

The Effect of Concentration, Exposure Time and Age of Plant on Uptake and Translocation of Two Systemic Fungicides in Soybeans

B. L. Kirkpatrick and J. B. Sinclair

Former Graduate Research Fellow and Professor, respectively, Department of Plant Pathology, University of Illinois, Urbana 61801. Senior author's present address: Department of Botany, University of Illinois, Urbana 61801. A portion of the senior author's M.S. Thesis. Supported in part by the Illinois Agricultural Experiment Station and the U.S. Agency for International Development Grant csd-1922.

Accepted for publication 20 July 1975.

ABSTRACT

When soybean (*Glycine max*) tissues were bioassayed by an agar diffusion method against *Penicillium atrovenerum*, the size of zones of inhibition was greater following a 24-hour root exposure to BD 18654 and thiophanate-methyl (TM) than with a 12-hour exposure. Thiophanate-methyl breaks down into methyl 2-benzimidazole-carbamate (MBC). Inhibition zones were greater around all tissues, except cotyledons, from seedlings treated with BD 18654 than TM. The higher the concentration of BD 18654 used, the greater the zones of inhibition for all tissue samples, except those

from trifoliolate leaves of 2-week-old seedlings. Increased concentrations of TM resulted in greater zones of inhibition only from hypocotyl tissues. Bioassays showed that the two fungicides were absorbed through the roots of 1- and 2-week-old seedlings, were translocated, and became more or less evenly distributed to all aboveground parts. The roots of seedlings exposed to the two fungicides for 24 hours were protected for 1 week against *Macrophomina phaseolina*.

Phytopathology 66:102-105

Additional key words: charcoal rot, *Rhizoctonia bataticola*.

The development and use of systemic fungicides for control of plant diseases has shown increasing promise on a variety of crops. However, there is no consistency in the translocation of a given systemic fungicide in different plant species or of different systemic fungicides in a given species (7, 12, 13). Studies on the uptake and translocation of systemic fungicides in soybean [*Glycine max* (L.) Merr.] seedlings have been confined to qualitative analysis and generally have been limited to whole plants treated for relatively long time periods (4, 5, 6, 13). This study reports on the effect of fungicide concentration, exposure time, and age of seedling on the uptake and translocation of two systemic fungicides in soybean seedlings and the possible use of these fungicides for control of charcoal rot.

MATERIALS AND METHODS.—The isolate of *Macrophomina phaseolina* (Tassi) Goid. [Sym. = *M. phaseoli* (Maubl.) Ashby, *Rhizoctonia bataticola* (Taub.) Butler] used was from silty-loam soil in a field cropped with soybeans for 6 consecutive years on the University of Illinois' South Farm. An isolate of *Penicillium atrovenerum* G. Smith was used for bioassays and bioautographs. Stock cultures of both organisms were maintained on Difco potato-dextrose agar (PDA) at room temperature (25 + 3 C). *Macrophomina phaseolina* inoculum was prepared using the method of Kirkpatrick and Sinclair (8).

The uptake and translocation of the following two systemic fungicides in soybean seedlings and their control of charcoal rot were investigated: BD 18654 [Methyl (1-((5-cyanopentyl)amino)carbonyl)-1H-benzimidazole-2-yl)-carbamate] (which is Chemagro's Bay Dam 18654 50WP) and thiophanate-methyl (TM) [1,2-bis-(3-methoxycarbonyl-2-thioureido) benzene] (which is Pennwalt's Topsin-M 70WP). Since TM converts to methyl 2-benzimidazole carbamate (MBC) (8), MBC is the active

component of this fungicide.

Amsoy soybean seeds, selected for uniformity from a certified seed lot, were used for all laboratory experiments. Seeds were planted in 7.5 × 10.5 × 2.0-cm plastic trays filled with 2,500 ml of vermiculite (Terralite) and moistened with 1,200 ml tap water. They were incubated in a growth chamber programmed for 25 C, with a wet-bulb depression of 7 C and a 14-hour day of 43,040 ± 2,152 lux. Two seedlings were transplanted 5 days later to each of several 475-ml (16-ounce) plastic (Styrofoam) cups filled with 400 cm³ of vermiculite and kept moist with tap water.

Root uptake studies.—The root uptake and translocation of the two fungicides in soybean was studied in 7- and 14-day-old seedlings. Eight seedlings per treatment were grown in plastic cups containing vermiculite, with two seedlings per cup. The cups were drenched with either 100 ml of distilled water (control) or 100-ml suspensions of either fungicide at 500 or 1,000 µg/ml. After 12 hours (8 hours light plus 4 hours dark) and 48 hours, four plants from each treatment were harvested. The plants were removed from the vermiculite, their roots washed with distilled water to remove excess fungicide, and the seedlings cut radially along the root-hypocotyl axis, 1.5 cm above the first lateral root. The plants were rinsed twice in sterile, distilled water, blotted dry and separated into: roots, hypocotyl, cotyledon, epicotyl and unifoliolate leaves for 7-day-old seedlings; plus the median leaflet of the first trifoliolate leaf for 14-day-old seedlings. The respective sections were placed into separate culture plates and stored at -15 C for at least 24 hours.

The tissues were bioassayed (8) by placing the following in plates containing 20 ml of PDA mixed with a conidial suspension (150,000 spores/ml agar) of *P. atrovenerum*: (i) 8-mm-diameter disks cut from the tips of unifoliolate

TABLE 1. Mean diameter^a of inhibition zones of *Penicillium atrovirens* in potato-dextrose agar formed by sections of 1- and 2-week-old soybean seedlings nontreated (control) or treated with a 100 ml drench BD 18654^b or thiophanate-methyl^c at 500 or 1,000 µg/ml for 12 or 48 hours

Exposure time (hours)	Chemical	Rate (µg/ml)	Mean diameter (mm) of inhibition zones for seedling sections										
			Root		Hypocotyl		Cotyledon		Epicotyl		Leaf		
			1 ^d	2 ^d	1	2	1	2	1	2	1	2	
12	Control	0	8 ^e	8	8	8	8	8	8	8	8	8	8
	BD 18654	500	28	32	11	15	8	8	8	11	10	9	8
		1,000	30	34	14	20	8	8	8	17	12	11	11
	Thiophanate-methyl	500	27	33	11	18	12	8	9	15	13	10	9
		1,000	29	35	14	19	10	8	8	16	14	11	10
	48	Control	0	8	8	8	8	8	8	8	8	8	8
BD 18654		500	36	43	25	35	30	15	24	34	33	36	36
		1,000	41	46	32	36	32	22	27	37	35	37	35
Thiophanate-methyl		500	30	43	21	31	28	18	15	31	33	34	36
		1,000	34	42	23	32	27	15	18	30	32	31	35

^aAverage of eight replicates.

^bBD 18654 = methyl (1-((5-cyanopentyl)amino)carbonyl)-1H-benzimidazole-2-yl)carbamate.

^cThiophanate-methyl = 1,2-bis-(3-methoxycarbonyl-2-thioureido) benzene.

^d1 = 1-week-old seedling; 2 = 2-week-old seedling.

^eRepresents no activity.

leaves, trifoliolate leaves and cotyledons; and (ii) 8-mm-long segments cut from the central region of hypocotyls and epicotyls, and from the primary root where the first lateral roots appeared. The mean diameter of inhibition zones was recorded after 18 hours at 8 C plus 2 days at 30 C. The experiment was repeated twice and analyzed as a three-factor factorial using a computer program (1). The control measurements were excluded from the analysis and the values for the two plants within a cup were averaged. Separate analyses were performed for the treatments within the different tissues. A priori single degree of freedom comparisons were made for treatment main effects and interactions at $P = 0.05$.

Extracts from treated plants were subjected to thin-layer chromatographic analysis (TLC) using a modification of Peterson and Edgington's technique (10). Extracts were obtained by massing the remaining portions of bioassayed sections from all plants of the same treatment, homogenizing them in a VirTis blender with 10 ml of chloroform for 2 minutes at high speed, followed by filtering the homogenate through eight layers of cheesecloth, and combining the homogenates of the same treatment from both repetitions of the original experiment. The volume of the homogenates was reduced to approximately 0.5 ml. Five and 10 µliters from each extract were spotted on silica chromatogram sheets (Eastman No. 6061) without fluorescent indicator. Five and 10 µl of 500-µg/ml solutions of the fungicides in chloroform and of chloroform extracts of BD 18654 in sterile, distilled water and TM in distilled water which was then autoclaved, also were spotted as standards. The sheets were developed for 13-14 cm in a chloroform-acetone (6:1,v/v) ascending solvent system. The developed chromatograms were bioautographed by placing them silica-side-down in sterilized enamel trays

containing PDA supplemented with 100,000 sclerotia of *M. phaseolina* from air-dried soybean-seed-extract (SSE) cultures (8), 40 mg streptomycin sulfate and 60 mg potassium penicillin/liter. The chromatograms were removed after 3 hours and incubated at room temperature. After 72 hours, R_f values of inhibition zones were recorded.

Control of charcoal rot on seedlings.—Control of charcoal rot was studied on soybean seedlings whose root systems were exposed to either of the two fungicides for 1 or 7 days. The plants were wound-inoculated with *M. phaseolina* and then placed in a growth chamber programmed for 14-hour days of 13,988 ± 2,152 lux at 34 ± 1.5 C and nights of 10 hours at 28 ± 1.5 C with both periods having a wet-bulb depression of 9 C. Nontreated, noninoculated seedlings served as controls.

For the first root-uptake experiment, 1-week-old seedlings were exposed for 1 day to either distilled water or 500-µg/ml drenches of the two fungicides, then removed, their roots washed under tap water and transplanted into plastic cups containing vermiculite and distilled water without fungicide. At 1, 3, and 7 days after replanting, six plants from each treatment were wound-inoculated (9) by cutting a 1-cm slit with a sterile scalpel immediately below cotyledonary nodes and placing wefts of mycelium from a 24-hour, SSE culture of *M. phaseolina* into the wound. Wounds were covered with sterilized petroleum jelly (Vaseline). Disease development was recorded after 7 days.

For the second root-uptake experiment, thirty-two 1-week-old seedlings were planted in pairs. Three sets of pairs were drenched with 100 ml of either distilled water (control) or suspensions of BD 18654 or TM at 100 or 500 µg/ml. The plants were wound-inoculated 12 hours after drenching, observed daily, and symptom development

recorded at 7 days. Hypocotyl and epicotyl portions were cut from each seedling, soaked in a 0.3% solution of sodium hypochlorite for 4 minutes and rinsed in sterile, distilled water. One-centimeter sections, with centers 1.5 cm above or below the center of the original point of inoculation, and 1.5-cm sections which included the wound-inoculated tissues, were placed on PDA supplemented with 40 mg streptomycin sulfate and 60 mg potassium penicillin/liter agar. Growth of *M. phaseolina* was recorded after 3 days at 30 C. One unifoliolate leaf from each plant was removed and bioassayed as previously described.

RESULTS AND DISCUSSION.—*Root uptake and translocation.*—When concentrations of BD 18654 and TM were increased in agar disks, inhibition zones for *P. atrovenetum* increased (8). The present bioassay technique seems quantitative for the fungitoxicants, but observed values were probably less than actual due to incomplete diffusion from the plant sections (2, 3). Due to the variation in weight between plant sections, larger sections may have contained greater amounts of fungitoxicant, as indicated by larger inhibition zones, than less massive ones; while actually having less fungitoxicant per gram of tissue. For this reason, the most meaningful comparisons are for differences within tissues rather than among them. All plant tissue from seedlings treated for 48 hours had significantly greater zones of inhibition than those from seedlings exposed for 12 hours, independent of fungicide and trifoliolate leaf tissues (Table 1). The increase in size of inhibition zones by all tissues, except cotyledons between 12- and 48-hour exposure was significantly greater for BD 18654 than for TM-treated seedlings. There was no significant effect due to age of plant. Higher concentrations of BD 18654 resulted in significantly greater zones of inhibition for all plant tissue, except those from the trifoliolate leaves of 2-week-old plants. Increased concentrations of TM resulted in a significantly greater inhibition zone only from hypocotyls.

Since the fungicide bioassay of plant sections with *P. atrovenetum* only indicated the presence of TM (MBC), BD 18654, and its breakdown product (8), extracts from treated plants were subjected to TLC analysis. MBC was present in plants treated with TM, while BD 18654 was the only fungitoxic compound present in plants treated with it. There were no zones of inhibition produced by extracts from control plants.

Bioassays of plant sections from treated seedlings showed that BD 18654 and TM were absorbed through the roots of 1- and 2-week-old seedlings and translocated to the hypocotyl, cotyledons, epicotyl, unifoliolate and trifoliolate leaves. Fungicides BD 18654 and TM were fairly evenly distributed in all plant tissues after 48 hours in both 1-week-old and 2-week-old plants in contrast to the reports of the localization of benomyl and chloroneb in cotyledons of soybean seedlings (12, 13). Their translocation was similar to that reported for thiabendazole (5) and carboxin (13) in soybean.

Greater amounts of the fungitoxicants were present in the roots, hypocotyls, and epicotyls of 2- rather than of 1-week-old plants. The opposite was true for cotyledons, which is probably related to the decrease in the active function of the cotyledons between the two plant ages. Greater amounts of BD 18654 and TM accumulated in all

tissues with increased exposure time. The higher rate of BD 18654 increased the amount of that chemical absorbed by the roots, and translocated to all other tissues except the trifoliolate leaves. Increasing the rate of thiophanate-methyl caused a significant increase of MBC only in the hypocotyl. The absolute amounts of fungitoxicants taken up and translocated might have been less if drenches had been applied to planting media containing organic matter (11).

Charcoal rot control on seedlings.—The fungitoxicants were successful in varying degrees in controlling charcoal rot. Seedlings whose roots were exposed for 24 hours to BD 18654 and TM were protected for 1 week against infection by *M. phaseoli* when inoculated 1 and 3 days after treatment, but not after 7 days. All nontreated-inoculated plants and those treated plants inoculated after 7 days developed charcoal rot symptoms. Noninoculated but wounded plants did not develop symptoms.

One-week-old seedlings treated with drenches of the two fungicides and inoculated 12 hours later developed no symptoms after 7 days, while all nontreated-inoculated control plants were diseased. *M. phaseolina* was recovered from the wounds of all fungicide-treated plants, but not from stem sections above and below the wound, except in those plants treated with 100 µg/ml BD 18654. The fungus was reisolated from all locations on all inoculated control plants. The fungitoxicants restricted the movement of *M. phaseolina* up and down the stem from the point of inoculation and were considered to be fungistatic.

The bioassays of unifoliolate leaves from all treated plants showed fungitoxic activity. The mean diameter (in mm) of inhibition zones for each treatment were: wound-inoculated control, 8; BD 18654 and TM at 100 and 500 µg/ml, 15.1 and 29.5; and 24.3, and 32.0, respectively. There was no activity from noninoculated controls.

These results indicate that the two fungicides were taken up by soybean seedling roots and translocated to aboveground tissues where they acted against *M. phaseolina* to control charcoal rot. The control exhibited by TM was due to MBC.

The rapid uptake and uniform translocation of BD 18654 and MBC in soybean seedlings following root drenches with BD 18654 and TM were effective in initially controlling charcoal rot of soybean when wound-inoculated. However, after 1 week the fungitoxicants were no longer present in amounts sufficient for control. Four possible reasons for a short control period are suggested: (i) BD 18654 and MBC were broken down to nontoxic compounds; (ii) the concentrations of fungitoxicants were diluted below that needed for control as the plants grew; (iii) the fungitoxicants accumulated in tissue away from the point of inoculation; or (iv) any combination of these. Due to their rapid uptake and uniform distribution, BD 18654 and TM may provide a successful means of controlling other fungal diseases on a short-term basis.

LITERATURE CITED

1. CRAMER, S. G., R. D. SEIF, and W. C. JACOB. 1964. A computer program for the analysis of factorial experiments. *Agron. J.* 56:363-364.
2. ERWIN, D. C. 1969. Methods of determination of the

- systemic and fungitoxic properties of chemicals applied to plants with emphasis on control of *Verticillium* wilt with Thiabendazole and Benlate. *World Rev. Pest Control* 8:6-22.
3. ERWIN, D. C., H. MEE, and J. J. SIMS. 1968. The systemic effect of 1-(butylcarbonyl)-2 benzimidazole carbamic acid, methyl ester, on *Verticillium* wilt of cotton. *Phytopathology* 58:528-529.
 4. GRAY, L. E., and J. B. SINCLAIR. 1970. Uptake and translocation of systemic fungicides by soybean seedlings. *Phytopathology* 60:1486-1488.
 5. GRAY, L. E., and J. B. SINCLAIR. 1971. Systemic uptake of ^{14}C -labeled 2-(4'-thiazolyl) benzimidazole in soybeans. *Phytopathology* 61:523-525.
 6. KHARBANDA, P. D. 1970. Systemicity of ^{14}C -labeled chloroneb in soybean tissues. Ph.D. Diss., Univ. of Illinois, Urbana. 46 p.
 7. KIRK, B. T., J. B. SINCLAIR, and E. N. LAMBREMONT. 1969. Translocation of ^{14}C -labeled chloroneb and DMOC in cotton seedlings. *Phytopathology* 59:1473-1476.
 8. KIRKPATRICK, B. L., and J. B. SINCLAIR. 1973. Uptake of two systemic fungicides and their breakdown products by soybean seed. *Phytopathology* 63:1532-1535.
 9. MEYER, W. A., J. B. SINCLAIR, and M. N. KHARE. 1973. Biology of *Macrophomina phaseoli* in soil studied with selective media. *Phytopathology* 63:613-620.
 10. PETERSON, C. S., and L. V. EDGINGTON. 1969. Quantitative estimation of the fungicide benomyl using a bioautograph technique. *J. Agric. Food Chem.* 17:898-899.
 11. SCHREIBER, L. R., W. K. HOCK, and B. R. ROBERTS. 1971. Influence of planting media and soil sterilization on the uptake of benomyl by American elm seedlings. *Phytopathology* 61:1512-1515.
 12. THAPLIYAL, P. N., and J. B. SINCLAIR. 1970. Uptake of three systemic fungicides by germinating soybean seed. *Phytopathology* 60:1373-1375.
 13. THAPLIYAL, P. N., and J. B. SINCLAIR. 1971. Translocation of benomyl, carboxin and chloroneb in soybean seedlings. *Phytopathology* 61:1301-1302.