

TRANSLOCATION OF BENOMYL IN ELM (Ulmus americana L.)

I. Effects of Hydrogen Ion Concentration (pH) on Absorption,
Distribution and Accumulation by Roots

by

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INTRODUCTION

Benomyl (methyl-1-butylcarbamoyl-2-benzimidazole carbamic acid) was first reported by Delp and Klopping (1966) of the DuPont Chemical Company, U.S.A. It was not until 1969, however, that a group of plant pathologists (Stipes 1969, Hock and Schreiber 1970) from Ohio, discovered its potentiality to control the vascular wilt of native elms (Ulmus americana L.) caused by the Dutch elm disease - DED - (Ceratocystus ulmi (Buismin) C. Moreau). When applied to stem, root and soil, in varying concentrations, benomyl conferred a significant degree of protection to the affected trees (Biehn and Diamond 1971; Hock and Schreiber 1971; Smalley 1971). However, the degree of control obtained by soil application was very variable and erratic because benomyl uptake by elm roots appeared to be limited by the physical characteristics of the soil (Hock, Schreiber, Roberts 1971; Pitblado and Edgington 1972). Using a mixture of sand, perlite and moss, under greenhouse conditions, Hock et al (1971) found no effect of pH and therefore concluded that pH did not exert any influence on the uptake and movement of benomyl into elm seedlings. Clearly, these experiments are subject to criticisms on the grounds that there was no rigorous control of pH in the nursery soil and that the range of pH employed was too narrow to discern any measurable response. On the other hand, working with elm leaves, Prasad (1971) noted greater penetration and transport of benomyl and thiabendazole at pH 2 than at pH 10. A similar finding was subsequently reported by Buchenauer and Erwin (1971) for cotton leaves. Thus, there is a need to reassess the role of pH on root absorption, particularly when it is known that many biologically active compounds penetrate plant tissues preferentially in the form of undissociated molecules (Simon and Beevers 1952).

The aim of the present investigation is to critically evaluate the influence of pH on uptake, translocation and accumulation after feeding roots with a C^{14} labelled benomyl (methyl-benzimidazole carbamic acid - MBC). In the next papers of the series, effects of adjuvants, host metabolites, environmental conditions (temperature, light, relative humidity), concentrations and methods of application on systemic activity of benomyl will be examined.

MATERIALS AND METHODS

Culture of Plants

Elm seedlings were raised from seeds treated with 30% "Javex" for three minutes in a greenhouse. In order to avoid excessive "damping-off" in the nursery, the soil-perlite mixture was also steam-sterilized. When the seedlings were ca. 15" high, they were transferred to a half-strength Hoagland solution (Hoagland and Arnon 1950) containing EDTA-iron and grown under constant conditions of light (3000 f.c. - 16 hours photoperiod) temperature ($25 \pm 1^{\circ}C$) and relative humidity (50-60%). To minimize contamination of roots by algae, the culture jars were covered with tinfoil and solutions changed on alternate days to replenish nutrients. This prevented undue drifts in pH (6.5 ± 0.3) and the plants grew excellently under these conditions. For treatment with radioactive benomyl, only healthy and uniform individuals were selected for the study.

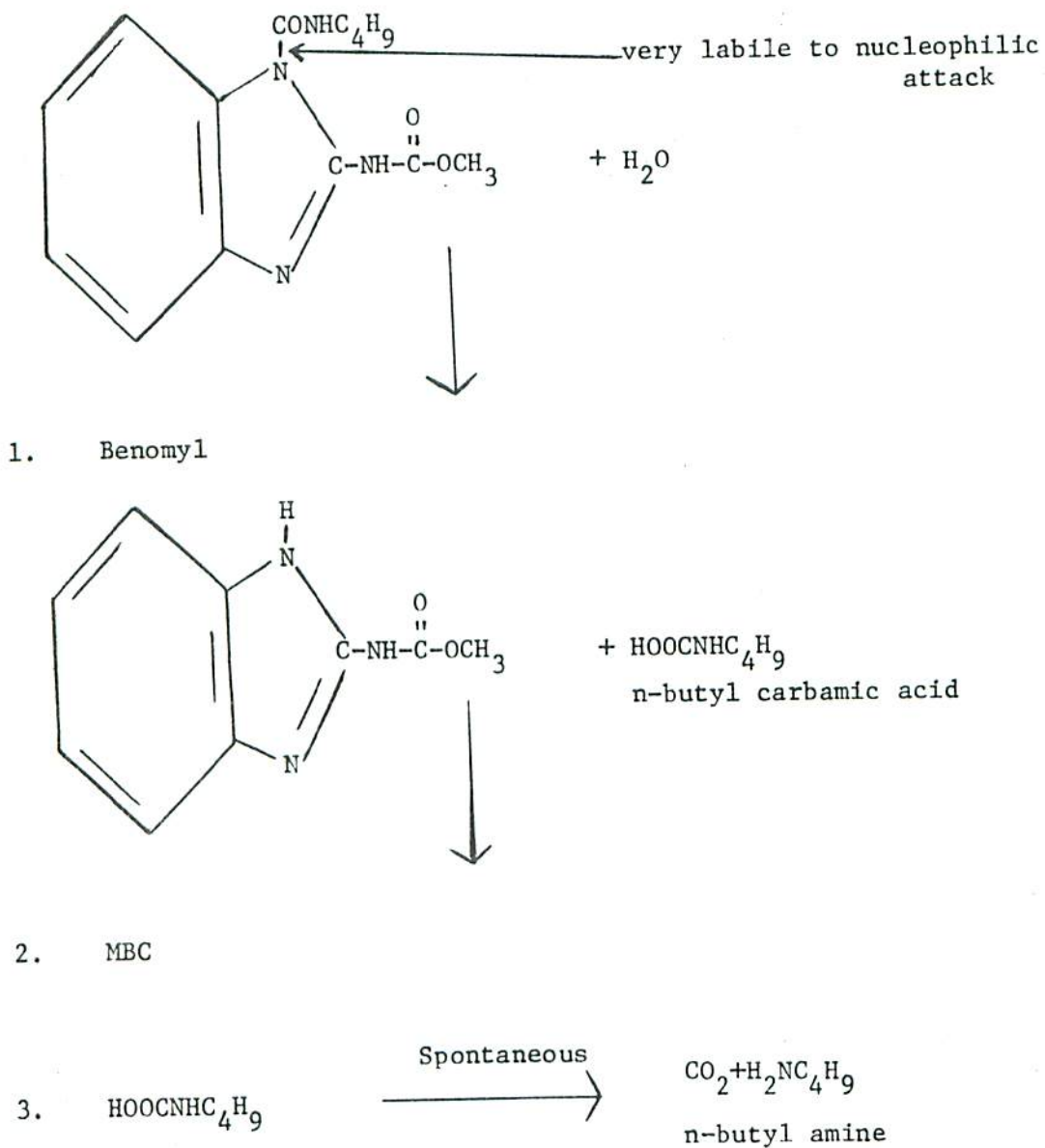
Chemistry of Pesticide

Benomyl readily decomposes to methyl-benzimidazole-carbamic acid (MBC) upon exposure to aqueous solution at room temperature (Kilgore and White 1970) and MBC thus formed (see Fig. 1) is the

actual fungitoxic principle that is responsible for suppression of the vascular wilt diseases (Siegel and Zabbia 1972, Peterson and Edgington 1971, Clemons and Sisler 1969 and Sims et al 1969). As can be seen from the chemical structure in Fig. 1, the butylcarbamoyl moiety of the benomyl molecule is highly labile to nucleophilic attack and cleaves out easily into products (carbon dioxide and butyl amine) that are seemingly innocuous to the host tissue. MBC is relatively insoluble in water but forms salts of acids which, in turn, are more soluble and hence possibly more potent. Therefore, only MBC has been used for most experiments and is denoted as benomyl in the remainder of the text here.

Radioactive benomyl (MBC-C¹⁴ labelled in the benzimidazole ring, specific activity 67 mc/mM) was purchased from the International Chemical and Nuclear Corporation, California and a portion of this was mixed with non-radioactive MBC to yield a concentration of 1500 ppm with 38×10^6 counts per minute. Since MBC is rather insoluble in water, a minimal amount of 85% lactic acid was used to dissolve it before preparing further dilutions from this stock solution (McWain and Gregory 1971). There were four pH levels (3.2, 5.2, 7.2 and 9.2); all solutions were buffered with potassium citrate and potassium dihydrogen phosphate according to Machlis & Torrey (1955). Thus there was a strict control of pH, and any drift that occurred was adjusted with 0.1 N HCL/KOH by using a microelectrode pH meter. To facilitate absorption, a surfactant (biofilm) was added @ 1.3 ml/L to all solutions before the final adjustments of pH were made.

Figure 1. Structural Formula of benomyl and its Principal Degradation Product MBC

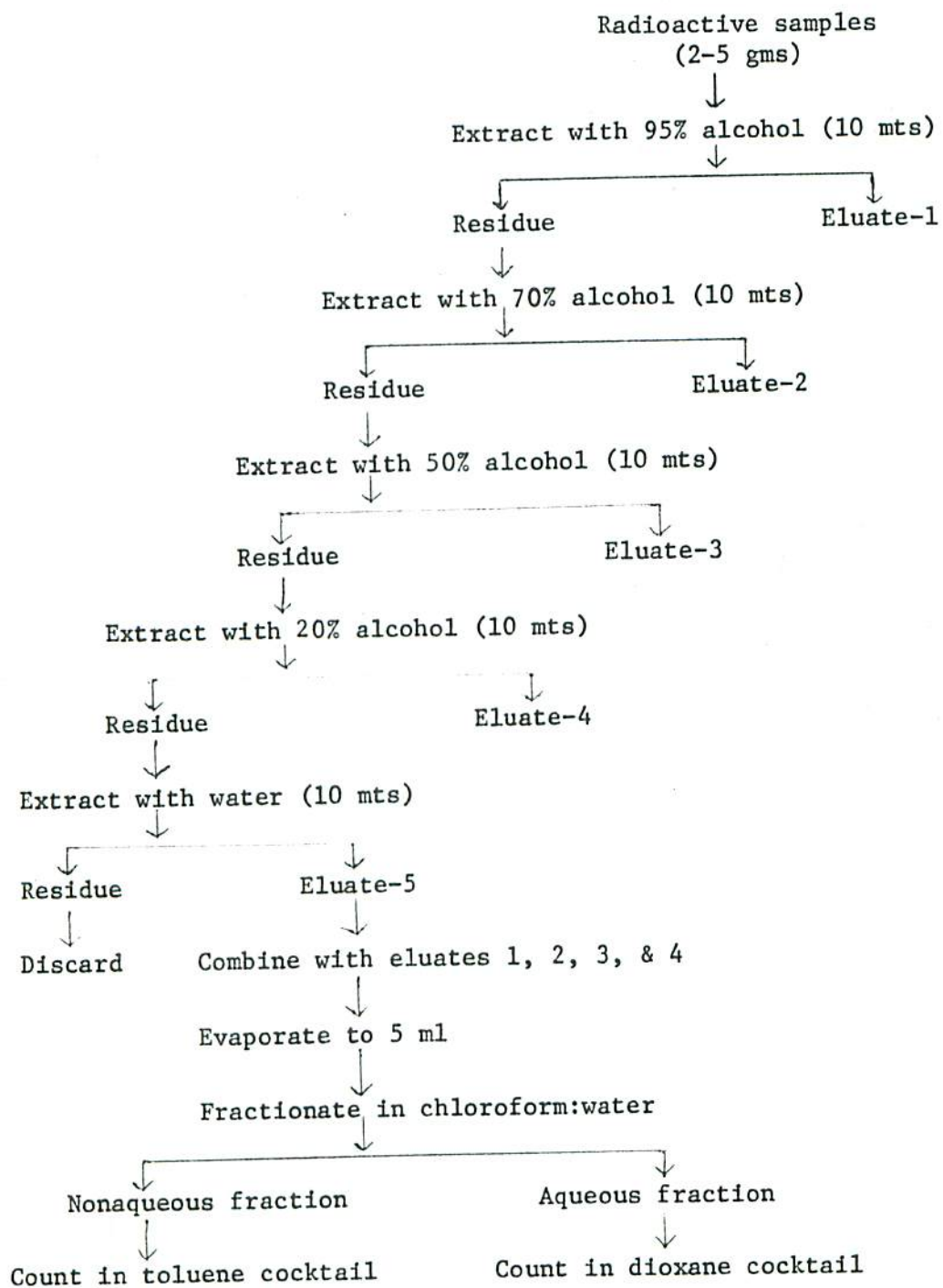


At each pH level, there were four replicates and each replicate contained the same concentration and radioactivity of MBC. The plants were allowed to absorb and translocate for three days before being harvested and sectioned into different parts (roots, stems and leaves). While harvesting, extreme care was taken to wash off all adhering radioactivity from the external surface of the roots and it was found that three quick rinses in a large volume of distilled water removed most of the contaminants.

Extraction and Assay of Radioactivity

The radioactivity of samples fed with MBC-C¹⁴ was determined by counting and autoradiography. For counting, plant segments of known weights were extracted with a series of hot ethanol (95, 70, 50 and 20%) and water (see Fig. 2). Extractions were repeated until all radioactivity was finally transferred to the liquid fractions and the residues were free from counts per minute. About 100 ml eluate was thus collected and then air dried to reduce the volume to 5 ml. This amount was then fractionated into chloroform and water soluble portions and aliquots were counted from both such fractions to yield the total radioactivity. While calculating the specific activity from samples, it was assumed that over a period of three days MBC is not appreciably metabolized by the plants. Aqueous fractions were counted in a standard dioxane cocktail while chloroform fractions were counted in a toluene based cocktail. After registering disintegration per minute (dpm) in a scintillation counter ("Ansitron" by Picker Nuclear Corp.), appropriate corrections were made for quenching of the samples.

Figure 2. Method of Extraction of Radioactivity from Elm Seedlings Fed with MBC-C¹⁴



In order to minimize variation, duplicate samples were counted usually for longer periods to yield statistically significant counts. For preparation of autoradiographs, duplicate samples were freeze-dried, mounted on a special paper, and exposed to X-ray film (non-screen) for 2-3 weeks. Further development of this film was carried out with the standard procedures as described by Crafts and Yamaguchi (1964). Where necessary some numerical data were analyzed statistically to test the level of significance, and for this, standard procedures as outlined by Snedecor (1956) were followed.

RESULTS

When the content of radioactivity of root, shoot and leaf is plotted against each pH level, it is very evident from Fig. 3, (a), (b), and (c) that the rate and extent of absorption and translocation is markedly influenced by the hydrogen ion concentration. Since roots are directly exposed to the fungitoxicant solution, there is even greater penetration and retention into this part of the seedling. Comparing radioactivity in all three parts it is evident that roots contain the greatest amount, and it is possible that active uptake mediated this accumulation. As can be seen from the curve, there is a profound effect of pH on the net accumulation rate at the most acidic medium (pH 3.2), and thereafter the rate declines rapidly resulting in about 10-fold reduction in contents at pH 5.2. It is also significant to note that beyond pH 5.2 there is very little accumulation into the roots save at pH 9.2 where the contents begin to rise again.

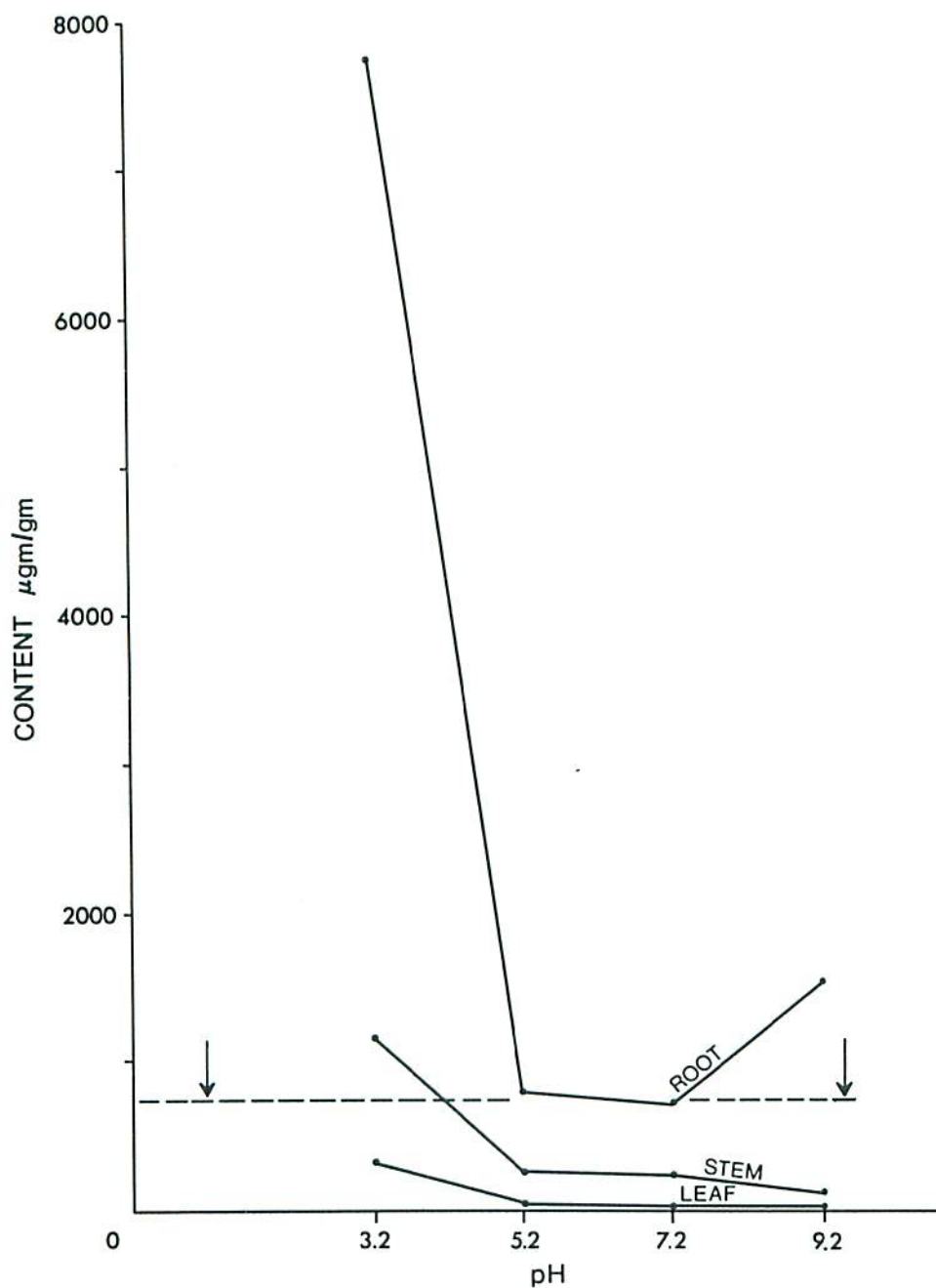


Figure 3. Effects of pH on absorption and translocation of MBC-C¹⁴ by elm seedlings roots. The contents expressed as μgm/gm fresh tissue weight reflect deposition of MBC inside the respective tissues. The arrow and dotted line approximately indicate the level of the external concentration (1500 ppm). Radioactivity 45×10^6 d.p.m. Absorption period - 3 days.

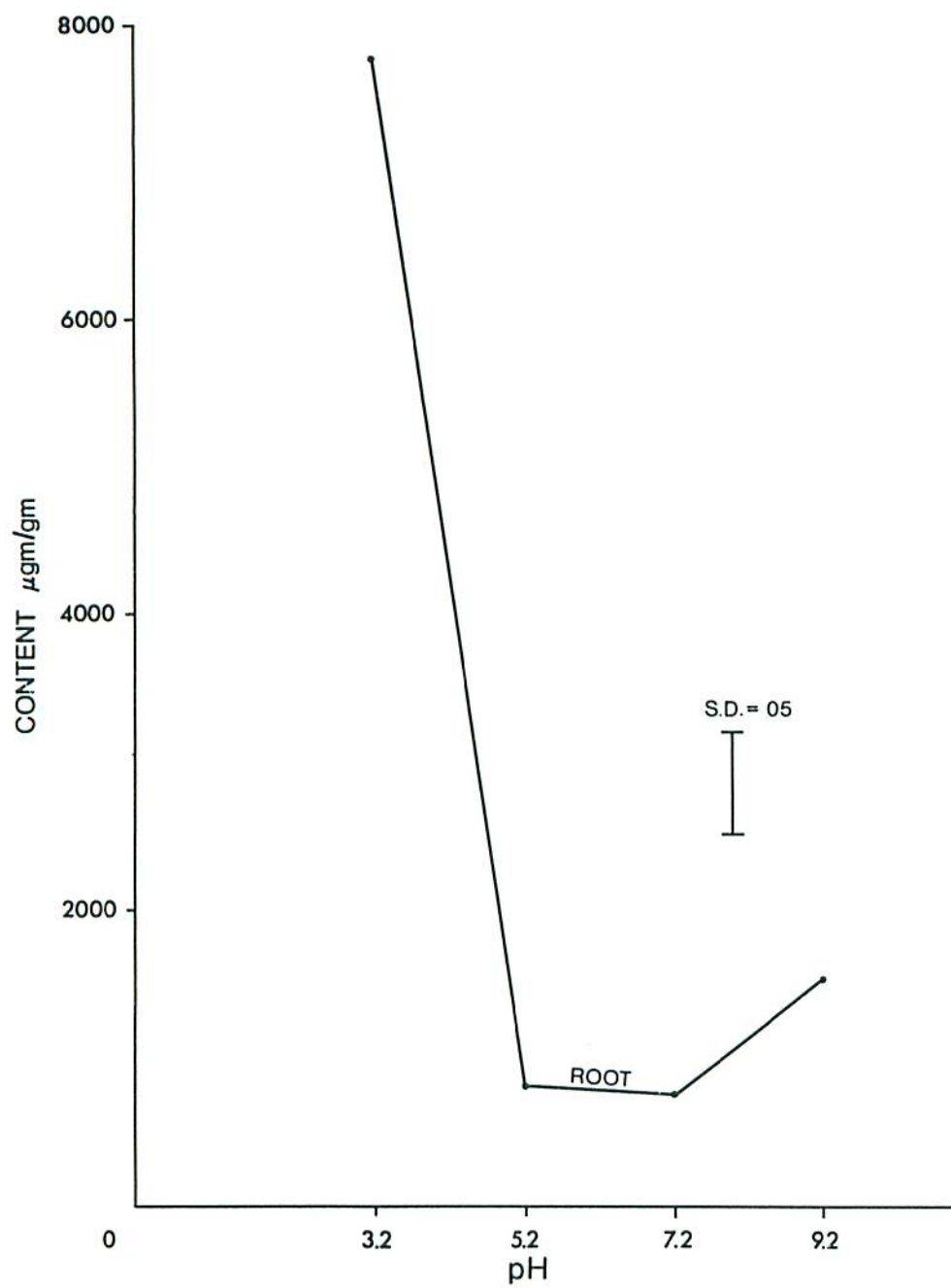


Figure 3(a). Effects of pH on absorption and accumulation of MBC-C^{14} into elm seedling roots alone. Concentration 1500 ppm. Activity 45×10^6 d.p.m. Absorption period - 3 days.

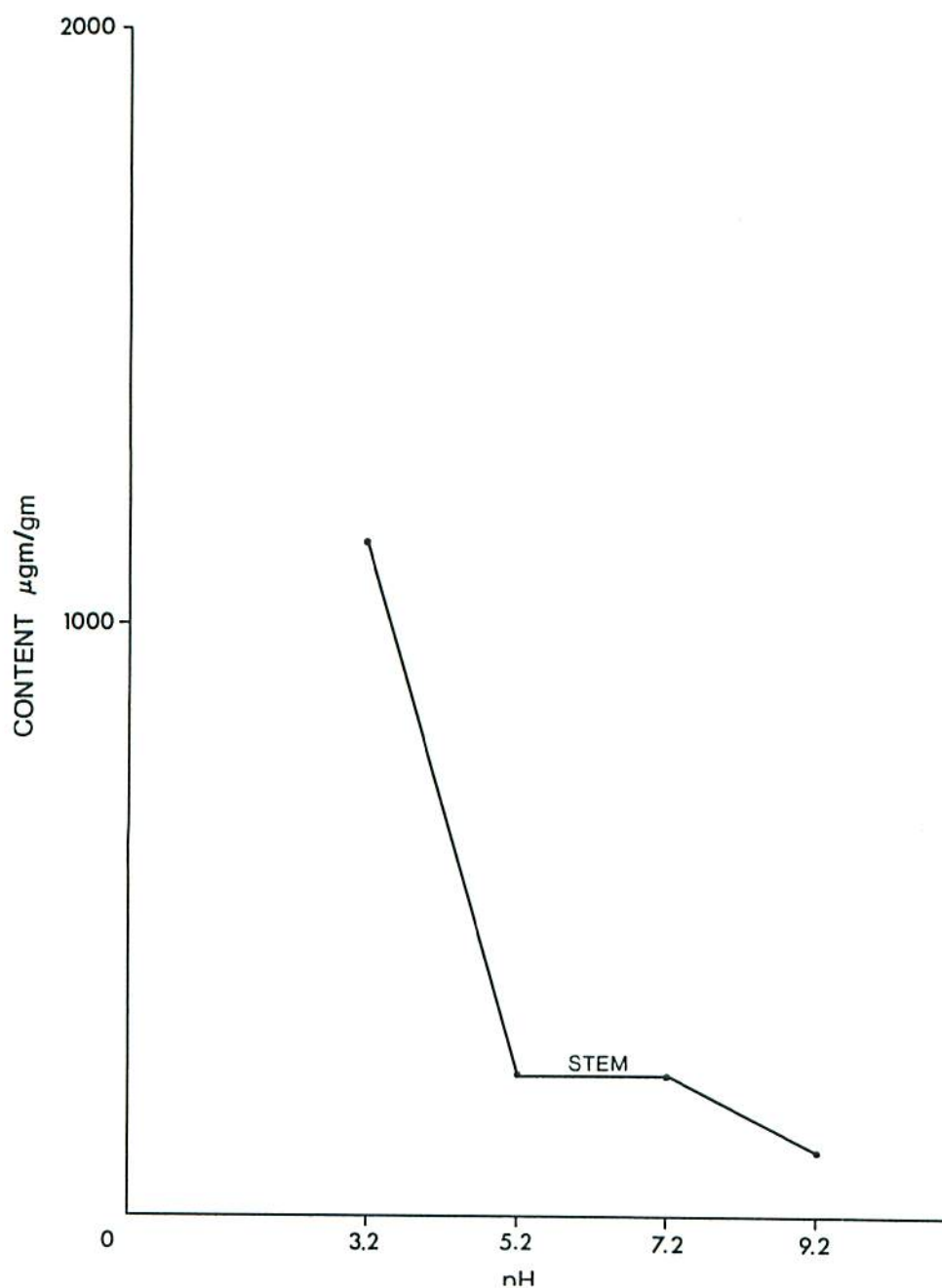


Figure 3(b). Effects of pH on translocation of MBC-C¹⁴ into stem of elm seedlings following uptake by the roots. Concentration 1500 ppm. Activity 45×10^6 d.p.m. Period of translocation - 3 days.

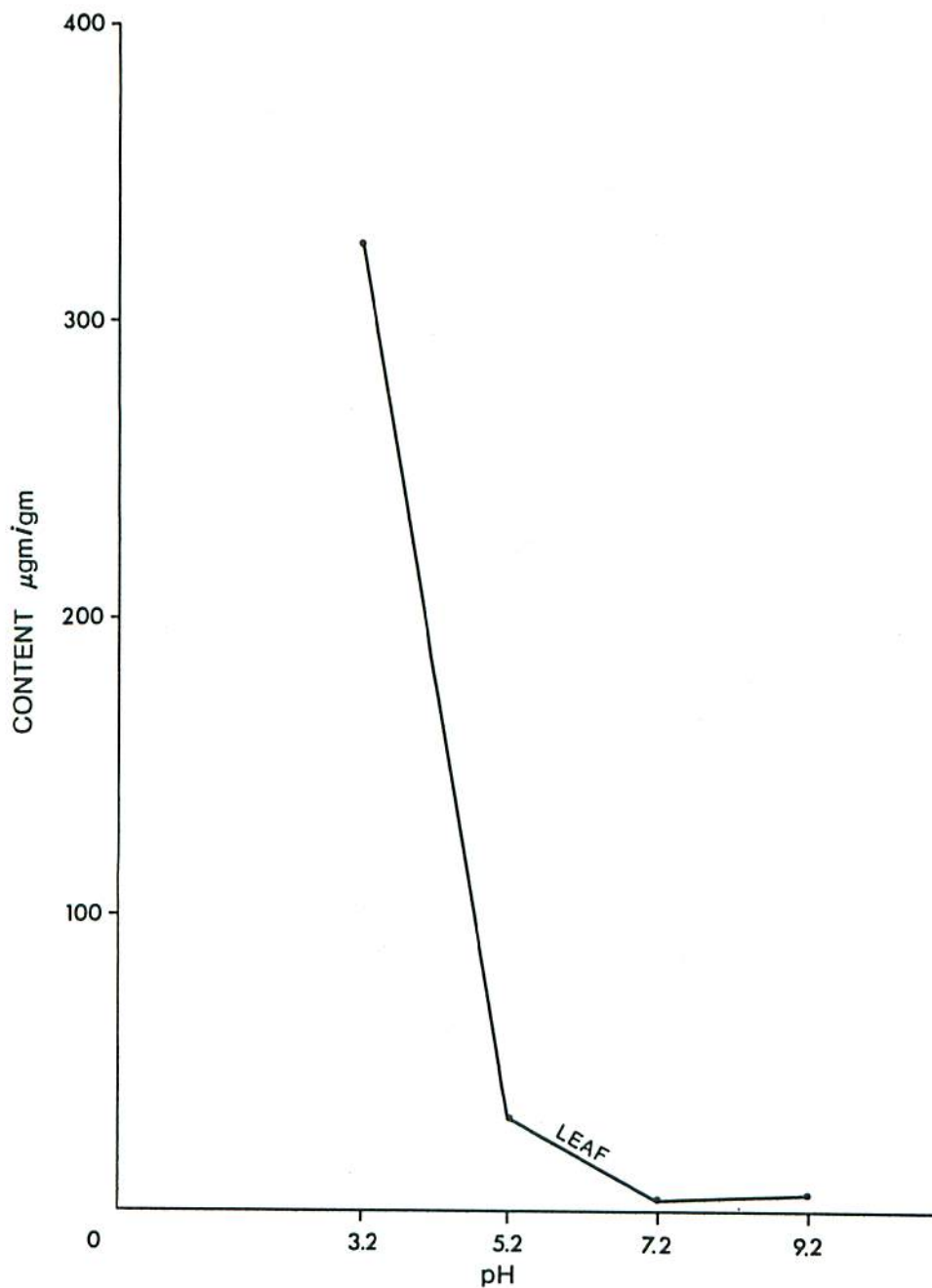


Figure 3(c). Effects of pH on translocation into the foliage of elm seedlings after root absorption of MBC-C^{14} . Concentration 1500 ppm. Activity 45×10^6 d.p.m. Period of translocation - 3 days.

The pattern of translocation into the stem and leaf is similar to that of root as indicated by the autoradiographs (Figs. 4 and 5). The dense labelling of root, stem, leaves and branches all tend to suggest that the radioactive species easily migrated to various parts of the seedling and that translocation to the shoot is not impeded by accumulation into the roots. This is somewhat contrary to findings of Siegel & Zabbia (1972) who reported preferential movement and localization into dwarf pea leaves 52 days after root treatment with MBC-C¹⁴. Predominantly apoplastic pattern of movement is again evident as judged from the diffusional manner of concentration of the tracer into margins, veins and other transpiring regions of the foliage. Centres of high metabolic activity such as growing tips, buds and young photosynthesizing leaves do not appreciably accumulate the label. This is again an indirect indication of the lack of phloem movement. Of significant interest is the slow rate of distribution and deposition into the leaves: at pH 9.2 the foliar contents approach infinitesimal values (see Fig. 6). This is further illustrated by Table I wherein the distribution of activity is expressed in terms of percentages.



Figure 4. Autoradiograph (right) and photograph of elm roots (left) fed with 1500 ppm of MBC-C^{14} (45×10^6 d.p.m.) at pH 3.2 for three days. Note the high degree of accumulation into roots. Before preparation of the autoradiograph, roots were thoroughly washed in water.

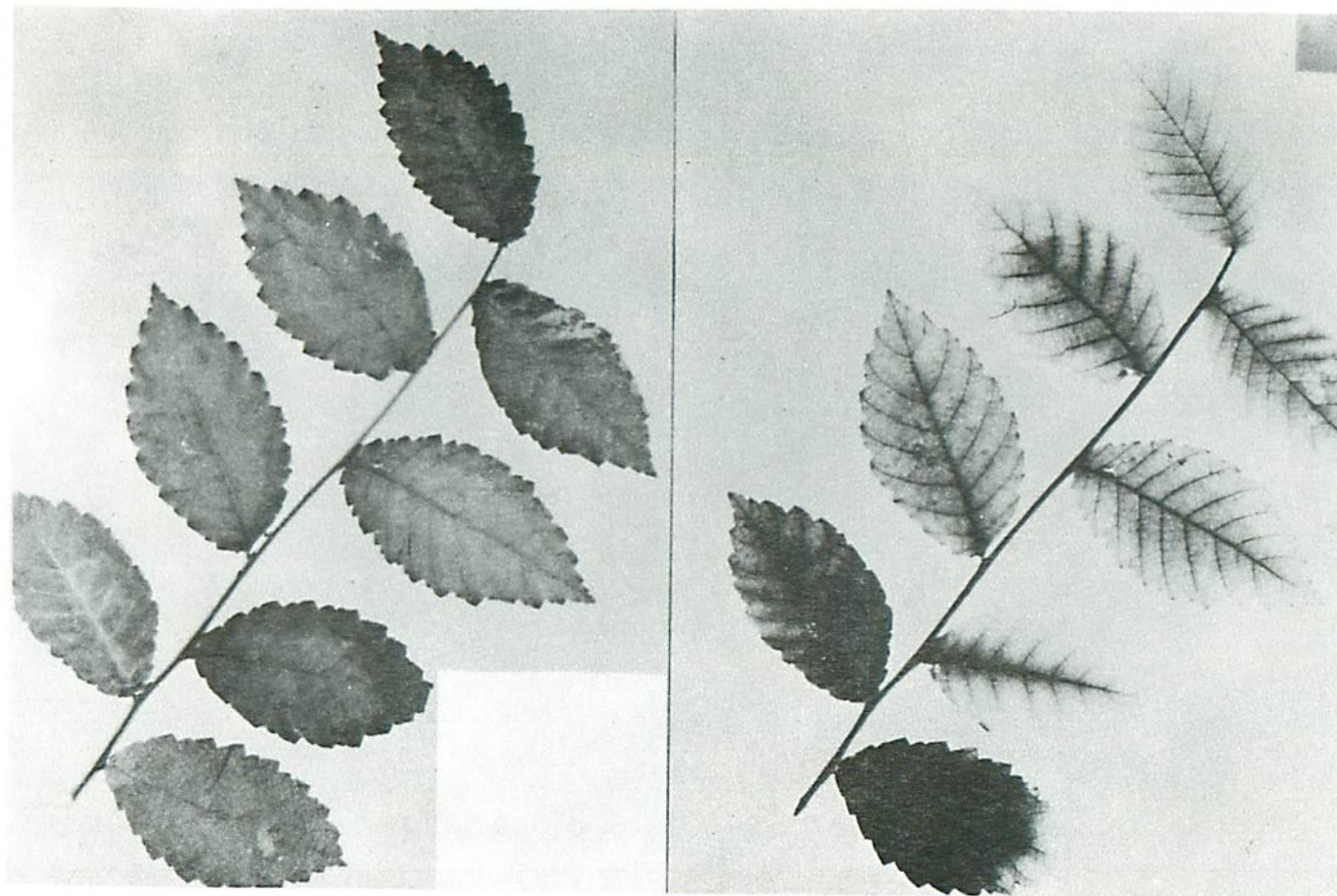


Figure 5. Autoradiograph (right) and photograph of elm shoot (left) whose roots were fed with 1500 ppm MBC- C^{14} (45×10^6 d.p.m.) at pH 3.2 for three days. Note the apoplastic type of movement into veins and margins of leaves. Older leaves and stem show dense labelling with radioactivity.

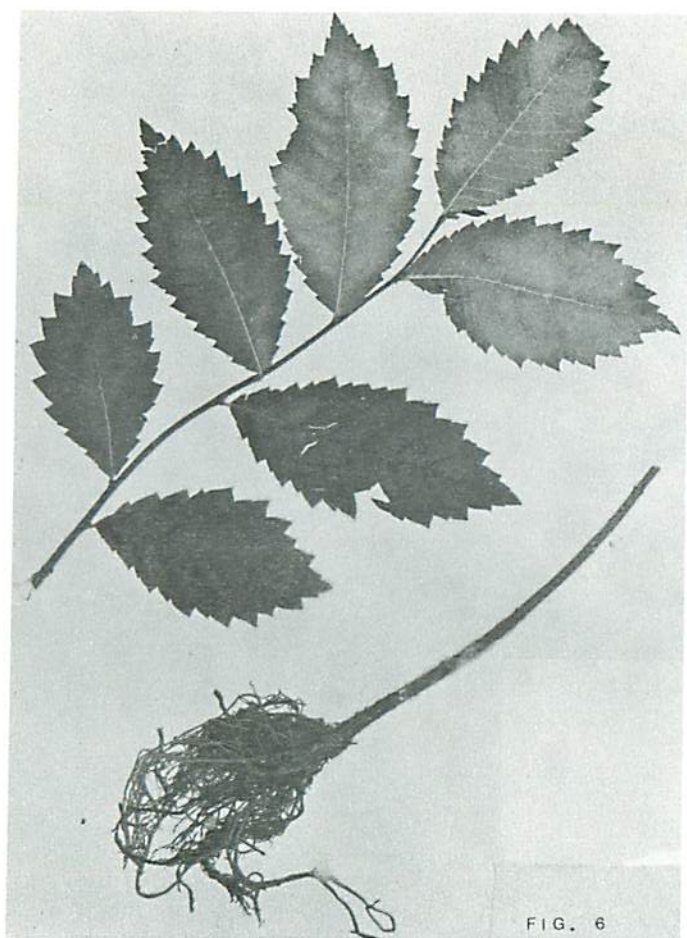


FIG. 6



Figure 6. Autoradiograph (right) and photograph of elm root and shoot (left). Roots were treated with 1500 ppm MBC-C¹⁴ (45×10^6 d.p.m.) at pH 9.2 for three days. They were then thoroughly washed in water before preparation of the autoradiographs. Note the slow migration of activity into stem.

TABLE I

Effect of pH on percentage distribution of radioactivity in entire elm seedling following root absorption of MBC-C¹⁴ for three days.
(Conc. 1500 ppm. Activity 45×10^6 d.p.m.)

Percentage of Applied Dosage

pH	Root	Stem	Leaf	Whole Plant
3.2	13.33	1.26	0.505	15.090
5.2	1.46	0.32	0.050	1.830
7.2	1.33	0.28	0.004	1.614
9.2	2.24	0.12	0.007	2.367

The observations that major part of the absorbed radioactivity was concentrated in the roots, raised the question of its site of localization. Therefore, to obtain some indication into which part of the cell the accumulation was taking place, the total activity was fractionated into water soluble and insoluble components.

Inspection of Table II reveals that the trend of distribution in these two fractions is similar to what has been described with whole organs but the gradient of accumulation from root to shoot is much more apparent here. The chloroform fractions, on the whole, possess higher counts than the corresponding water fractions and this could be partly attributable to the selective solubility of MBC in organic solvents (Clemons & Sisler 1971).

TABLE II

Distribution of radioactivity in chloroform (CHCl_3) and water (H_2O) fractions following extraction from root, stem and leaf of elm seedlings fed with MBC-C^{14} for three days. (Conc. 1500 ppm. Activity 45×10^6 d.p.m.)

DPM/gm Fresh Weight

pH	Root		Stem		Leaf	
	CHCl_3	H_2O	CHCl_3	H_2O	CHCl_3	H_2O
3.2	1,530,208	615,433	147,383	166,633	67,100	25,167
5.2	162,302	59,174	42,105	25,720	8,200	1,233
7.2	167,209	41,096	41,650	25,717	750	33
9.2	332,100	92,600	24,025	8,633	533	898

DISCUSSION

From the foregoing results it is apparent that pH has a strong influence on the absorption and translocation of MBC-C^{14} by the roots. There are several ways through which pH could exert such effects and some of the most probable modes of its action are discussed below:-

(1) Effect of pH on the solubility of MBC. It is known from the work of several investigators, that benomyl is more soluble in acidic than in alkaline media (Erwin et al 1971; Prasad 1971; McWain & Gregory 1971; Pitblado & Edgington 1972) and it is possible that the enhanced effect at pH 3.2 resulted simply by a greater solubility of MBC. That this may be so was indicated by the fluidity of the test solutions. During the course of the experiments for three days all MBC solutions remained clear and liquid at pH 3.2 while they started turning cloudy and foggy as soon as the pH was increased above 3.2, to the extent that considerable quantities of the pesticide precipitated out of solution at pH 5.2, 7.2 and 9.2. This would be expected if the chemical configuration of benomyl is critically examined (see Fig. 1, p. 4). For, acidity would tend to induce excessive protonation of the basic imidazole ring containing nitrogen moiety and thus cause greater solubility of the molecule. In this connection it is of some pertinence to note that Pitblado and Edgington (1972) have arrived at a similar conclusion in order to explain greater mobility of benomyl into soil treated with acidic surfactants. In fact, they went a step further to postulate that it is not only the excessive protonation that is responsible for solubility but also the formation of micelles by the surfactants that hold benomyl molecule in solution. It is possible that a similar mechanism operated in the present situation as well since all solutions of varying pH contained the same concentration of a surfactant (biofilm). It must be emphasized, however, that the role of micelle formation by sur-

factants and the consequent causation of solubility of pesticides is a controversial issue and unless direct evidence is obtained to substantiate the claim, the theory of Pitblado & Edgington (1972) should be treated with caution. Therefore it seems more possible that the acids reacted with MBC/benomyl to form salts (hydrochlorides, chlorides or lactates) which on a priori ground would be more water soluble than the parent molecule. This may explain why Erwin et al (1971) recorded higher systemic activity after spraying cotton foliage with benomyl solubilized in concentrated HCl than in water and it is likely that hydrochlorides, chlorides or other acid derivatives of the fungitoxicant are more potent simply because of higher solubility.

(ii) Effect of pH on root permeability. The effect of pH on biological membranes is well documented (Simon & Beevers 1952) and it can be safely advanced that lower pH accelerated absorption of MBC probably by influencing the permeability of root membranes. Because it is a well established fact that the uptake and entry of many inorganic and organic pesticide molecules in plant tissues is regulated by subtle changes in permeability of root membranes (Bukovac & Norris 1972) it follows as a corollary that entry of benomyl into elm roots would not be an exception to this generalization.

(iii) Effect of pH on dissociation of MBC. Another fundamental property of hydrogen ion concentration is to affect the dissociation of various weak acid and bases into ions and molecules, and thus evoke a preferential penetration of undissociated molecules into biological systems (Simon and Beevers 1952). However, it seemed

unlikely that MBC salts would be unduly subject to dissociation at lower pH because MBC or benomyl is, intrinsically, a non-electrolyte type of pesticide.

(iv) Effect of pH on differential rate of metabolism of MBC.

Lastly, there existed a possibility that varying pH levels influenced the decomposition of MBC differentially in such a manner that the metabolites thus formed had greater penetrability at lower pH. But according to Peterson & Edgington (1971) MBC is relatively stable to degradation in bean and pea plants and only a fraction (1-8%) is metabolized after 4 days (Siegel & Zabbia 1972). However without chromatographic identification of the nature of MBC-C¹⁴ from elm roots it would be premature to draw conclusions. Therefore, it is reasonable to conclude that pH operates principally by influencing the solubility of MBC.

Turning to the pattern and site of accumulation, it is apparent from Tables I & II and Figs. 4 & 5, that most of the radioactivity is concentrated into roots. Roots actually accumulate the pesticide (or a product thereof) to the tune of ca. 7-fold at pH 3.2 (see Fig. 3(a)) above the external concentration (1500 ppm). Similarly, the stem contains far more at lower than higher pH levels. This extent of concentration into root and stem is of considerable significance. Transpirational forces and apoplastic movement alone cannot account for this accumulation into the root as has been demonstrated by several workers (Peterson & Edgington 1971; Hock, Roberts & Schriber 1971). It appears metabolic processes (active uptake) are at play to transport concentrations against gradients and there may be some metabolism of MBC at these sites. Therefore,

some translocation into the symplasm seems to be involved (Prasad 1972). Secondly, these data point out that such high concentration of MBC would surely suppress or eradicate the pathogen (DED) from these sites of pathogenesis. Even if metabolic degradation takes place only to the extent of 8% as reported by Siegel & Zabbia (1972) in roots and shoots, there would still be substantial concentration of MBC left over to inhibit the proliferation of the disease. In this connection it should be recalled that vascular systems (xylem and cambial layers) are the sites of pathogenesis in stems and concentrations higher than 50 ppm are usually needed to arrest the pathogen advancement (Kondo 1972). That some movement takes place through symplasm can also be deduced from Table II where activity was fractionated into chloroform and water fractions. Considerable quantities resided in the chloroform portions and this suggests that MBC is partitioned into lipids, fats and pigments of the cytoplasmic components of the cell.

It is somewhat surprising that accumulation into the leaf is minimal. Inspection of Fig. 3(c) and Table III (page 22) reveal that, save for pH 3.2, MBC is not even present in effective concentrations in foliage to be able to inhibit the pathogen, whereas the amount concentrating into the stem and root is highly significant (Table III). At any rate, undue accumulation into leaves is undesirable because they are not the primary sites of pathogenesis and thus the pesticide may not be of value there. Then again, since elm leaves are deciduous, undue stockpiling into foliar components may lead to unnecessary contamination of the environment, when such leaves fall to the ground in the autumn.

TABLE III

Accumulation of MBC-C¹⁴ in root, stem and leaf of elm in terms of internal concentrations based on appropriate water content of the respective tissues. Accumulation Ratio (A.R.) is a ratio between internal and external concentration and signifies net accumulation rate. (External conc. 1500 ppm. Activity 45×10^6 d.p.m.)

Internal Concentration (ppm)					Accumulation Ratio
pH	Root	Stem	Leaf	Whole Plant	
3.2	10,392	2,266	394	13,052	8.70
5.2	1,071	489	40	1,601	1.07
7.2	1,006	488	3	1,497	1.00
9.2	2,054	236	6	2,296	1.53

The preferential accumulation of MBC into chloroform fractions does not necessarily carry the implication that very little fungitoxicant is available for water transport (xylem) systems. Actually it is the transpiration stream wherein the pathogen exerts its maximum effect. Therefore it is important that substantial amount of MBC must travel and persist in xylem to arrest further development of the disease or to eradicate it altogether. Assuming MBC is uniformly distributed in the water of the plant, the effective concentrations can be calculated

from Table II. Thus the amount of MBC in water fractions of the stems at pH 3.2 and 5.2, 7.2 and 9.2 are 1202, 187, 186 and 62 ppm, respectively. Clearly, these levels are, by and large, above the critical concentration (50-100 ppm) that are normally required to inhibit the DED pathogen. Further cogent evidence is provided by Table III where the internal concentrations of the whole plant is presented. It is apparent that MBC is distributed in more than sufficient concentration to offer at least prophylactic, if not therapeutic, protection in the event these plants are infected with the disease.

Finally, the effects of pH on growth and development of roots and seedlings need some discussion. Judging from the changes in fresh weight over a period of three days (see Appendix III), apparently there is no deleterious effect of pH on the roots and this was confirmed by the visual observations that all experimental plants looked green and healthy. To ascertain the long term effects of pH, a similar experiment was laid out for investigating uptake and accumulation at pH 3.2, 7.2 and 9.2 for nine days. Results indicated that the pattern of translocation, even after nine days, remained similar to the three-day-experiment, except that more activity concentrated into the foliar parts. Roots still contained the maximum amount and there were no adverse effects of pH on the growth of plants. This led to a conclusion that elms can tolerate wide fluctuations in soil conditions ranging from low land and swampy (acidic) to upland and salty (alkaline) habitats of the urban

environments. Perhaps this adaptability to varying site condition has been one of the most important factor in distribution and survival of elms throughout North America. Evidence that pH influences movement and persistence of benomyl in field soils is forthcoming from the experiments of Pitblado and Edgington (1972). These workers concluded that the mobility of the fungicide in field soil is dependent upon both water solubility caused by acidification and by surfactant actions of spray additives.

PRACTICAL APPLICATION

The results presented above suggest several applications:--

- (1) Benomyl would be more effective in water soluble than in insoluble form and this is a function of pH of the formulations. Acidic formulations would be more water soluble and hence are likely to be more potent in combatting the Dutch Elm Disease than basic formulations.
- (2) Probably water soluble salts of benomyl can be manufactured by reacting it with weak (lactic) or strong (hydrochloric) acids.
- (3) Applications of benomyl in somewhat acidic soils (swampy and low land sites) are likely to produce better results than those in alkaline soils.
- (4) Relatively low dosages of benomyl would be required for effectiveness on acidic sites.
- (5) Surfactants (biofilm) seem to aid in penetration and translocation of benomyl and should be included in formulations.
- (6) Further dissemination of the disease through root grafts may be halted by applications of benomyl to root or soil. This is because benomyl concentrates in the roots.

SUMMARY AND CONCLUSIONS

Controlled laboratory experiments employing elm seedlings, grown in nutrient solution were carried out to test the effects of pH on penetration and translocation and hence systemic action of radioactive benomyl (MBC-C¹⁴) by roots. Results demonstrated that benomyl attains maximum systemic action (absorption and translocation) at a pH 3.2 and lesser and lesser as the pH is raised beyond this level. At pH 9.2 there was no significant translocation and accumulation into leaves. The principal site of accumulation is root where the concentration level approaches several fold after three days of continuous immersion in the pesticide solution (conc. 1500 ppm). Stem and leaves contained less fungicide than the roots. Much of the movement seems to occur in xylem (apoplasm) and only a slight amount seems to translocate into the phloem (symplasm). The acidic pH cause greater solubility of benomyl probably by protonation and micelle (salt) formation.

It is concluded that acidic formulations would be more effective for combatting Dutch Elm Disease because they penetrate and accumulate rapidly at the site of pathogenesis and thereby lower the dosages to be applied. It is also suggested that benomyl might afford a higher degree of protection to affected trees growing in acidic soils. Water solubility of benomyl is apparently an important factor in the transport, accumulation and systemicity.

ACKNOWLEDGEMENTS

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A P P E N D I X - I

DISTRIBUTION OF RADIOACTIVITY IN ELM SEEDLINGS TREATED WITH MBC-C¹⁴

Upper Figures - dpm/Plant

Lower Figures - Percent of Applied Dosage/Plant

pH	ROOT			STEM			LEAF			Plant
	CHCl ₃	H ₂ O	TOTAL	CHCl ₃	H ₂ O	TOTAL	CHCl ₃	H ₂ O	TOTAL	
3.2	4,330,489	1,741,675	6,072,164	269,711	304,938	574,649	165,737	62,162	227,899	6,874,712
	9.51	3.82	13.33	0.59	0.67	1.26	0.36	0.14	0.50	15.09
5.2	486,906	177,522	664,428	91,630	56,577	148,207	19,926	2,996	22,922	835,557
	1.07	0.39	1.46	0.20	0.12	0.32	0.04	0.01	0.05	
7.2	484,906	119,178	604,084	79,135	48,862	127,997	1,912	8	1,920	734,001
	1.06	0.27	1.33	0.17	0.11	0.28	0.004	0	0.004	1.61
9.2	797,040	222,240	1,019,280	40,842	14,676	55,518	1,146	1,935	3,081	1,077,879
	1.75	0.49	2.24	0.09	0.03	0.12	0.003	0.004	0.007	2.37

Dosage Applied - 100 ml MBC-C¹⁴ Solution = 45 x 10⁶ dpm

Conc. - 1500 ppm

Period of Uptake - 3 days

APPENDIX - I I

DISTRIBUTION OF RADIOACTIVITY IN ELM SEEDLINGS TREATED WITH MBC-C¹⁴

Micrograms/Plant

pH	ROOT			STEM			LEAF			Plant
	CHCl ₃	H ₂ O	TOTAL	CHCl ₃	H ₂ O	TOTAL	CHCl ₃	H ₂ O	TOTAL	
3.2	15,711	6,319	22,030	979	1,106	2,085	601	226	1,827	24,942
5.2	1,766	644	2,410	232	206	538	72	11	83	3,031
7.2	1,759	433	2,192	287	177	464	7	0	7	2,663
9.2	2,892	806	3,698	148	53	201	4	7	11	3,910

Dosage Applied - MBC-C¹⁴ μg/g Conversion Factor = 0.003628 for dpm/g

Conc. - 1500 ppm

Period of Absorption - 3 days

A P P E N D I X - I I I

DISTRIBUTION OF AVERAGE FRESH WEIGHT IN ELM SEEDLINGS IN GMS

FW - Fresh Weight

Water Content = FW-Dry Wt.

pH	ROOT		STEM		LEAF		PLANT	
	F.W.	75% H ₂ O	F.W.	50% H ₂ O	F.W.	85% H ₂ O	F.W.	70% H ₂ O
3.2	2.83	2.12	1.83	0.92	2.47	2.10	7.13	4.99
5.2	3.00	2.25	2.20	1.10	2.43	2.07	7.63	5.34
7.2	2.90	2.18	1.90	0.95	2.55	2.17	7.35	5.14
9.2	2.40	1.80	1.70	0.85	2.15	1.83	6.25	4.38

APPENDIX - I V

DISTRIBUTION OF RADIOACTIVITY IN ELM SEEDLINGS TREATED WITH MBC-C¹⁴

Upper Figures - dpm/gms Fresh Weight

Lower Figures - Percent of Applied Dosage/gm Fresh Weight

pH	ROOT			STEM			LEAF			Plant/g
	CHCl ₃	H ₂ O	TOTAL	CHCl ₃	H ₂ O	TOTAL	CHCl ₃	H ₂ O	TOTAL	
3.2	1,530,208 3.36	615,433 1.35	2,145,641 4.71	147,383 0.32	166,633 0.37	314,016 0.69	67,100 0.15	25,167 0.05	92,267 0.20	850,641 1.87
5.2	162,302 0.36	59,174 0.13	221,476 0.49	42,105 0.09	25,720 0.06	67,825 0.15	8,200 0.02	1,233 0.002	9,433 0.02	99,578 0.22
7.2	167,209 0.37	41,096 0.09	208,305 0.46	41,650 0.09	25,717 0.06	67,367 0.15	750 0.002	3 0	753 0.002	92,142 0.20
9.2	332,100 0.73	92,600 0.20	424,700 0.93	24,025 0.05	8,633 0.02	32,658 0.07	533 0.001	900 0.002	1,433 0.003	152,930 0.33

Dosage Applied - 100 ml of MBC-C¹⁴ Solution = 45×10^6 dpm

Conc. - 1500 ppm

Period of Uptake - 3 days

A P P E N D I X - V

DISTRIBUTION OF RADIOACTIVITY IN ELM SEEDLINGS TREATED WITH MBC-C¹⁴

ppm/Plant

pH	ROOT			STEM			LEAF			Plant
	CHCl ₃	H ₂ O	TOTAL	CHCl ₃	H ₂ O	TOTAL	CHCl ₃	H ₂ O	TOTAL	
3.2	7,411	2,981	10,392	1,064	1,202	2,266	286	108	394	3,104
5.2	785	286	1,071	302	187	489	35	5	40	315
7.2	807	199	1,006	302	186	488	3	0	3	344
9.2	1,607	447	2,054	174	62	236	2	4	6	491

Dosage Applied - MBC-C¹⁴ µg/g Conversion Factor = 0.003628 for dpm/g

Conc. - 1500 ppm

Period of Absorption - 3 days