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Effects of Benefin Vaporizing from Soils on Tobacco (*Nicotiana tabacum*) Foliage[1]

Y. YAMASUE and A. D. WORSHAM[2]

Abstract. In closed vapor exposure chambers, foliar injury on tobacco (*Nicotiana tabacum* L. 'Speight G-28') seedlings was caused by vapors arising from soils in which technical grade benefin (*N*-butyl-*N*-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine) or its commercially-formulated emulsifiable concentrate had been incorporated. The emulsifier alone did not cause injury. Dosages of the herbicide ranged from 0.84 to 6.72 kg/ha or 13.1 to 104.8 ppmw in the concentration in soils, and the lowest dosage was sufficient to induce the foliar injury. Leaves which developed during the exposure were markedly shortened, narrowed, and thickened, distorted in shape, and had an extremely poor lamina expansion and abnormal venation pattern. Severity of the foliar injury increased as the herbicide dosage and soil moisture increased, and as the soil organic matter content decreased. Leaves which were partially expanded before the exposure were affected much less. When exposed to vapors of ^{14}C-benefin, plant seedlings readily absorbed radioactive materials by its entire foliage including the stem tip with a few tiny developing leaves. More than half of the ^{14}C absorbed and adsorbed during a 3-day exposure was recovered by $CHCl_3$ wash of leaves and appeared to be retained by the wax and cutin layers, and also by lipoidal secretion substances of the numerous trichomes on the leaf surface. There was a small fraction of unabsorbed or loosely retained ^{14}C material on the surface of the leaves and stem tip, which was removed by water. That not recovered from H_2O and $CHCl_3$ washes appeared to be distributed in the internal tissues. Little acropetal translocation appeared to occur from the expanded leaves toward the stem tip. There was no translocation of ^{14}C-benefin into roots of plants where only a single leaf was exposed to the radioactive vapors.
Additional index words. Volatile herbicides, herbicide vapor injury.

INTRODUCTION

Benefin is a selective, soil-incorporated, preemergence herbicide for agronomic and horticultural crops. The herbicide is an analog of trifluralin (α,α,α-trifluroro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine), with chemical and biological properties that are similar to trifluralin. Benefin and trifluralin are slightly volatile, with vapor pressures of 3.89×10^{-5} mm Hg at 30 C and 1.99×10^{-4} mm Hg at 29.5 C, respectively (17).

Trifluralin has no foliar contact activity or may not be absorbed by plants if so applied, although instances of foliar distortion have been observed in tobacco and summer squash (*Cucurbita pepomelon* L.) (17). Injury to tobacco foliage has been observed when benefin was used as a preplant, soil-surface application. In these cases, the crop was grown for a time after transplanting, being either capped with a paraffin-paper cone or covered with a transparent polyethylene row cover. Benefin vapors from the soil was suggested as a possible cause of the foliar injury in tobacco[3].

Objectives of experiments reported here were to determine if foliar injury could be induced on tobacco exposed to vapors of the herbicide from treated soils, and to characterize absorption of ^{14}C-benefin vapors and translocation of radioactivity in tobacco seedlings.

MATERIALS AND METHODS

Vapor exposure chamber. The apparatus used to expose tobacco foliage to benefin vapors consisted of a 23-L plastic container, a round salad-mold with 19-cm diam, a 237-ml Styrofoam cup with a plant seedling and a 19-L glass dome (Figure 1). The container was filled with Norfolk sandy loam moistened to 75% field capacity at the beginning of all experiments, thus giving support to the mold as well as preventing vapor leakage through bottom of the glass dome. The mold contained benefin-incorporated soil or aqueous ^{14}C-benefin solution as the source of herbicide vapors, and the Styrofoam cup with the bottom removed for plant root growth was inserted into the central space of the mold. The glass dome was then placed on the surface of the soil thus sealing the atmosphere. The herbicide dosages were calculated based on bottom area (506.5 cm^2) of the dome and concentration was 15.6 ppmw for the soil incorporation, equivalent to one kg/ha.

Plant and soil preparation. Uniform-sized tobacco seedlings, 60 to 70 days old, were transplanted in the Styrofoam cup and cultured for 7 to 14 days until treatment in the greenhouse. Every two days, plants were watered with 30 ml of a nutrient solution having a pH 5.9 and containing 10 mM KNO_3, 3 mM $Ca(NO_3)_2$, 2 mM KH_2PO_4, and 2 mM $MgSO_4$. When treated, the seedlings had seven to eight leaves and were 4 to 5 cm in height.

The soils used in the experiments were collected from research stations in North Carolina, and were air-dried, screened through a 0.32-cm mesh screen, and kept in a ventilated room until treatment. Characteristics of these soils are listed in Table 1. For all treatments excluding those with ^{14}C-benefin, the herbicide was incorporated in 300 g of the air-dried soils with a volume of water which would bring the soils to 75% field capacity unless otherwise noted.

Foliar effects. To differentiate effects of benefin from possible effects of the other constituents in the commercially emulsified formulation (0.18 kg/L), plants were exposed to vapors arising from the Norfolk sandy loam incorporated with the technical grade benefin (99% purity), emulsifier alone, and the commercial formulation, all at an equivalent dosage of 5.04 kg/ha. When the technical grade material was used, it was first diluted ten times with acetone and incorporated into the soil. After the odor of acetone was no longer detectable, distilled water was added to the soil to bring it to the designated moisture level.

Magnitude of the foliar injury on tobacco was determined with various herbicide rates, soil types, soil organic matter contents and soil moisture levels. Benefin was applied in aqueous

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[3] Personal communication with research scientists of the Japan Monopoly Corp., Japan.

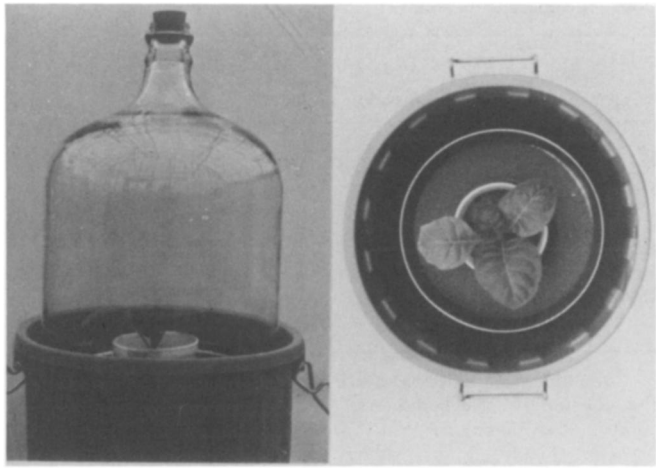

Figure 1. Vapor exposure chamber used for greenhouse experiments; a front view (left) and axial view (right).

solutions prepared from the commercial formulation. Herbicide dosages employed were 0.0, 0.84, 1.68, 3.36, and 6.72 kg/ha in Norfolk sandy loam. Soil types included Durham loamy sand, Norfolk sandy loam and Cecil clay loam. The herbicide was incorporated into these soils at 1.68 kg/ha. Soils of 2.7, 7.8, and 11.0% (w/w) organic matter content were prepared by thoroughly mixing Norfolk sandy loam with a histosol (muck) with 93% organic matter, and treated with the herbicide at 3.36 kg/ha. The moisture levels selected were 15, 75, and 150% (w/w) field capacity in Norfolk sandy loam. Volume and concentration of the aqueous benefin was adjusted to obtain these soil moisture levels and a common dosage of 1.68 kg/ha. Salad-molds of treated soil were then placed on soil of container.

The chambers were placed on a greenhouse bench in a completely randomized design with three replications. The atmospheric temperatures were 28.6 ± 3.6 C during the day and 22.8 ± 7.2 C at night. Temperature inside the chambers was 36.5 ± 7.5 C at 1 to 2 p.m.

The photoperiod was maintained at 14 h light, with incandescent lamps as the supplementary light source, and 10 h dark. For the first 5 days, plants were allowed to grow under the glass domes, and exposed to vapors arising from the treated soils. The plants required no water for this period. For the next 10 days, plants were grown under normal greenhouse conditions by removing both the glass domes and the treated soil. Observation and measurement of growth in height and leaf area of the plants were made at the tenth day after removal of the domes. Height of each plant had been recorded at the beginning of the treatment. The leaf area determined was the total area of the leaves becoming visible after treatment and was obtained by using an electronic area calculator. The extent of foliar injury was rated from 0 to 5: 0 = none, 1 = very slight, 2 = little, 3 = moderate, 4 = severe, and 5 = very severe malformation.

^{14}C-benefin movement. Absorption of ^{14}C-benefin vapors by tobacco seedlings and the subsequent translocation of the radioactivity within the plants were studied by use of uniformly ring labeled ^{14}C-benefin, 3.91 μCi/mg specific activity. Stock solution was prepared by dissolving the radioactive material and the commercially emulsified formulation into acetone to contain 4.0 μCi radioactivity and 20.5 mg benefin active ingredient per ml of the solution.

In the absorption study, plants were exposed for 3 days to radioactive vapors arising from a 43.2-ml aliquot containing 6.0 μCi ^{14}C-benefin, and immediately sampled. After being thoroughly rinsed under running water for about 3 min, stems, leaves, and roots were separated and then pressed between pieces of plywood. The samples were held at −10 C in a freezer until autoradiographic assay. In making autoradiographs, the plant samples were air-dried and mounted on white, sized bond paper with rubber cement, and exposed to 20- by 25-cm X-ray films for a month at −10 C.

To determine whether the radioactive compounds were on or in the tobacco seedlings, six plants were exposed to the vapors arising from a 33.7-ml aliquot containing 4.7 μCi ^{14}C-

Table 1. Characteristics of the soils used in experiments.

Soil code and source	Scientific name	Soil types	Field capacity	pH	Organic matter
			(% w/w)		(%)
Clayton, Wake Co.	Typic paleudult; fine loamy, siliceous, thermic	Norfolk sandy loam	23.4	5.5	2.9
Oxford, Granville Co.	Typic hapludult; fine loamy, siliceous, thermic	Durham loamy sand	17.4	5.6	0.7
Reidsville, Rockingham Co.	Typic hapludult; clayey, kaolinitic, thermic	Cecil clay loam	27.4	5.8	1.2
Smithfield, Johnston Co.	Typic paleudult; fine loamy, siliceous, thermic	Norfolk sandy loam	19.4	5.4	0.6
Willow Spring, Wake Co.	Typic paleaqualt; fine loamy, siliceous, thermic	Rains fine sandy loam	20.3	5.5	1.1

benefin in the chamber. After exposure for 3 days, foliage of the three plants was immediately sampled, and three other plants were allowed to grow for an additional 2 days under the greenhouse conditions. The plants sampled were separated into stem tips (about 1 cm in length) and leaves, gently washed with 100 ml distilled water for 3 min to remove the labeled materials loosely retained or unabsorbed on the surface, then washed with 100 ml chloroform for 5 min to dissolve the wax and cutin layers. In this context, fractions recovered by the water wash and chloroform wash were defined as external retention and wax-cuticular absorption, respectively, and the fraction not recovered by these washes was defined as internal absorption. This technique was previously used by Wathana et al. (16) to study the distribution of ^{14}C-2,4-DB [4-(2,4-dichlorophenoxy)butyric acid] in soybean [*Glycine max* (L.) Merr.] and cocklebur (*Xanthium* sp.). Radioassays were made from 1 ml of the aliquots used for the water wash and chloroform wash by adding 15 ml of a scintillation cocktail each in counting vials. Ingredients of the cocktail per 22-ml vial were: 4 ml redistilled ethanolamine, 9 ml methanol and 6 ml scintillator containing 5% PPO (2,5-diphenyloxazole) and 0.5% POPOP [1,4-bis-2-(5-phenyloxazolyl)benzene] in toluene. Counts were corrected by the channel ratio method. In case of the chloroform aliquots, the cocktail was added after evaporating chloroform to dryness. The plant samples washed with both water and chloroform were wrapped with dialysis tubing, combusted in a sample oxidizer, and the $^{14}CO_2$ radioactivity assayed.

In the translocation study, a single expanded leaf of each plant was exposed to the radioactive vapors from a 3-ml aqueous solution containing 1.2 μCi ^{14}C-benefin and 6.15 mg benefin active ingredient. The treatment solution was put into a waxed paper dish (3 cm in diam and 1 cm in height) and placed on the upper surface of a single leaf of each seedling, and then covered with an inverted 250-ml glass beaker and sealed with lanolin. After a 1-day exposure, both the ^{14}C-benefin solution and the beaker were removed and the seedlings were grown under greenhouse conditions. The plants were sampled at 0, 2, and 4 days after treatment, washed 3 min under running water, detached into the stem tips (about 1.5 cm in length), leaves, and roots and stored at lower than −10 C with dry ice until detection of radioactivity by liquid scintillation in the same manner as mentioned above.

RESULTS AND DISCUSSION

Foliar effects. Foliar injury was observed only on the plants treated with technical grade benefin and the commercial formulation and were rated 3.2 and 4.0, respectively (Table 2). The leaves injured by these treatments were commonly narrowed and shortened, distorted in shape and with an irregular vein pattern and extremely poor lamina expansion (Figure 2).

Leaf area was significantly reduced but there was no induction of foliar injury on the plants exposed to emulsifier alone. Since the commercial formulation is a mixture of benefin and the emulsifier, this result suggested that the cause of foliar injury on tobacco was vapors from benefin itself and not those from the other constituents of the commercial formulation.

Table 2. The effects of benefin formulations on growth of tobacco exposed to vapors from soil.

Treatment[a]	Dosage	Growth[b]		Foliar injury rating[c]
		Height[c]	Leaf area[c]	
	(kg/ha)	(%)		
Distilled water	...	100a (3.5cm)[d]	100a (164.0cm^2)[d]	0.0a
Benefin	5.04	69a	43c	3.2b
Emulsifier	...	91a	71b	0.0a
Commercially-formulated benefin	5.04	54a	29d	4.0b

[a]Benefin was applied with acetone at 1:10 ratio. The emulsifier was the one used in the commercial formulation of benefin, and treated equivalent at 5.04 kg/ha.

[b]Growth is expressed as % inhibition of the treated plants in height and overall leaf area after treatment, compared to those of the control 100.

[c]Values in a column followed by a common letter are not significant at the 5% level according to Duncan's multiple range test. Statistical analyses were run on the actual measurements taken.

[d]Values in parenthesis indicate actual data taken, means of three replications, in the control plants.

The foliar injury from the commercial formulation appeared to be greater than that of benefin alone. This may indicate that the emulsifier present in the commercial formulation facilitated movement of benefin vapors causing the foliar injury on tobacco by increasing volatilization of the herbicide. Danielson and Gentner (3) reported that technical grade

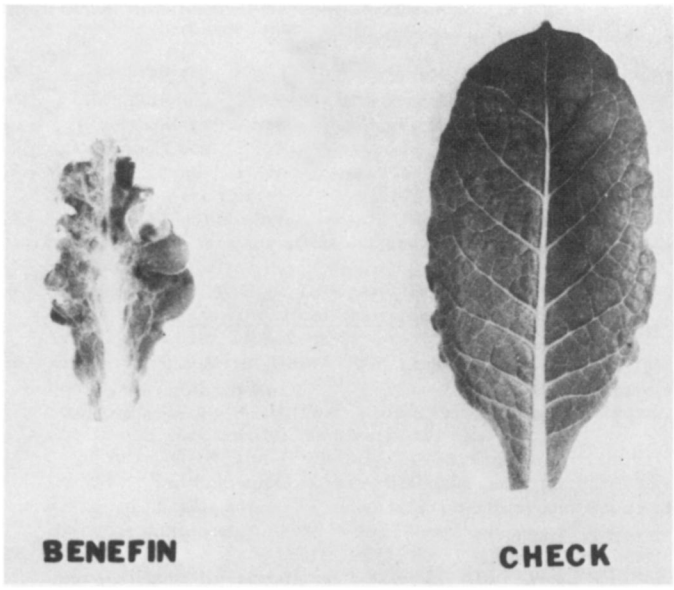

Figure 2. Typical appearance of a tobacco leaf exposed to vapors of benefin in a closed container. Leaves shown were taken from near top of plant 30 days after treatment. Left, exposed to benefin vapors from soil treated with 5.04 kg/ha; right from non-treated control.

EPTC (*S*-ethyl dipropylthiocarbamate) incorporated into soil vaporized less rapidly than when applied in the commercial emulsifiable concentrate formulation. However, an additive or synergistic effect of benefin plus emulsifier is conceivable. Considering the emulsifier reduced the leaf area, the plants might have been in a weakened state when the emulsifier was present.

Injury on tobacco foliage occurred with all dosages studied under our experimental conditions (Table 3). Reduction in plant height and leaf area increased with the increase in herbicide dosages. The treatment of 6.72 kg/ha induced 90 and 92% growth reduction in height and leaf area, respectively, while the 0.84-kg/ha dosage reduced height and the leaf area by 46 and 44%, respectively, compared to the control. There was much variation in growth of height and leaf area between experiments, which were conducted separately for herbicide rates, soil types, soil organic matter contents and soil moisture levels. Variation in foliar injury rating was, however, much less between experiments.

The severe foliar injury with the Durham loamy sand was the highest among the three types of soils (Table 3). Moderate and slight injury were noted for treatments of the sandy loam and clay loam soils, respectively. Degree of foliar injury was inversely related to organic matter content of the soils treated with benefin at 3.36 kg/ha (Table 3). The soil with 2.9% organic matter showed moderate to severe foliar injury, while benefin in the 11.0% organic matter soil caused no significant foliar injury. The adsorption of benefin in soils is probably increased by an increase of the organic matter content as reported for oryzalin[4] (3,5-dinitro-N^4,N^4-dipropylsulfanilamide) and trifluralin (1). There was greater foliar injury on the plants exposed to benefin-incorporated soil at 150% field capacity moisture than those at 15 and 75% field capacity (Table 3). The foliar injuries were slight at 75% and very slight at 15% field capacity moisture. These results are in agreement with reports that volatilization of water-insoluble, volatile herbicides increased from soils as the moisture level and the volume of application increased. Those herbicides include CDAA (*N*,*N*-diallyl-2-chloroacetamide) (4), EPTC (5, 7, 9), benefin (13) and trifluralin (2, 11, 13, 14). Upchurch (15) attributed the increase in volatilization to a competitive adsorption between water and the herbicide for binding sites on soil surfaces, so that the preferential binding of water to soil surfaces leaves the herbicide exposed to the air whereupon vapor loss is great.

^{14}C-benefin movement. Autoradiographs of the tobacco seedlings immediately after 3-day exposure to ^{14}C-benefin vapors showed the image of ^{14}C materials throughout the aerial parts including the stem tip with a few young leaves, but not in the roots (Figure 3). Leaves which were sufficiently expanded at the time of treatment were densely and uniformly imaged, indicating large amounts of ^{14}C presence on or in the plant parts. Close observation of the image suggested that some of the radioactive materials appeared to be retained with abundant trichomes on the outer leaf surface. Mid and lateral veins in the leaves contained little or no label at the proximal

Table 3. Effects of benefin dosages, soil types, organic matter content and moisture levels on tobacco foliar injury by benefin vapors.

Soil conditions at treatment				Growth[de]		Foliar[e] injury rating
Benefin	Types	O.M. content	Moisture level	Height	Leaf area	
(kg/ha)		[% w/w]	(% F.C.)	—— (%) ——		
0.0	NSL[a]	2.9	75	100a	100a	0.0a
6.72	NSL	2.9	75	10c	8b	3.0bc
3.36	NSL	2.9	75	15c	12b	2.7bc
1.68	NSL	2.9	75	44bc	38b	4.0c
0.84	NSL	2.9	75	54b	56ab	2.3b
0.0	NSL	2.9	75	100a	100a	0.0a
1.68	DLS[b]	0.7	75	50a	56a	4.0d
1.68	NSL	2.9	75	85a	69a	3.0c
1.68	CCL[c]	1.2	75	103a	81a	2.0b
0.0	NSL	2.9	75	100a	100ab	0.0a
3.36	NSL	2.9	75	58c	60c	3.3b
3.36	NSL	7.9	75	75b	73bc	3.0b
3.36	NSL	11.0	75	86b	114a	0.3a
0.0	NSL	2.9	75	100a	100a	0.0a
1.68	NSL	2.9	15	49b	46b	1.0b
1.68	NSL	2.9	75	44b	26bc	2.0c
1.68	NSL	2.9	150	33b	17c	3.7d

[a]Norfolk sandy loam.

[b]Durham loamy sand.

[c]Cecil clay loam.

[d]Growth is expressed relative to the control = 100.

[e]Values in a column are means of three replications on the 10th day after completion of 5-day exposure, and when followed by a common letter, there is no significant difference at 5% level according to Duncan's multiple range test.

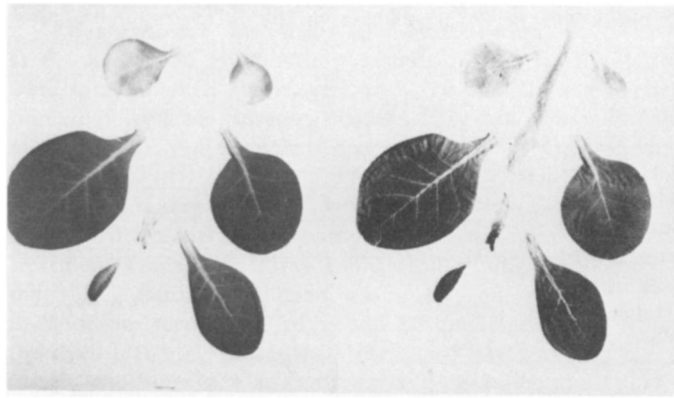

Figure 3. Plant (left) and autoradiograph (right) of tobacco harvested immediately after 3-day exposure to vapors from aqueous ^{14}C-benefin solution.

[4]McLaughlin, R. 1970. The phytotoxicity of EL-119 and trifluralin as influenced by soil constituents. M.S. thesis, Dep. Crop. Sci., North Carolina State Univ., Raleigh. 69 pp.

Table 4. External and internal distribution of ^{14}C in tobacco seedlings.

Fraction	Plant part	Recovered radioactivity[a]			
		Immediately after 3-day exposure		2 days after 3-day exposure	
		dpm/plant part (%)	dpm/mg fr. wt.	dpm/plant part (%)	dpm/mg fr. wt.
H_2O wash[b]	Stem tip[d] + leaves	3,690 (4.1)	0.8a	2,170 (4.1)	0.6a
$CHCl_3$ wash	Stem tip[d] + leaves	49,857 (56.0)	10.8b	26,287 (50.0)	6.7b
Internal[c]	Stem tip[d]	563 (0.6)	12.4b	472 (0.9)	7.3b
Internal[c]	Leaves	34,950 (39.3)	7.7ab	23,655 (45.0)	6.1b
Total		89,060 (100)	...	52,584 (100)	...

[a]Means of three replicates, single plant each. Values in a dpm column followed by a common letter are not significantly different at 5% level according to Duncan's multiple range test.

[b]The entire leaves and stem tip washed with H_2O for 3 min and then $CHCl_3$ for 5 min.

[c]Those components determined after H_2O and $CHCl_3$ washes.

[d]Stem tip 1 cm long segment with a few tiny leaves

and basal portions. Absorption of benefin vapors was characterized by exposing tobacco seedlings for 3 days to vapors arising from a ^{14}C-benefin aliquot and sampled immediately and 2 days later. Their leaves and stem tips were washed with water and with chloroform. The plants sampled immediately after the exposure showed a large amount, 89,060 dpm, of ^{14}C collectively in the leaves and stem tip (Table 4). Of this radioactivity, 4.1 and 56.0% was recovered from the water wash and chloroform wash, respectively. Wathana et al. (16) considered in their research on soybean and cocklebur that a water wash of a 2,4-DB-treated leaf removed the unabsorbed chemical on the surface, and the chloroform wash dissolved the cuticular fraction without extracting the internal cellular components. The leaf and stem of tobacco are covered with numerous glandular trichomes, making the surfaces sticky from the secretions of lipoidal substances (6). The surfaces are also coated with a cuticular layer. The ^{14}C recovered from the chloroform washes was probably dissolved in the lipoidal secretions as well as the cuticular layer including the cuticle extending into the stomatal chambers. Strang and Rogers (12) showed by microradioautographs that the accumulation of ^{14}C-trifluralin in cotton and soybean roots is primarily due to an adsorption to the cuticle, epidermis and less extent, cell wall.

The leaves and stem tips, after washing with water and chloroform, contained collectively 39.9% of the total ^{14}C-activity absorbed (Table 4). The small fraction (472 dpm) of ^{14}C which was recovered from the stem tissue 2 days after completion of exposure was 17.0 ppmw as benefin when computed on a basis of mean fresh weight of the stem tip (64.1 mg). These labeled materials apparently penetrated the lipoidal secretions and cuticular layer and were distributed in the internal tissues. There was less accumulation of the radioactivity in the plants grown for 2 days in an open atmosphere after the 3-day exposure to vapors from ^{14}C-benefin. Reduction in disintegration rates during the 2 days was larger from the water and chloroform washes than from the internal tissues. Possibly the radioactive materials on or in external portions diffused out more readily than the internally absorbed materials. Yamaguchi (18) demonstrated a loss of EPTC-^{35}S vapors through the leaves of red kidney beans (*Phaseolus vulgaris* L.).

Translocation of ^{14}C in tobacco was determined by exposing a single expanded leaf to vapors from a ^{14}C-benefin aliquot. The treated leaf of plants sampled immediately after 1-day exposure to the ^{14}C vapors showed a disintegration rate

Table 5. Translocation of ^{14}C-benefin in tobacco seedlings.

Plant part	Radioactivity in plant part[a]					
	Immediately after 1-day exposure		2 days after 1-day exposure		4 days after 1-day exposure	
	dpm/plant part	dpm/mg fr. wt.	dpm/plant part	dpm/mg fr. wt.	dpm/plant part	dpm/mg fr. wt.
Stem tip[b]	0	0.0a	0	0.0a	10	0.1a
Treated leaf	13,271	55.7b	11,317	43.1b	10,209	14.4b
Roots	0	0.0a	0	0.0a	0	0.0a

[a]Mean values of three replications. There were no significant differences among treaments in dpm and dpm/mg at 5% level. Values in a column followed by a common letter are not significantly different at 5% level according to Duncan's multiple range test.

[b]Stem tip 1.5 cm long with a young leaf developing after the exposure.

of 13,271 dpm, indicating significant ^{14}C absorption (Table 5). This was equivalent to 0.60% of the ^{14}C-benefin available. There was no ^{14}C activity in the stem tip (1.5 cm segment) or the roots. In plants sampled 2 days after the exposure, all of the radioactivity was again limited to the treated leaf. Stem tips of plants sampled 4 days after the exposure revealed presence of some radioactive materials; however, no radioactivity was evident in the roots. The presence of radioactive materials in the stem tips was insignificant, but may indicate an extremely minor acropetal movement of benefin and/or its derivatives from the single treated leaf as shown in the ^{14}C movement along the veins of radioautographed leaves in the absorption study. A slight acropetal movement of benefin from roots has been confirmed in peanut and alfalfa by Golab et al. (8) and in tobacco by Long et al. (10).

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