

TRANSLOCATION OF BENOMYL IN ELM (Ulmus americana L.)

IX. SOME ECOLOGICAL CONSEQUENCES OF THE TREATMENTS ON POPULATION

DYNAMICS OF EARTHWORMS (Lumbricus terrestris L.)

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ABSTRACT

Earthworm populations were greatly reduced in forest soil treated with benomyl and MBC. There was 100% mortality of worms, (Lumbricus terrestris L.) held in soil drenched with 100,000 ppm and 10,000 ppm suspensions of benomyl and MBC after 14 days exposure in the laboratory. Mortality rate was higher in the MBC treated soil than in soil drenched with benomyl. The LD₅₀ values for benomyl and MBC were 3000 ppm and 300 ppm, respectively. Thin layer chromatographic (TLC) analysis of the benomyl drenched soil showed that after 14 days the fungicide molecule had broken down into 78% benomyl, 21.3% MBC, and 0.7% AB. Radioactive tracer experiments showed that MBC-C¹⁴ was most concentrated in the digestive system of treated worms.

RÉSUMÉ

Les populations de vers de terre ont considérablement baissé dans les sols forestiers traités au bénomyl et au MBC. Après 14 jours d'exposition en laboratoire, la mortalité chez les vers (Lumbricus terrestris) gardés dans un sol saturé avec des suspensions de bénomyl et de MBC à 100,000 p.p.m. et 10,000 p.p.m. a atteint 100 p. 100. Le taux de mortalité était beaucoup plus élevé dans le sol traité au MBC que dans le sol saturé de bénomyl. Les DL₅₀ pour le bénomyl et le MBC ont été de 3,000 p.p.m. et 300 p.p.m. respectivement. La chromatographie en couche mince du sol saturé de bénomyl a montré qu'après 14 jours, la dégradation chimique de la molécule de fongicide avait produit 78 p. 100 de bénomyl, 21.3 p. 100 de MBC, et 0.7 p. 100 de AB. Des dosages par radio-activité ont montré que la concentration la plus forte en ¹⁴C du MBC marqué se trouvait dans le système digestif des vers (L. terrestris) traités.

INTRODUCTION

Benomyl, 1-(butylcarbamoyl)-2-benzimidazole carbamic acid, methyl ester, is a systemic fungicide which is presently employed in combating a wide variety of plant fungal diseases. Clemons and Sisler¹ demonstrated the highly unstable nature of benomyl in aqueous suspensions and isolated the fungitoxic breakdown product, 2-benzimidazole carbamic acid, methyl ester (MBC). Large quantities of benomyl are being employed in an attempt to control Dutch elm disease both by foliar² and soil³ application. This is being done without adequate information on the long term effects of this highly persistent⁴ fungicide in the forest ecosystem. A reduction of mite population has been observed following benomyl treatment^{5,6}. The inhibitory effect of benomyl and MBC on earthworm populations has been observed in both field and laboratory soils^{7,8,9}. The present paper gives further evidence of reduction of earthworm populations in treated forest soil, and describes a laboratory study of the effect of benomyl and MBC on the earthworm, Lumbricus terrestris.

MATERIALS AND METHODS

A. FIELD STUDIES

(i) Field Site

Earthworm populations surveys were performed at Shirley's Bay, near Ottawa at four sites. Plot WB had been sprayed with a benomyl - water suspension (2,500 ppm) in the spring of 1972. Plot YM had been sprayed with MBC-C1 (2,500 ppm). These areas contained elm trees (Ulmus americanus L.) that had been sprayed during a Dutch elm disease control program. Three quadrats (2 X 2') were marked out at the base of each tree as shown in Fig. 1. Two untreated areas were selected for controls.

(ii) Earthworm Collection

Estimation of earthworm populations in forest soils was carried out by the method of Raw¹⁰. A solution of formalin (50 ml of 40% formaldehyde/10 litre of water) was poured on each quadrat (2 x 2') of soil and the number of emerging worms was recorded for twenty minutes after application.

Worms (L. terrestris) for laboratory use were obtained commercially from Alves Worm Wholesales, Ottawa. These were about 15 cm long and held in captivity for at least two weeks before use. Food consisted of a mixture of dried deciduous leaves that had been ground in a Wareing blender.

(iii) Fungicide Treatment

Benlate, (50% benomyl W.P.) obtained from the Dupont Company, was used for all fungicide preparations. Benomyl and water suspensions were prepared without heating and used immediately to prevent decomposition. MBC-Cl was prepared by heating a benomyl-water suspension with HCl(0.1N) at 90° C for one hour. The fungicidal formulations (benomyl and MBC-Cl) had a pH of 7.

B. LABORATORY STUDIES

(i) Soil Drenching

Worms were held in plastic 2-litre pots filled with soil and contained 10 worms per plot. Aqueous suspensions of benomyl and MBC-Cl were poured on the soil surface of each pot. Fifty ml of each concentration (10, 100, 1000, 10,000, 1000,000 ppm) were applied to each pot in three replicates. The number of living worms was counted after 3, 6, 10, and 14 days and food was given subsequent to each count.

(ii) Thin Layer Chromatography (TLC)

To determine exact composition, the benomyl and MBC suspensions prepared for soil drenching were first analysed by TLC following the method of Siegel and Zabbia¹¹. A solution of chloroform, ethyl acetate, and acetic acid (1:1:0.4) was employed to develop the TLC plates*. The fate of benomyl from the drenched soil, in the 100,000 ppm pots, was also analysed after 14 days when the experiment was concluded. Cold chloroform was stirred with the soil for one hour, and after filtering the solvent was concentrated by evaporation and spotted on the TLC plates.

(iii) Radioactive Labelling

C¹⁴ labelled MBC was obtained from the International Chemical and Nuclear Corporation, California, with a specific activity of 5 mc/mml. Six worms were immersed in an aqueous solution of MBC-C¹⁴ (725 DPM/ μ l) for two minutes. These treated worms were kept separately in 250 ml Erlenmeyer flasks for 4 days in the dark at 25°C and 90% humidity. Then the radioactive solution used for immersion purposes was mixed into 100 cc of soil in a 3 liter beaker and six untreated worms were added to this beaker containing soil, drenched with MBC-C¹⁴ and kept under the same environmental condition as immersed worms. After 4 days the worms were killed with chloroform vapour and rinsed briefly under running water to remove any MBC-C¹⁴ adhering on the exterior surface. Three worms from each treatment type (immersed and soil-drenched) were carefully dissected and separated into digestive system, reproductive organs, remaining skin and musculature. The remaining six worms were cut into thirds by incision just anterior to the clitellum and by halving the posterior section. The components were extracted separately in a series of hot ethanol-water solutions (95%, 70%, 50%, 20%, 0% ethanol). The extracts

*Glass TLC plates (5 x 20 cm) precoated with 0.25 ml layer of silica Gel F-254 (Brinkman Instruments Inc., Westbury, N.Y.) and developed to 10 cm.

were concentrated by evaporating and counted in a Picker Nuclear (Ansitron II), liquid scintillation counter using the appropriate dioxane/toluene cocktails and the external standards for correction of quenching and efficiency of the radioactive samples.

RESULTS AND DISCUSSION

A. FIELD STUDIES

Following foliar sprays, the fluctuations in earthworm populations at Shirley's Bay plots were examined after three months and the results are shown in Table I.

Table I

Earthworm populations at Shirley's Bay on Treated and Check Areas .

<u>Treatment</u>	<u>Ave. No. of worms/quadrat, mean of 30 replicates</u>		
	<u>L. terrestris</u>	<u>Other</u>	<u>Total</u>
Plot WB (Benomyl, 2500 ppm)	0.03	2.30	2.33
Plot YM (MBC, 2500 ppm)	0	1.25	1.25
Controls (Check)	2.41	12.70	15.11

Thus it is quite apparent from the data that the population was greatly reduced in the sprayed plots and the results are consistent with those obtained by Stringer and Wright⁹ during their examination of the treated soil in apple orchards. In general, worms were more numerous in the benomyl plot than in the MBC plot and this might be related to the decomposition of benomyl to MBC. Another interesting feature to note is the acute toxicity of the treatments to L. terrestris.

B. LABORATORY STUDIES ON SOIL DRENCH

The results from this study are presented in Fig. 2 and as can be

seen from the graphs it is clear that toxicity is related to the level of concentration of the fungicides. As before, the MBC treated pots showed much greater mortality than benomyl drenched-soil. The LD_{50} for the two fungicides was calculated from the 6th day data and was found to be 3000 ppm and 300 ppm for benomyl and MBC respectively which corroborate the findings that MBC is more toxic to L. terrestris than benomyl. (Stringer and Wright⁹).

TLC analysis of benomyl drenched soil revealed a partial breakdown of benomyl into MBC and AB (2-aminobenzimidazole) and the R_f values obtained were 0.62, 0.37, and 0.01, for benomyl, MBC, and AB respectively (see Fig. 3). These values are similar to those obtained by Siegel and Zabbia¹¹. It may be that MBC was the agent responsible for eliciting mortality, and that benomyl must first break down into MBC before it becomes toxic to earthworms. This would also explain the slower rate of killing in the benomyl drenched-soil.

(iv) Radioactive Experiment

Further evidence of the mechanism and locale of toxicity was obtained by feeding of $MBC-C^{14}$ to live worms. As can be seen from Table II the concentration of radioactivity is largely in the digestive tracts of the worms.

Table II

Distribution of radioactivity in various parts of earthworms
after feeding with $MBC-C^{14}$

<u>Body Parts</u>	<u>(DPM/gm fresh material)</u>			
	<u>Immersed worms</u>	<u>% Activity</u>	<u>Soil drenched worms</u>	<u>%Activity</u>
Reproductive System	22,241	21.6	20,712	18.7
Digestive System	69,206	67.3	64,587	58.3
Skin and Musculature	11,387	11.1	25,554	23.0
Anterior	33,511	26.9	28,655	22.2
Middle	51,988	41.7	47.652	36.9
Posterior	39,136	31.4	52,904	41.0

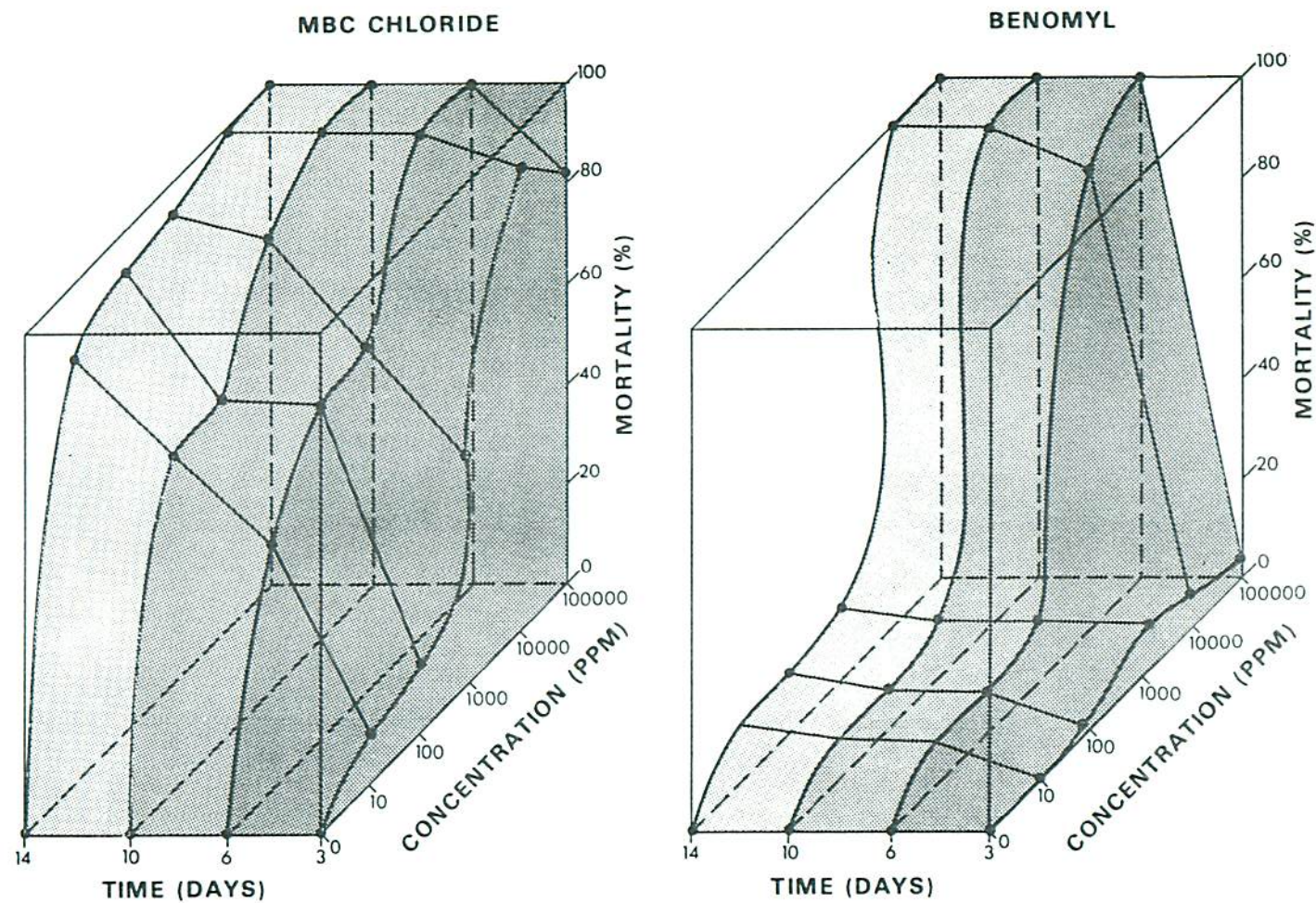


Fig. 2 Three dimensional graphs illustrating the effects of concentration of MBC chloride and benomyl and the period of treatment on mortality rate of earthworms.

Under both conditions of treatments, the results indicate that more activity was present in the digestive system than in the body wall. The activity was however weakest in the anterior section. It is interesting to note that the total amount of activity was not appreciably higher in the soil-treated worms which received both external and internal dosage than the immersed worms. Stringer and Wright⁹ demonstrated the repellent nature of minute quantities of benomyl on food material and thus lethal effect of benomyl can result solely from skin contact as opposed to ingestion. This experiment did not separate the digestive system into internal (feces) and external components. Hence it is not possible to determine whether the fungicide was transported to the digestive system to be excreted with the waste products, or was, in fact, being accumulated in the musculature of the intestine. The latter condition would more readily explain mortality. However, it was observed that the amount of feces present in the immersed worms was small probably due to the starvation of these worms during the experiment. It was also noted that the killing by use of chloroform vapour precipitated evacuation. Considering this it seems more probably that the activity was actually present in the tissue of the digestive system.

Conclusion

Both field data and laboratory soil-drench experiments confirm the findings of Stringer and Wright⁹ that benomyl in minute quantity is lethal to earthworms; Lumbricus terrestris being a very susceptible species. MBC is much more toxic to worms than benomyl and it is possible that the lethal effect of benomyl is due to the formation of MBC as a breakdown product in the soil environment.

The radioactive tracer studies reveal that MBC diffuses readily through the earthworm skin and accumulates within the digestive system. Further radioisotope studies employing the histoautoradiographic technique should be carried out to establish the localization of MBC-C¹⁴ at the cellular level.

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