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

II. SOME ENVIRONMENTAL FACTORS AFFECTING UPTAKE AND DISTRIBUTION BY ROOTS

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TABLE OF CONTENTS

INTRODUCTION

The efficacy of systemic pesticides, in general, is greatly modified by the environmental conditions under which they are applied. For instance, drying out of spray droplets on foliar surfaces is a common phenomenon associated with the ambient relative humidity and many investigators (Crafts and Crisp 1971, Franke 1971, Hull 1970, Prasad, Foy and Crafts 1967) have demonstrated that pesticides, by and large, penetrate faster under conditions of early morning or on calm days when the relative humidity, wind velocity, light and temperature conditions are most favourable for uptake. For this reason alone, many pesticidal formulations contain various additives (oils, humectants, spreader-stickers, etc.) that are designed to enhance effectiveness by minimizing the loss of residues from evaporation and volatilization (Hull 1970, Crafts and Crisp 1971).

The weather conditions equally well affect the systemicity of pesticides applied through soil or roots. In a study of root injection of water soluble materials into mature elm trees, Kondo (1972) observed that climatic factors play an important role in directing the movement of methyl-2-benzimidazole carbamic acid-phosphate (MBC-PO4). He noted that sunny and windy days favoured upward movement while cold and rainy days suppressed the translocation. Similarly in an attempt to control the Dutch elm disease (DED) with benomyl (Benlate^{R}), several arborists and researchers (Biehn and Diamond 1971, Smalley 1971, Hock & Schreiber 1972) have reported differential action of fungicides in elm trees following soil applications from different geographical locations across the U.S.A. and Canada. The purpose of this study is to determine the relationship of three prominent climatic

factors (light intensity, temperature and relative humidity) on the movement of benomyl in elm trees. In this connection, benomyl has recently been found to control the DED caused by Ceratocystis ulmi (Buism) Moreau in elms and this is partly described in the first report of this series (Prasad 1972). Also since DED is a vascular parasite and produces pathogenesis in the shoot of the trees, it is of some significance to know how the translocation and accumulation pattern of a fungitoxicant is influenced by the environmental factors. If the protection of the tree results from a deposition of effective dosages of benomyl at the site of pathogenesis and if by chance environmental factors interfere with the accumulatory process, then a knowledge of their mechanisms becomes essential to understanding of disease prevention and therapy.

MATERIALS AND METHODS

i) Culture of Plants

The method of cultivation of elm seedlings was essentially similar to that described in the first report (Prasad 1972). Briefly, four month old seedlings were raised hydroponically in the greenhouse under constant conditions of light intensity (3000 ± 500 f.c.; 16 hr. photoperiod) temperature (76 ± 2°F) and relative humidity (50 ± 10%). The seedling trees were cultured in a half strength Hoagland solution adjusted to a pH 6.5 and these solutions were changed regularly to replenish nutrients and to minimize drifts in pH. Finally the mason jars containing the Hoagland solution and seedlings were covered with a tinfoil to prevent growth of algae in the culture solution. The

plants grew normally under these conditions and produced profuse roots and shoot. As before, radioactive fungicide (MBC-C^{14}) was then fed to such plants through the roots by mixing with the Hoagland solution. The concentration of MBC was 375 ppm and the radioactivity level to 30 x 10^6 d.p.m.

ii) Environmental Conditions

All experiments involving varying regimes of light intensity, temperature and relative humidity were conducted in controlled growth cabinets and while studying the influence of one physical factor (e.g. light intensity), only this factor was altered and the rest were held constant during the entire experimental period. Briefly, before the start of an experiment, all greenhouse plants were grown in the controlled growth cabinets at 76 ± 2°F, 50-70% relative humidity and 3000 ± 200 f.c. for 2 weeks so as to acclimatize them to this condition. Then after the addition of appropriate dosage (375 ppm; 30 x 10^6 d.p.m.) of the pesticide, batches of plants were transferred to varying levels of the environmental conditions and allowed to absorb and translocate for 3 days. There were two sets of light intensity (0 and 3000 f.c.), two levels of temperature (68 and 88°F) and two extremes of relative humidity (25-35% and 85-95%). To minimize variation, four replicates (1 replicate = 1 seedling) were used per treatment. After the absorption period, the plants were harvested, sectioned into root, shoot and leaf and frozen immediately for subsequent determination of radioactivity. In few cases, some plants were retained at each level of the environment for microscopic examination of stomatal movement.

iii) Radiometric Determination

This was carried out essentially in the same manner as described earlier (Prasad 1972). For autoradiography, duplicate samples of an experimental materialwere first freeze-dried and then exposed to non-screen X-ray film for 2-3 weeks. The plates were then developed and printed according to a standard procedure (Crafts and Yamaguchi 1962).

For quantitative assay of radioactivity, duplicate samples were extracted with both hot ethanol and water in a graded series as described earlier (Prasad 1972) and then the total activity was fractionated into chloroform and water soluble portions. Radioactivity was monitored by a Picker Nuclear Scintillation Spectrometer after making necessary corrections for quenching. For water soluble fractions a standard cocktail containing napthalene, POPOP, PPO, methanol, ethylene glycol and dioxane was used while for chloroform fractions a cocktail mixture containing toluene was employed. Statistical analyses were performed on some samples and for this, the method of least square (Snedecor 1956) was usually used.

iv) Determination of Stomatal Behaviour

For studying the distribution and behaviour of stomata under each set of environmental condition, attempts were made to use the silicone-rubber technique (Sampson 1961) for preparation of imprints but because elm leaves are extremely pubescent, the plastic material did not yield clear replicas of the epidermis. Therefore leaves were examined under a microscope (X250) for the width of the stomatal aperture and the status of opening and closing. Based on this, stomata were classified as fully open, partially open and fully

closed under any set of environmental condition. Altogether, one hundred stomata drawn from four replicates of elm leaves were counted and out of this, the proportions of fully open, partially open and fully closed status recorded on a percentage basis.

RESULTS

For the sake of convenience, the findings are arranged in three categories and each factor is examined in detail:

i) Effect of Light Intensity on Root Absorption and Translocation

The data obtained from the light and dark experiments are presented in Tables I and II and indicate that light has a positive effect on the uptake and transport of MBC. Accordingly there was considerable reduction in the rate of penetration and subsequent translocation under conditions of complete darkness (zero light) and leaves manifested this response to the greatest extent. Apparently there was no significant difference in the manner of translocation under light and no light conditions because MBC moved to stem and leaf without restriction. Thus the differences in the action of light were rather of a quantitative nature. (vide Figs 1 & 4). The chloroform and water fraction (Table II) exhibited a similar pattern of distribution. It is significant to note that chloroform fractions concentrated higher radioactivity than the water fractions. On a percentage basis the accumulation was greatest in the root and least in the leaves.

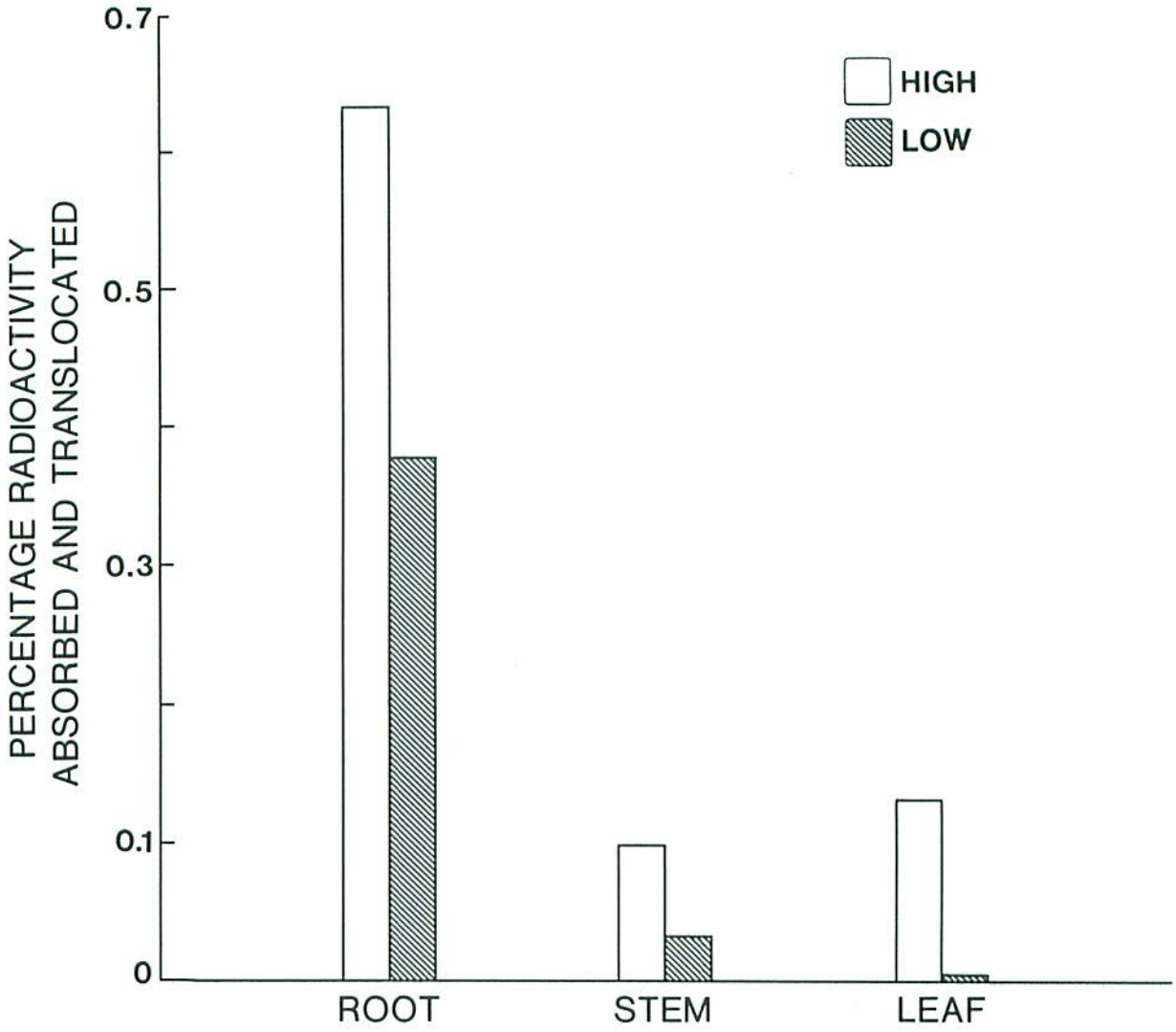

Fig. 1. Influence of light intensity (0 and 3000 foot candles) on uptake and distribution of MBC-C^{14} in elm seedlings following root application for 3 days. Greater absorption and translocation takes place under high light intensity.

TABLE I

Effects of light and dark on absorption and transport of MBC-C^{14} by elm seedlings roots. *

Contents - µg MBC/gm Fresh Weight

Plant Part	Light	Percent	Dark	Percent
Root	117.00	73.63	67.49	90.64
Stem	18.20	11.45	6.24	8.38
Leaf	23.71	14.92	0.73	0.98

* Concn. 375 ppm; activity 30 x 10^6 d.p.m.; uptake period 3 days.

TABLE II

Distribution of radioactivity in chloroform ($CHCl_3$) and water (H_2O) fractions after roots were fed with 375 ppm MBC-C^{14} in light and dark and extracted with hot ethanol and water. *

Contents - µg/gm

Plant Part	Light		Dark	
	$CHCl_3$	H_2O	$CHCl_3$	H_2O
Root	82.00	35.00	45.97	21.52
Stem	13.42	4.78	3.54	2.70
Leaf	18.10	5.61	0.52	0.21

* Activity applied 30 x 10^6 d.p.m.; uptake period 3 days.

ii) Effect of Temperature on Root Absorption and Translocation

There is a wide fluctuation in temperature during the growing period of elms as well as during the spraying operations with pesticides. Temperature affects both the growth as well as the effectiveness of any chemical treatment that is superimposed on the tree because the rate of chemical reactions is controlled by the thermal energy (Glasstone 1946). Therefore, influence of two levels of temperature ($68^{o}F$ and $88^{o}F$) were tested. Tables III and IV show the distribution of activity into various parts of the plant at these temperatures and Figs. 2, 5 and 6 show the translocation pattern into root, shoot and leaves.

TABLE III

Effects of temperature on distribution of activity in entire seedlings after feeding the roots with MBC-C^{14} 375 ppm. *

Contents - µg/gm

Plant part	$68^{o}F$	Percent	$88^{o}F$	Percent
Root	63.75	90.41	174.36	86.53
Stem	5.62	7.97	23.30	11.56
Leaf	1.14	1.62	3.85	1.91

* Activity applied, 30×10^{6} d.p.m.; Period of uptake 3 days.

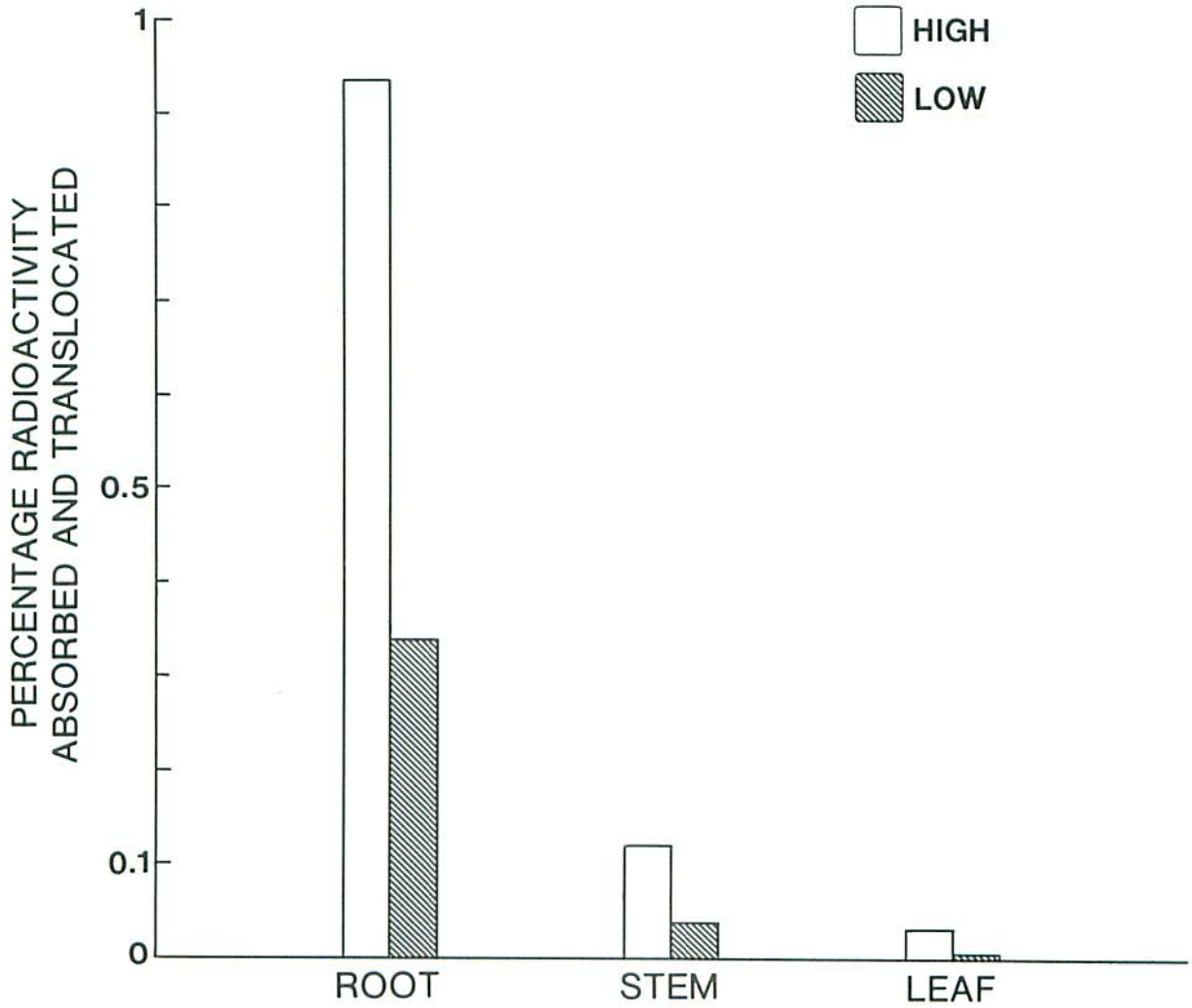

Fig. 2. Influence of temperature (68, 88°F) on uptake and distribution of MBC-C^{14} in elm seedlings following root absorption for 3 days. Note greater absorption and translocation at high temperature.

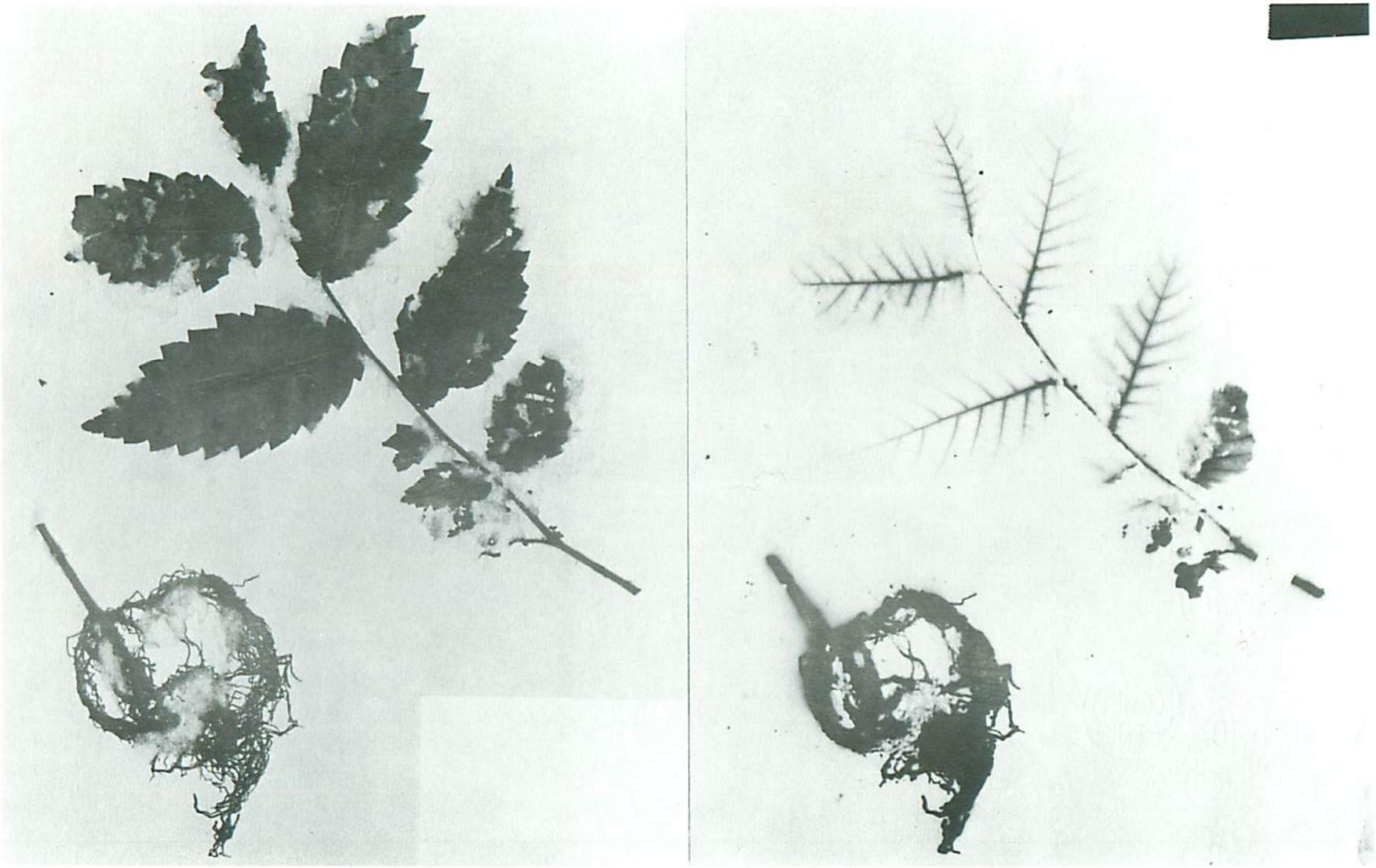

Fig. 5. Distribution pattern of MBC-C^{14} in elm seedlings following root uptake at low temperature (68°F) for 3 days. Photograph of seedling on left and autoradiograph on right. Note slower movement into veins, margin and laminae of leaves.

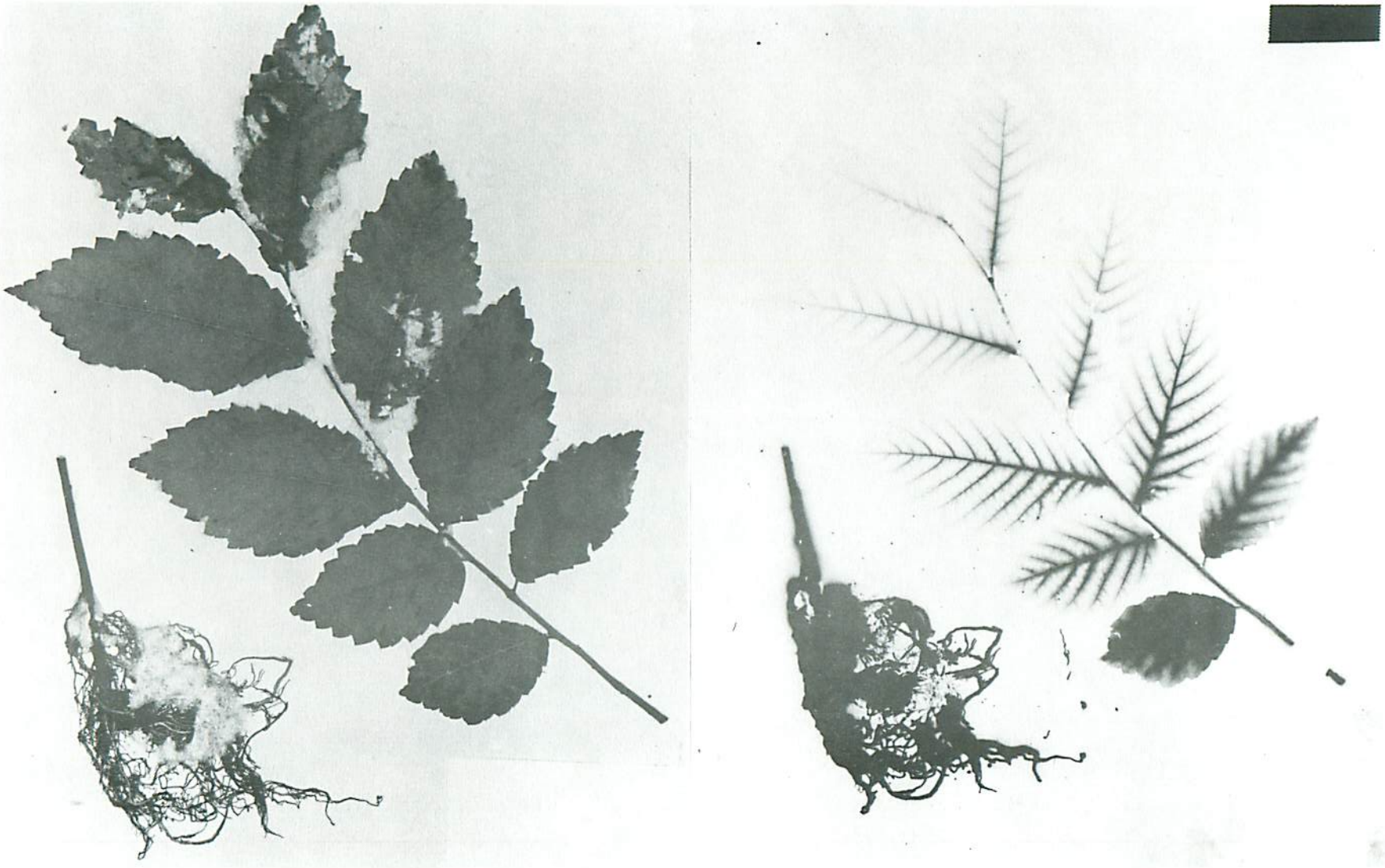

Fig. 6. Distribution pattern of MBC-C^{14} in elm seedlings following root uptake at high temperature (88°F) for 3 days. Note the greater movement and localization of activity in roots, old leaves, veins and stem.

TABLE IV

Distribution of radioactivity in chloroform ($CHCl_3$) and water (H_2O) fraction after feeding roots with 375 ppm MBC-C^{14}. *

Content - µg/g

Plant part	68°F		88°F	
	$CHCl_3$	H_2O	$CHCl_3$	H_2O
Root	35.49	28.26	143.05	31.31
Stem	3.50	2.12	19.35	3.95
Leaf	0.87	0.27	3.43	0.42

* Activity applied 30 x 10^6 d.p.m. Uptake period 3 days.

Evidently a rise in temperature by 20°F more than doubles the rate of absorption by roots and its further translocation into the shoot. This suggested involvement of some metabolic factors that mediated these processes and therefore temperature-coefficients (Q_{10}) were calculated from the Vant's Hoff (Glasstone 1946) equation in the following manner:

$$2.303 \log \frac{K_2}{K_1} = \frac{E}{R} \left[\frac{1}{T_1} - \frac{1}{T_2} \right]$$

where K = rate of absorption or translocation per gm of fresh weight per day, E = energy of activation, R = Arrhenius constant, and T = absolute temperature.

The values so deduced are presented in Table V and inspection confirms that Q_{10} are more than two.

TABLE V

Temperature coefficient (Q_{10}) values of MBC absorption and translocation by roots.

Plant part	Q_{10}	Temperature Range
Root	2.24	$68^{o}F$ - $88^{o}F$
Stem	2.61	
Leaf	2.60	

iii) Effect of Relative Humidity (R.H.) on Root Absorption and Translocation

The movement of solutes and organic materials has been found to be influenced by the transpiration stream of plants (Crafts 1961) and this, in turn, is directly affected by the relative humidity of the ambient environment. To test whether absorption and redistribution of MBC is controlled by humidity, batches of plants were exposed to low and high relative humidity for three days. Since roots were continuously immersed in nutrient solutions containing the pesticide, any effect of humidity that would accrue would operate on the shoot only. Therefore, it is not surprising from Table VI that this is actually so. For example, the transpiring organs viz leaf (see Fig. 3) contain almost double the amount of fungitoxicant at low relative humidity. As expected the effect of humidity on stem and root is less clearly defined.

The trends in distribution of activity in chloroform and water fractions remain similar to Table II. In general, the chloroform

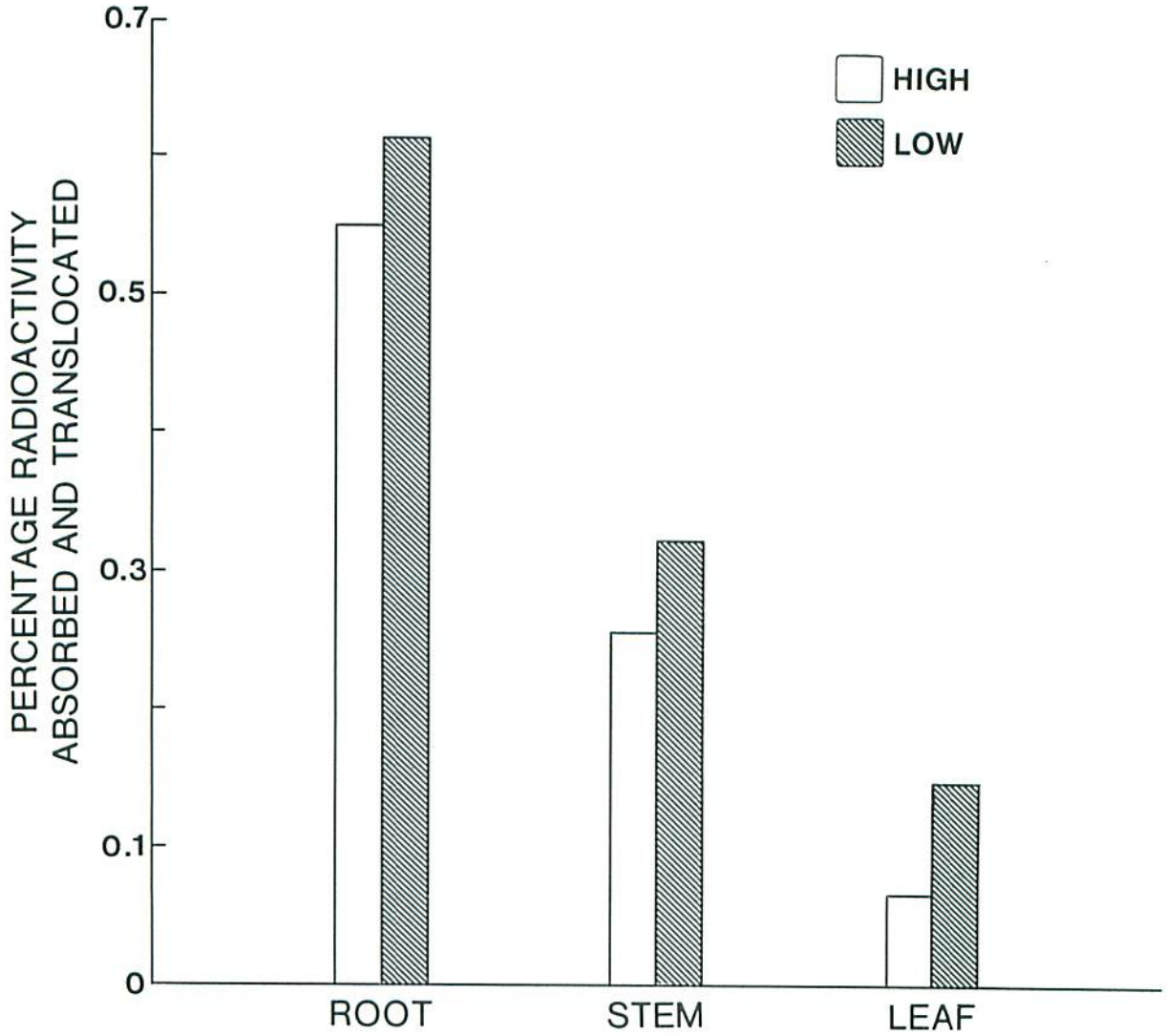

Fig. 3. Influence of relative humidity (20 and 90%) on uptake and distribution of MBC-C^{14} in elm seedlings following root absorption for 3 days. Apparently low humidity favours enhanced absorption and translocation.

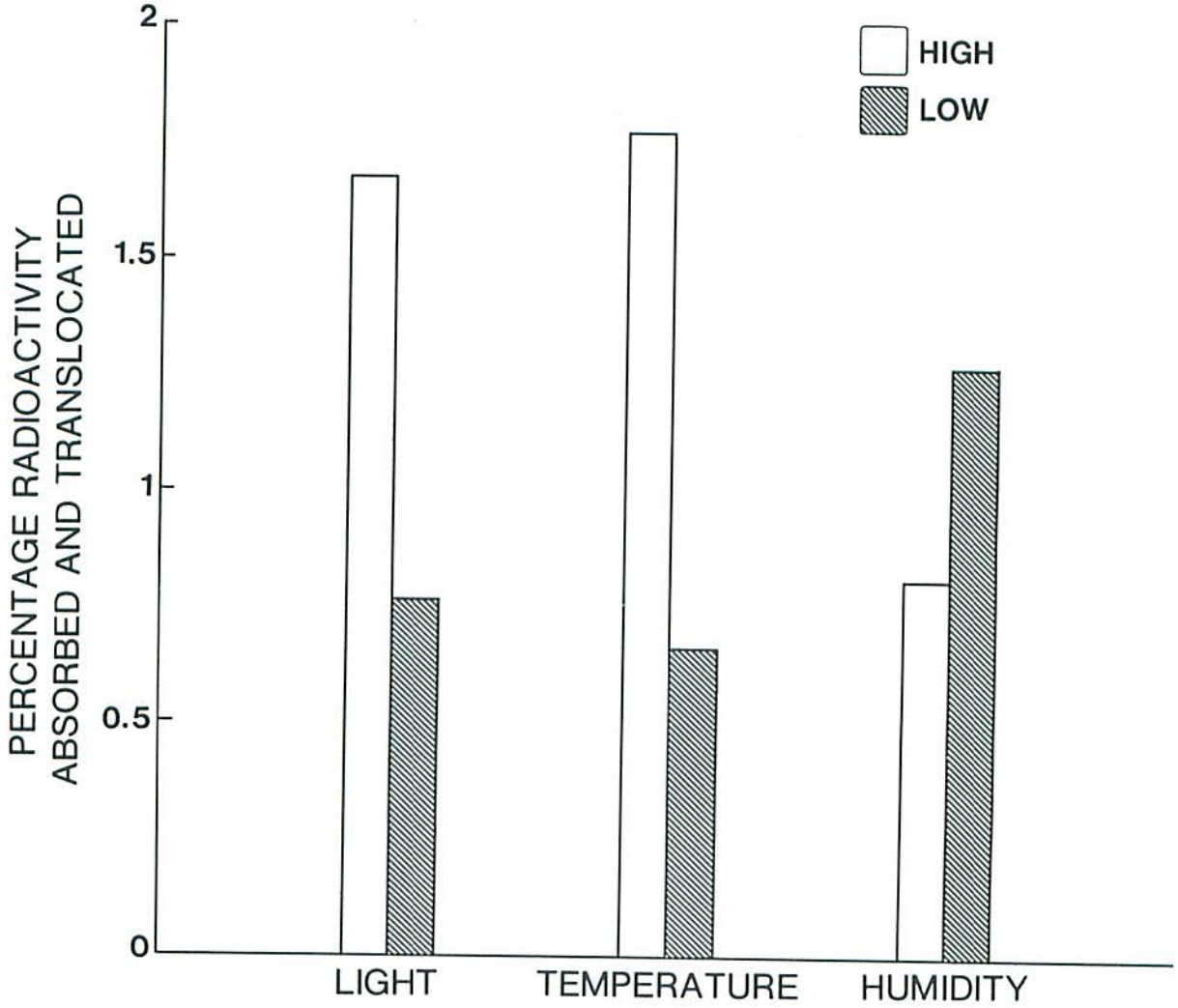

Fig. 4. Comparative effects of light, temperature and relative humidity on absorption and translocation of MBC-C^{14} by elm roots after a 3 day uptake. Maximum effects are brought about first by temperature, then by light intensity and then by relative humidity.

fractions contain higher amounts of MBC than the corresponding water fractions (see Table VII).

TABLE VI

Influence of relative humidity on uptake and distribution of MBC-C^{14} following root absorption for 3 days. *

Contents - µg/gm

Plant part	Low Humidity	Percent	High Humidity	Percent
Root	113.30	57.18	103.06	63.57
Stem	58.93	29.74	47.32	29.18
Leaf	25.90	13.08	11.75	7.25

* Concn. 375 ppm; activity 30 x 10^6 d.p.m.

TABLE VII

Effect of relative humidity on distribution of radioactivity in water and chloroform fractions.

Contents - µg/gm

Plant part	Low R.H.		High R.H.	
	$CHCl_3$	H_2O	$CHCl_3$	H_2O
Root	84.81	28.49	83.93	19.13
Stem	47.72	11.21	37.83	9.49
Leaf	20.28	5.62	9.05	2.70

iv) Effects of Environmental Factors on Stomatal Behaviour

The degree of stomatal opening and closing on the abaxial surface of elm leaves was examined under each set of environmental conditions and the data are presented in Table VIII.

TABLE VIII

Relationship between the degree of opening of elm stomata and varying levels of environmental factors.

Environmental Conditions	Degree of Opening (Percent)		
	fully open	partially open	fully closed
Highlight 3000 f.c., 76°F, 60% R.H.	84	4	12
Zero light 0 f.c., 76°F, 60% R.H.	2	3	95
High temp 88°F, 3000 f.c., 60% R.H.	65	15	20
Low temp 68°F, 3000 f.c., 60% R.H.	5	10	85
High R.H. 90%, 3000 f.c., 76°F	75	7	18
Low R.H. 20%, 3000 f.c., 76°F	4	24	72

DISCUSSION

The results clearly indicate that all three environmental factors (light, temperature and relative humidity) influence the rate of uptake and transport of the MBC into elm seedlings. In discussing the role of these factors it is important to distinguish their effects on morphological features from the physiological effects. For example, as far as light intensity is concerned, it is bound to exert control

on stomatal apparatus and the photosynthetic processes. The reduction in absorption and translocation at zero light intensity is a clear indication of reduced transpiration rate resulting probably from closed stomatal condition. On the other hand photosynthetic processes would also cease or slow down in zero light and this might restrict translocation of photosynthate and accompanying MBC or a product thereof. However, since much of the activity seems to move in the xylem (see Fig. 6), it seems unlikely that photosynthesis has been governing the rate of movement of MBC into elm seedlings. Therefore, it seems more plausible that retardations in uptake are brought about by a lack of vigorous transpiration via shutting off stomatal apertures. Further cogent evidence of this claim can be produced from the foliar contents of MBC under light and dark in Table I and from stomatal behaviour in Table VIII. Most stomata were closed on the abaxial leaf surface under darkness, the transpiration rate declined and consequently the accumulation into the leaf also diminished. These findings are in accordance with those of other workers (Peterson & Edgington 1972, Hock, Roberts & Schreiber 1972 and Prasad 1972) that in many plant species benomyl and MBC move apoplastically.

Turning to effects of temperature, the rate of uptake and translocation, as expected, were doubled when the Q_{10} values (see Table V) are calculated. This is not surprising since many biological and chemical reactions are controlled by temperature and it is possible that the transport and metabolism of benomyl by elm roots is mediated by some metabolic (enzymic) processes. However, it would be interesting to know whether temperature caused any decomposition of the pesticide in the culture solution or in the tissues. To this end, Kilgore & White

(1970) carried out controlled experiments on the rate of degradation of pure benomyl solutions and found temperature to be a cardinal factor in influencing the hydrolysis of benomyl to MBC, butylamine, carbon dioxide and water. MBC is also subject to degradation in pea plants at room temperatures but the rate is very slow: only 8% decomposes in 4 days (Siegel and Zabbia 1972). Finally Stumpf & Nieland (1965) pointed out that too much emphasis must not be placed on the Q_{10} values since some physical reactions such as adsorption and diffusion, at times exhibit unduly large values and as such the conclusions drawn could be misleading. They stressed the use of other parameters (metabolic inhibitor studies) as well to differentiate between physical and metabolic components of absorption. That there was an effect of temperature on the transpiration rate cannot be completely ruled out. Stomatal behaviour in Table VIII confirms that opening and closing of stomata is indeed regulated by temperature.

The manner by which relative humidity exerts its effect on pesticide absorption is not clearly understood. According to Prasad, Foy & Crafts (1967) and Crafts & Crisp (1971) relative humidity operates largely by regulating the stomatal movements on leaves and this, in turn, influences the transpiration stream. However, inspection of Table VIII revealsto the contrary and therefore the stomatal hypothesis seems untenable. It could be that cuticular and lenticular transpiration are more significant in elms under conditions of low humidity than under other situations. On the other hand the distribution of absorbed material would be more uniform under conditions of high humidity because

there is no rapid loss of water from the plant surface and this would give rise to a multidirectional movement and distribution. How precisely transpiration and the flow of solutes and pesticides are regulated by various environmental factors, particularly relative humidity, is discussed in greater details by Crafts & Crisp (1971), Hull (1970), Kozlowski (1964), Crafts, Currier & Stocking (1945).

Recent discoveries by Heath (1965) and Heath and Meidner (1963), point out that stomatal opening and closing is actually regulated by the concentration of CO_2 and that the modus operandus of most environmental factors is to influence the content of CO_2 in the intercellular spaces of leaves. These workers explained closing of stomata in darkness on the basis of undue accumulation of CO_2 into the intercellular spaces, whereas during periods of illumination most of the CO_2 is consumed in photosynthesis. Similarly higher temperature cause greater utilization of CO_2 for photoassimilation than for accumulation into intercellular spaces with the result that stomata remain open at higher temperatures (Table VIII). Likewise growth regulators and nutrients (Fischer 1968, Sawhney and Zelitch 1971), also influence the stomata. Despite this, since most of the stomata are distributed on abaxial rather than adaxial surface of leaves, it seems cuticular and lenticular transpiration would also be efficiently operative in elm trees.

PRACTICAL APPLICATION

These findings suggest that physical factors of the environment such as light intensity, temperature and relative humidity are of significance during benomyl application to elm roots. Warmer days with sunny periods would favour greater systemic action because the

rate of absorption and translocation of benomyl is accelerated at that time. Accordingly root injection or soil incorporation of benomyl should be carried out at a time when the transpiration rates are higher, i.e. when the weather is warm, sunny and the relative humidity low. Thus summer application of benomyl to soil and root are likely to be more effective than the spring or autumn applications. Fluctuations in weather conditions might also account for differential effectiveness of benomyl treatments from one geographical area to another.

SUMMARY AND CONCLUSION

Controlled laboratory experiments employing elm seedlings, grown in Hoagland solution, were carried out to test the effects of three climatic factors (light intensity, temperature and relative humidity) on absorption, translocation and accumulation of benomyl (MBC-C^{14}) by roots. Autoradiographic and scintillation counting data demonstrated that all three factors influenced the rate of uptake and distribution. Maximum absorption and transport occurred under conditions of high light intensity (3000 f.c.), high temperature (88^{o}F) and low relative humidity (25%). Even though much of the activity was retained by the roots during a 3 day absorption period, the translocation pattern was apoplastic and hence factors that regulated transpiration probably controlled the pattern of movement of benomyl as well. It is suggested that stomatal behaviour (opening and closing and size of the stomatal aperture) together with cuticles and lenticels regulate the transpiration and translocation patterns of root/soil applied fungicides.

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REFERENCES

Biehn, W.L. and A.E. Diamond. 1971. Prophylactic action of benomyl against Dutch elm disease. Plant Disease Report 55: 179-182.

Crafts, A.S., H.B. Currier and C.R. Stocking. 1949. Water in the physiology of plants. Chronic Botanica. Waltham, Massachusettes.

Crafts, A.S. 1961. Translocation in plants. Holt, Rinehart and Winston.

Crafts, A.S. and C. Crisp. 1971. Phloem translocation in plants, W.H. Freeman & Co., San Francisco.

Crafts, A.S. and Yamaguchi, S. 1964. Autoradiography of plant materials. Calif. Agric. Expt. Station Bull. 35:143 pp.

Franke, W. 1971. The entry of residues into plants via ectodesmata. Residue Reviews 38: 81-115.

Fischer, R.A. 1968. Stomatal opening: role of potassium by guard cells. Science 160: 784-85.

Glasstone, S. 1946. Text book of physical chemistry. 2nd Edition Van Nostrand Company.

Heath, O.V.S. 1959. Water relations of stomatal cells and the mechanisms of stomatal movement. In "Plant Physiology". Edit. F.C. Steward, 1959.

Heath, O.V.S. and H. Meidner. 1957. Mid-day closure of stomata. Effects of CO_2 and temperature on stomata of Allium cepa. Nature 180 (181).

Hoagland, D.R. and D.I. Arnon. 1952. Water culture of plants. Calif. Agric. Station, Bull., 31 pp.

Hull, H.M. 1970. Leaf structure as relatedto absorption of pesticide and other compounds. Residue Reviews 31, 1.

Kilgore, W.K. and E.R. White. 1970. Decomposition of the systemic fungicide 1991 (Benlate). Bull. Env. Contam. & Toxicol. 5: 67-69.

Kondo, E.S. 1972. A method for introducing water soluble chemicals into mature elms. Information Report, CFS 0-X-171, 11 pp.

Kozlowski, T.T. 1964. Water metabolism in plants. Harper and Row Publishers.

Peterson, C.A. and L.V. Edgington. 1970. Transport of the systemic fungicide benomyl in bean plants. Phytopathology 60: 475-478.

Prasad, R. 1972. Translocation of benomyl in elm. I. Effects of pH on absorption, distribution and accumulation by roots. Information Report, CC-X-32, 33 pp.

Prasad, R., C.L. Foy and A.S. Crafts. 1967. Effect of relative humidity and solution additives on foliar uptake and translocation of Dalapon-C^{14} by bean and cotton plants. Weeds 49(7): 149-156.

Sampson, Joan. 1961. A method of replicating dry or moist surfaces for examination by light microscopy, Nature 191, 932-933.

Sawhney, B.L., and I. Zelitch. 1969. Direct determination of K^+ accumulation in guard cells in relation to stomatal opening in light, Plant Physiology 44: 1350-54.

Siegel, M.R. and A.J. Zabbia. 1972. Distribution and metabolic fate of the fungicide benomyl in dwarf pea. Phytopathology 62: 630-634.

Smalley, E.B. 1971. Prevention of Dutch elm disease in large nursery elms by soil treatment with benomyl. Phytopathology 61: 1351-54.