PROTECTION OF ELM TREES AGAINST THE DUTCH ELM DISEASE (CERATOCYSTIS ULMI BUISM/MOREAU) IN THE URBAN ENVIRONMENTS:

Some Factors Affecting Uptake and Translocation of the Systemic Fungicide, Benomyl, by Roots

by

Raj Prasad

Chemical Control Research Institute
Canadian Forestry Service
Department of the Environment
Ottawa, Canada

CC-X-133

A Volunteer Paper Presented at the Second FAO/IUFRO World Technical Consultation on Forest Diseases and Insects

New Delhi, India

April 7 - 12, 1975

SUMMARY

Controlled laboratory experiments employing elm seedlings, grown in Hoagland solution, were carried out to test the effects of pH and 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 four factors influenced the rate of uptake and distribution. Maximum absorption and transport occurred under conditions of low pH, 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.

RÉSUMÉ

On a réalisé des expériences en laboratoire sur des jeunes pousses d'orme croissant dans une solution de Hoagland, afin de déterminer les effets de pH et trois facteurs climatiques (l'intensité lumineuse, la température et l'humidité relative) sur l'absorption, le transport et l'accumulation du bénomyle (MBC marqué au C^{14}) par les racines. Les données provenant de l'autoradiographie et de la scintigraphie ont révélé que les quatre facteurs influençaient la vitesse d'absorption et de distribution. Les niveaux maximums d'absorption et de transport ont été enregistrés à base pH à forte intensité lumineuse (3000 f.c.), à température élevée (88°F) et à faible taux d'humidité relative (25%). Même si, sur une période d'absorption de trois jours, une grosse part de l'activité s'est concentrée au niveau des racines, le transport était apoplastique et, par conséquent, les facteurs dont dépendait la transpiration avaient probablement une influence directe sur le cheminement du bénomyle. Nous croyons donc que l'action des stomates (l'ouverture, la fermeture et la grandeur des ostioles), ainsi que celle des lenticelles et de la cuticule, gouvernent les mécanismes de la transpiration et du transport des fongicides absorbés au niveau des racines.

RESUMEN

Se realizaron experimentos controlados de laboratorio con
plántulas de olmo cultivadas en una solución Hoagland, para
verificar los efectos de tres factores climáticos (intensidad
de la luz, temperatura y humedad relativa) en la absorción,
desplazamiento y acumulación de benomilo (MBC-C^{14}) en las raíces.
Los datos autoradiográficos y de recuento de escintilaciones
demonstraron que los tres factores influyeron en la tasa de
absorción y distribusión. La absorción y distribución máximas
se experimentaron bajo condiciones de gran intensidad lumínica
(3000 f.c.), elevada temperatura (31^{o}) y humedad relativa baja
(25%). Aunque las raíces acapararon gran parte de la actividad
durante un período de absorción de tres días, et patrón despla-
zamiento fue apoplástico, por lo que los elementos reguladores
de la transpiración probablemente controlaron también las ten-
dencias de distribución del benomilo. Se sugiere que los patrones
de transpiración y desplazamiento de fungicidas aplicados en las
raíces y la tierra regulan, junto con las cutículas y lenticelas,
el proceso estomático (apertura, cierre y tamaño del orificio
estomático)

INTRODUCTION

Elm trees belonging to the family *Ulmaceae* are widely distributed in Asia, Europe and America. While some members (*Ulmus wallichiana*) of this family are timber species in India (Champion and Seth 1968), others (*Ulmus americana, U. pumila, U. parvifolia, U. hollandica, U. carpinifolia, U. procera, U. laevis, U. Thomasii*) are used as shade and ornamental trees in urban and suburban environs of many parts of the world. White elm (*U. americana* L.) is one of the most popular shade trees in North America and its protection against the Dutch elm disease (DED) caused by *(Ceratocystis ulmi* Buism/Moreau) is urgently needed. In 1969, Stipes discovered the chemotherapeutic properties of benomyl (1-butyl carbamoyl-2-benzimidazole carbamic acid, methyl ester) against the DED. Since then much research has progressed in this area and apparently elm trees can now be protected from the ravages of this fatal disease (Biehn and Dimond 1972, Kondo 1973, Jones et al 1973, Smalley et al 1973, Prasad 1974, Gibbs and Clifford 1974). Several methods of application of benomyl and its solubilized formulations (MBC-chloride, MBC-phosphate) to elm trees have been investigated and it seems root application is the most efficient method of treatment. However, several arborists and researchers (Biehn and Dimond 1971, Smalley 1971, Hock, Roberts and Schreiber 1972) have reported differential responses of soil-applications of benomyl and it appears that root uptake from soils is usually poor and erratic. Therefore, a detailed study was carried out to examine the influence of light intensity, temperature, relative humidity and pH on the rate and pattern of absorption and translocation of benomyl by roots under controlled laboratory conditions.

MATERIAL AND METHODS

Elm seedlings (6 months old) were cultivated hydroponically in a greenhouse under constant conditions of light intensity (3000 ± 500 foot candles; 16 hr photoperiod), temperature (76 ± 2°F) and relative humidity (50 ± 10%) using Hoagland solution (adjusted to pH 6.5) as a nutrient source. Radioactive benomyl (MBC-C^{14} conc. 375 or 1500 ppm) was fed to roots by mixing with the culture solution and after 3 days, plants were harvested and processed for autoradiography and scintillation counting as previously described (Prasad, 1972). The latter experiments were carried out in controlled growth-cabinets at: light intensity of 0 and 3000 foot candles, temperature at 68 and 88°F, relative humidity of 25 and 95% and pH at 3.2, 5.2, 7.2 and 9.2

RESULTS AND DISCUSSION

(i) Effects of Light

The data obtained from the light and dark experiments are presented in Table I and indicate that light has a positive effect on uptake and transport of benomyl. Thus low light or cloudy days are likely to impede the rate of absorption from the roots and this in turn may affect systemic action at the site of pathogenesis.

TABLE I

Effects of light and dark on root absorption and translocation of MBC-C^{14} by elm seedlings

Contents - µg MBC/g fresh weight

Plant part	Light	Percent of Total	Dark	Percent of Total
Root	117.0	73.63	67.49	90.64
Stem	18.20	11.45	6.24	8.38
Leaf	23.71	14.92	0.73	0.98
Total	158.91	100.00	74.46	100.00

(ii) Effects of Temperature

Under natural conditions there is a wide fluctuation in temperature during the growing periods of elms. Temperature affects both the growth and the effectiveness of the chemical treatment. Table II shows the distribution into various parts of the plant at these temperatures and Figs. 1 & 2 show the translocation pattern into root, shoot and leaves.

TABLE II

Effects of temperature on distribution of activity in elm seedlings following absorption of MBC-C^{14} by roots

Contents - µg/g fresh weight

Plant part	68°F	Percent of Total	88°F	Percent of Total
Root	63.75	90.42	174.36	86.53
Stem	5.61	7.96	23.30	11.56
Leaf	1.14	1.62	3.85	1.91
Total	70.50	100.00	201.51	100.00

(iii) Effects of Relative Humidity

The movement of water and solutes in plants is generally affected by changes in relative humidity of the ambient environments (Crafts and Crisp 1971). The translocation of benomyl also seems to be influenced by relative humidity as can be gauged from Table III. Since roots were immersed in nutrient solution, the effects of relative humidity are manifested on the shoot. Low humidity seem to favour greater uptake and distribution and this may be related to stomatal behaviour and number on the leaves (Prasad et al 1967).

TABLE III

Influence of relative humidity on root absorption and translocation of MBC-C^{14} by elm seedlings

Contents - µg/g fresh weight

Plant part	Low Humidity	Percent of Total	High Humidity	Percent of Total
Root	113.30	57.19	103.06	63.57
Stem	58.90	29.73	47.32	29.18
Leaf	25.90	13.08	11.75	7.25
Total	198.10	100.00	162.13	109.00

These results indicate that all three environmental factors (light, temperature and relative humidity) influence the rate of uptake and movement of benomyl into the tree. These factors could operate by modifying the morphological features (stomatal movements) or by influencing the physiological processes such as photosynthesis etc. Therefore, the degree of stomatal opening and closing on the abaxial surface of elm leaves were examined microscopically (x200) under each set of environmental conditions and the data are presented in Table IV.

TABLE IV

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

Environmental conditions	Percentage of stomata observed at various degrees of opening		
	fully open	partially open	fully closed
High Light - 3000 fc (76^{o}F, 60% R.H.)	84	4	12
No Light - 0 fc (76^{o}F, 60% R.H.)	2	3	95
High Temp. - 88^{o}F (3000 fc, 60% R.H.)	65	15	20
Low Temp. - 68^{o}F (3000 fc, 60% R.H.)	5	10	85
High R.H. - 95% (3000 fc 76^{o}F)	75	7	18
Low R.H. - 25% (3000 fc - 76oF)	4	24	72

Thus, these findings demonstrate that climatic and edaphic factors (light, temperature, relative humidity and pH) are of significance during benomyl application to elm roots (Figs. 3 & 4). Accordingly warmer days with sunny periods are likely to favour greater systemic action because the rate of absorption and translocation of benomyl would be accelerated at that time. This seems to be largely accomplished by the stomatal movements and transpiration rates of the leaves. Likewise, summer application of benomyl to soil and roots are likely to be more effective than autumn applications. Fluctuations in weather conditions might also account for differential effectiveness of benomyl treatments from one geographical area to another. Similarly variations in soil conditions (pH) and benomyl formulation (acidic-alkaline) are likely to affect the uptake from roots. Generally, acidic formulations and somewhat acidic soils would promote greater penetration and translocation of benomyl than alkaline formulations or basic soils (Prasad 1972).

LITERATURE CITED

1. Biehn, W.L. and A.E. Dimond (1971). Plant Dis. Reptr. 55:179-82.

2. Champion, H.G. and S.K. Seth (1968). Forest Types of India, Gov't India Publication, Delhi - 6, 404 pp.

3. Crafts, A.S. and C. Crisp (1971). Phloem Transport in Plants. Holt, Rhinehart and Winston 481 pp.

4. Gibbs, J.N. and D.R. Clifford (1974). Ann. Appl. Biol. 78, 309-18, U.K.

5. Gregory, G.F., T.W. Jones and P. McWain (1973). U.S.D.A. For. Ser. Res. Notes NE-176 9 pp.

6. Hock, W.K., B.R. Roberts and L.R. Schreiber (1970). Phytopath. 60:1619-22.

7. Kondo, E.S. and G.D. Huntley (1973). Canada Dept. of Env. Inf. Rept. 0-X-182, 11 pp.

8. Prasad, R. (1972). Canada Dept. of Environ. Inf. Rept. CC-X-32. 33 pp.

9. __________ (1972). Canada Dept. of Environ. Inf. Rept. CC-X-35. 23 pp.

10. __________ (1974). Canada Dept. of Environ. Inf. Rept. CC-X-73. 14 pp.

11. Prasad, R., C.L. Foy and A.S. Crafts (1967). Weed Science 49(7): 149-156.

12. Smalley, E.G. (1974). Phytopath. 61: 1351-54.

13. Stipes, R.J. (1969). Phytopath. 59: 1560.

14. Van Alfen, N.K. and G.S. Walton (174). Phytopath. 64: 1231-34.

APPENDIX I

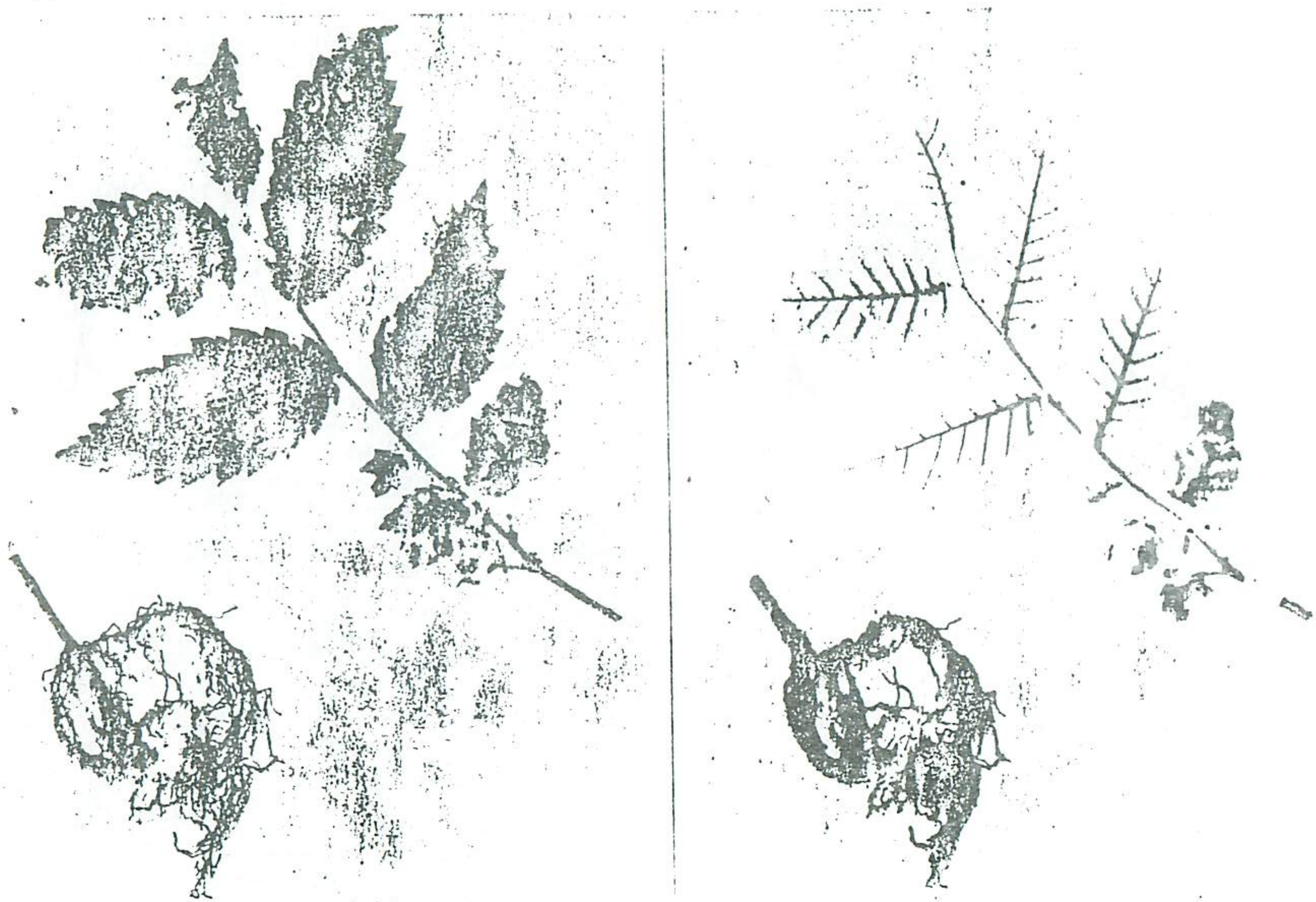

Fig. 1. Distribution pattern of MBC-C^{14} in elm seedlings following root uptake at low temperature (68°F) for 3 days. Photograph of seedling is on left and autoradiograph on right. Note slower movement into veins, margin and laminae of leaves.

APPENDIX II

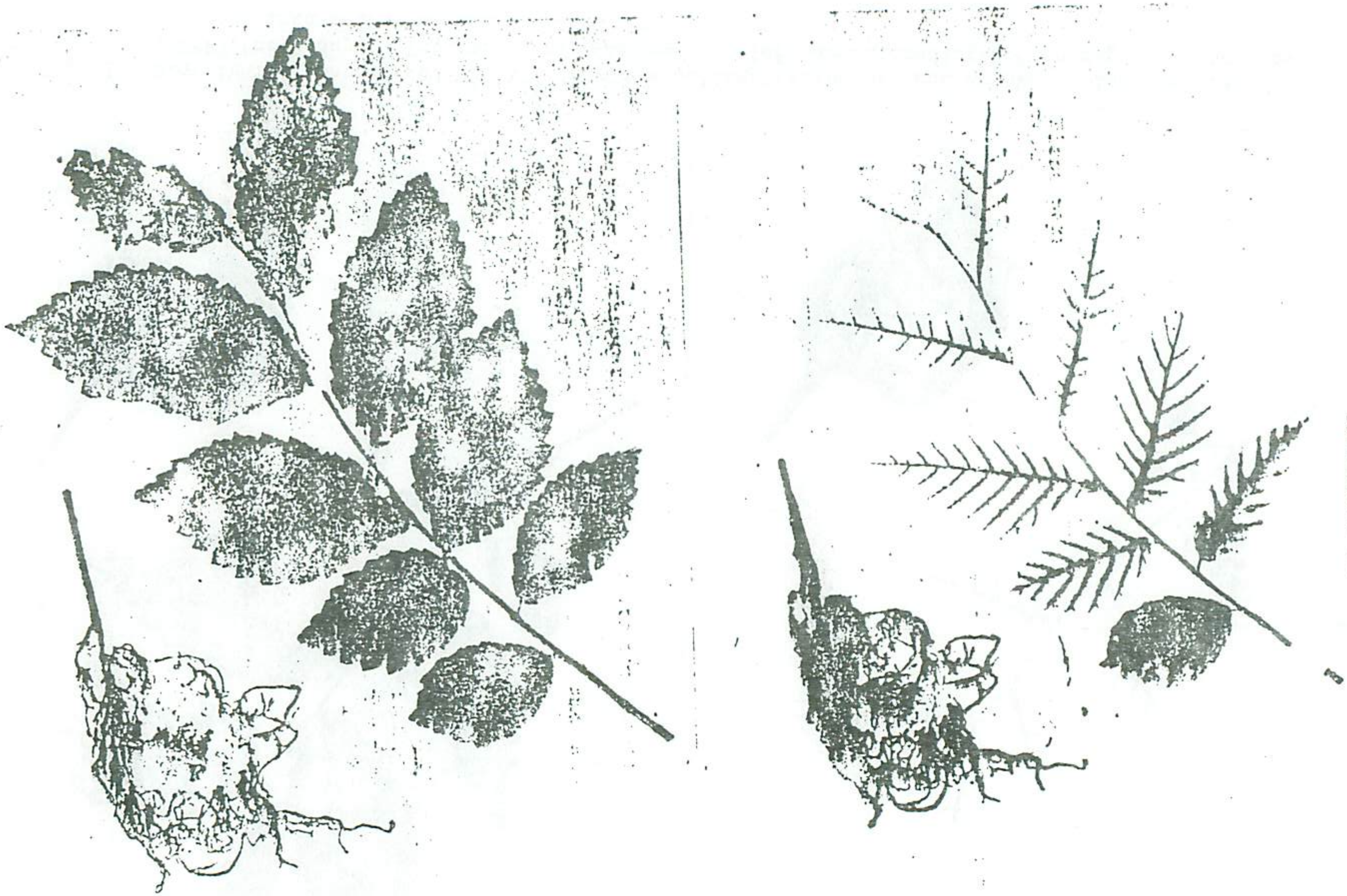

Fig. 2. Distribution pattern of MBC-C^{14} in elm seedlings following root uptake at high temperature (88°F) for 3 days. Note the greater movement and localization of activity in roots, old leaves, veins and stem. Autoradiograph of the treated plant is on the right hand side.

APPENDIX III

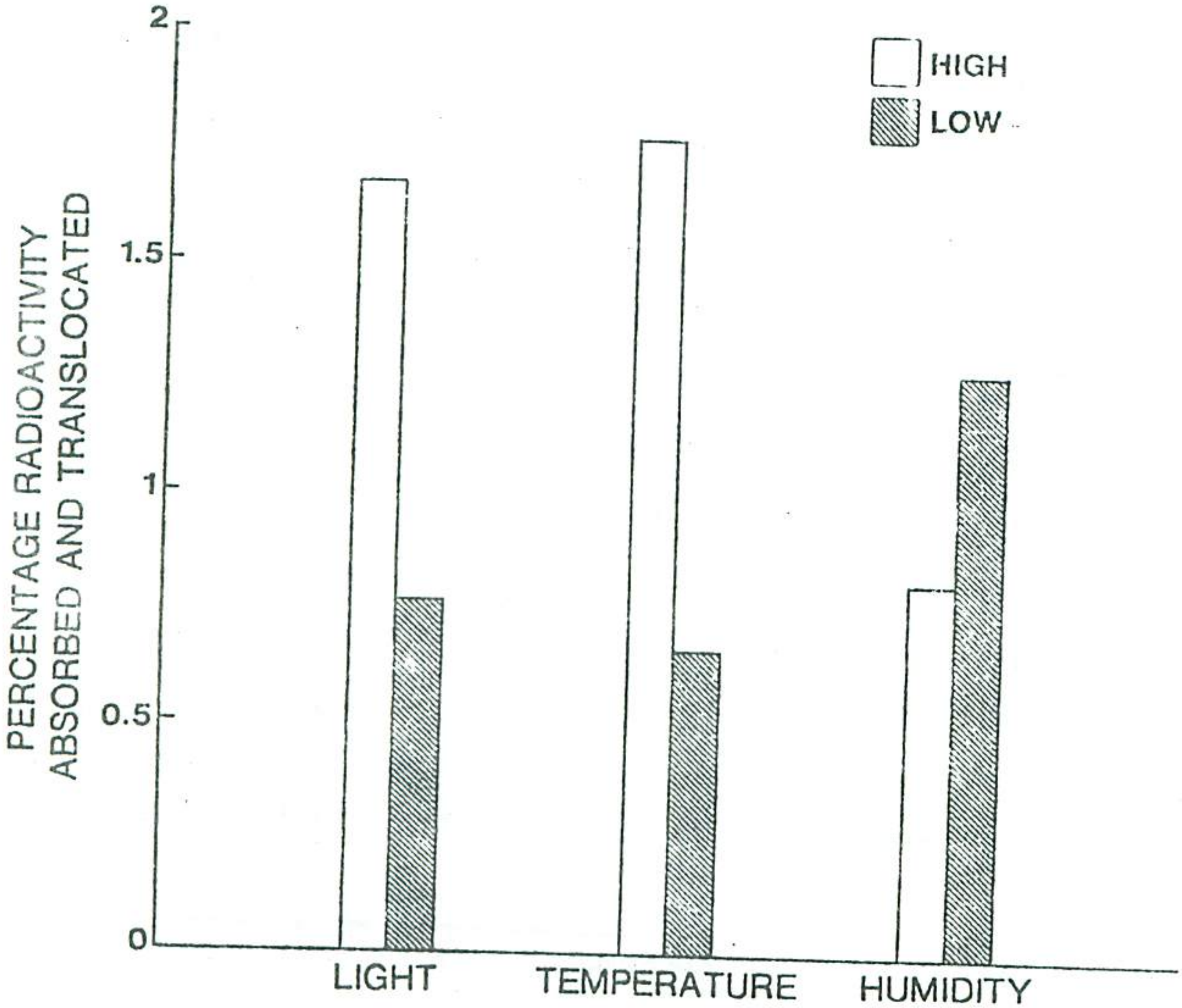

Fig. 3. Comparative effects of light, temperature and relative humidity on absorption and translocation of $MBC\text{-}C^{14}$ by elm roots after a 3 day uptake. Maximum effects are brought about first by temperature, then by light intensity and then by relative humidity.

APPENDIX IV

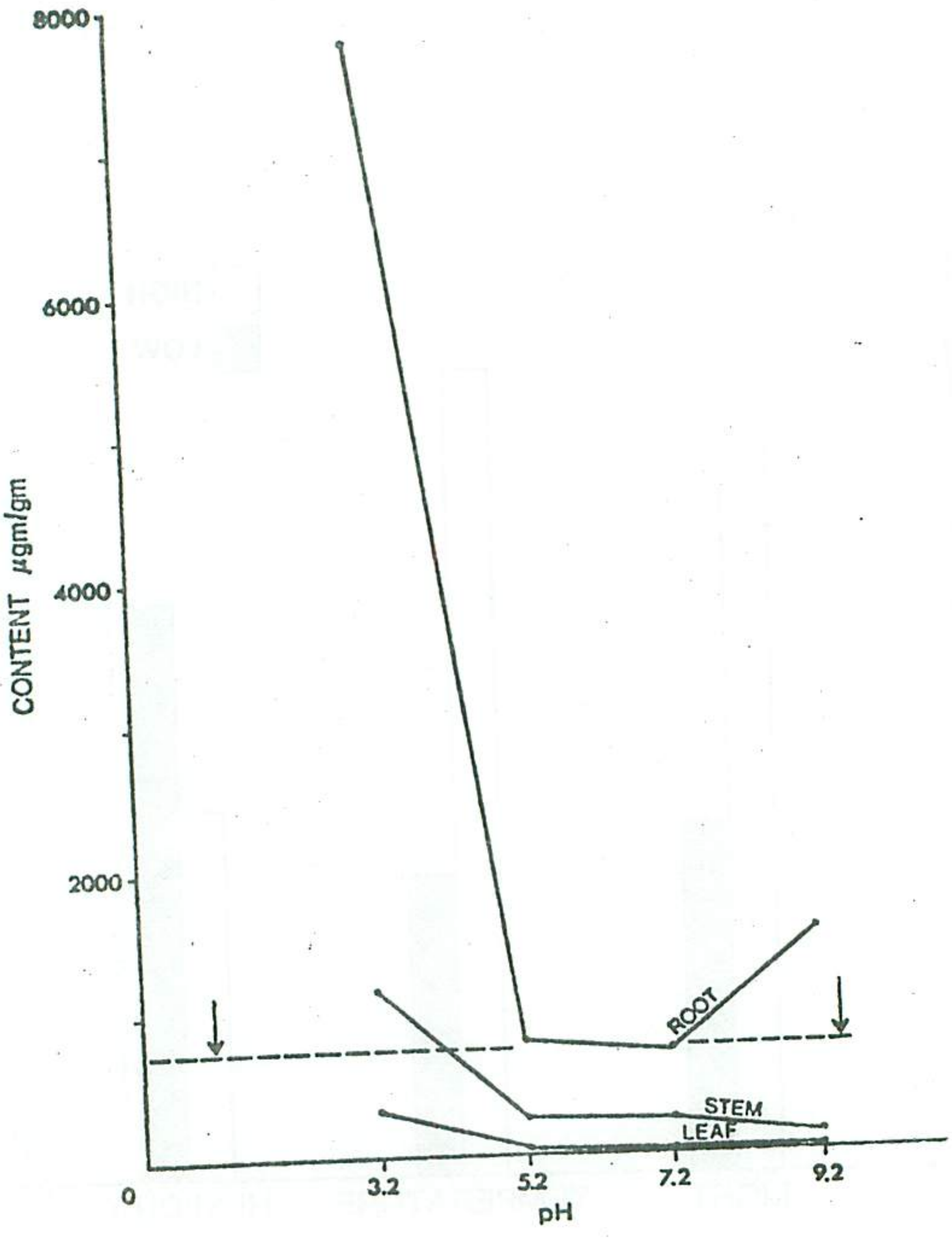

Figure 4. Effects of pH on absorption and translocation of MBC-C^{14} by elm seedlings roots. The contents expressed as µg/gm fresh tissue weight reflect deposition of MBC inside the respective tissues. The arrow and dotted line approximately indicate the level of the external concentration (1500 ppm). Radioactivity 0.45 µc/ml. Absorption period - 3 days.