of these adjuvants.

PRACTICAL APPLICATION

The findings that bark and associated tissues retain excessive quantities of benomyl or MBC-C^{14} and the fact that some penetration and translocation does take place across the bark, could be used to devise practical control measures against the DED. Spraying bark surfaces with benomyl formulations containing potent adjuvants and spreader-stickers is likely to increase the concentration and persistence of the fungicide on the bark and possibly into the tree. This, in turn, may offer protection to healthy trees against new infections brought about by the vectors every year. Because there is greater persistence and movement through the young bark than through the old bark, application of benomyl in conjunction with methoxychlor in early spring are likely to yield better results than one pesticide alone. Also, since bark spraying is more convenient and economical than root applications, spraying operations could be easily carried out before leaf flush and beetle activity for the purpose of preventive control. Thus large groves of elms could be easily treated by helicopter or ground sprayers.

SUMMARY AND CONCLUSION

Employing seedling and mature elm trees the retention and penetration of benomyl (MBC-C^{14}) through bark tissues was investigated. It was found that both old and young bark retain benomyl to a considerable degree and only slight amounts penetrate the inner tissues. Movement through the young bark appeared faster than from the old bark and this seems to be related to the nature of the bark rather than to distribution

or size of lenticels. Rupture of the bark surfaces enchances penetration and translocation but not of sufficient magnitude to lend itself to therapeutic control measures. It is suggested that bark treatment with benomyl may provide a prophylactic cure and that a mixture with methoxychlor might augment its effectiveness.

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