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Uptake, Translocation, and Metabolism of [14C]Thuringiensin (β-Exotoxin) in Corn

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The absorption, translocation, and metabolism of [14C]thuringiensin (β-exotoxin), an insecticide, derived from *Bacillus thuringiensis* was investigated in corn. Corn was harvested 3 and 7 days after its roots or leaves were exposed to thuringiensin. Corn absorbed more thuringiensin at 7 than 3 days of root exposure. Less than 10% of the applied thuringiensin was absorbed after 7 days of exposure. Only 12% of the foliar-applied thuringiensin was detected in the whole plant, and amounts absorbed at 3 and 7 days were similar. About 80% of the applied radioactivity was found in the leaf wash at both times of harvest, and only 20% of the absorbed was translocated out of the treated leaf. More than 95% of the absorbed radioactivity remained in the root. Time did not affect the distribution pattern of root- or foliar-applied thuringiensin in different parts of corn. In this study, thuringiensin was not readily absorbed by root or leaves of corn and had limited mobility in the plant. The insecticide was also not metabolized by corn shoot after 3 and 7 days of exposures. The implications of these results are discussed.

Thuringiensin (β-exotoxin) is an insecticide produced by the entomopathogen bacteria *Bacillus thuringiensis*. The insecticide is produced commercially by fermentation and can be formulated as stabilized emulsion, wettable powder, or dust (Burges, 1982). It is a nucleotidic ATP analogue (Benz,1966; Bond et al., 1969) that inhibits the production of DNA-dependent RNA polymerase and consequently the production of ribosomal RNA (Lecadet and De Barjac, 1981). The insecticide has shown potential for the control of insects on field crops, trees, ornamentals, vegetables, and stored grain and grain products (Miller et al., 1983). It is most effective to immature Lepidoptera, Diptera, Coleoptera, Hymenoptera, Isoptera, and Orthoptera (Burgerjon and Martouret, 1971).

In corn, it has given effective control of the larvae as well as the adults of *Heliothis zea* (Herbert and Harper, 1985;

Ignoffo and Gregory, 1972). Although its effect on *Heliothis* and other insects has been documented extensively (Burgerjon and Martouret, 1971; Herbert and Harper, 1985; Sebasta et al., 1981), there are no studies on its behavior in plant species. In order to maximize the efficacy of thuringiensin aimed at insects, it is important to understand how much of the insecticide is absorbed and the extent of its translocation out of the site of application in plants. The objectives of this research were to investigate thuringiensin absorption, translocation, and metabolism when applied to roots or leaves of corn.

METHODS AND MATERIALS

Plant Culture. Corn (*Zea mays* L. cv. Sunbelt 1860) was grown from seed in a greenhouse (24/20 °C day/night) in planter flats (Styrofoam flats of 66 × 33 × 12.7 cm) containing sand. Plants were watered and fertilized with half-strength Hoagland and Arnon (1950) nutrient solution as needed.

[14C]Thuringiensin Application. At the three-leaf stage of corn, uniform plants were selected and transferred to 1-qt darkened jars with 900 mL of half-strenth Hoagland and Arnon solution, pH 7–7.5. The jars were then trans-

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Table I. [^{14}C]Thuringiensin Found in Hoagland's Solution, Root Wash, and Corn after Root Application[a]

harvesting time, days	^{14}C in Hoagland's soln[b]		^{14}C in root washings[b]		^{14}C absorbed[b]		recovered,[b]
	dpm	% appl	dpm	% appl	dpm	% appl	% appl
3	59119 a	73.2 a	13146 a	16.3 a	3174.4 b	3.9 b	93 a
7	57655 a	71.3 a	13432 a	16.6 a	6442.3 a	7.9 a	96 a

[a] Data are averages of two experiments (six replications). [b] Values in a column followed by the same letter are not significantly different as determined by the LSD test at the 0.05 level of probability.

ferred to a laboratory with 30/20 °C (day/night) temperature and photosynthetic photon flux density (PPFD) of 200 μE·M^{-2}·s^{-1} for 14 h. After 48 h of acclimatization in the above nutrient solution, 100 μL of [^{14}C]thuringiensin (80000 dpm, specific activity 0.72 μCi/mg, 92% radiochemical purity) was added to each jar. The thuringiensin used in this work was supplied by Abbott Laboratories (North Chicago, IL) as ABG 6162, Lot XDZ41. Hoagland and Arnon solution was added to the jars every 2 days to maintain the original volume of the liquid, and the roots were aerated by bubbling air through the solution. For foliar application, 100 μL of [^{14}C]thuringiensin (80000 dpm) in 0.1% (v/v) X-77 (surfactant) was spotted on the first true leaf.

Uptake and Translocation. Absorption and translocation of [^{14}C]thuringiensin in corn was determined quantitatively by combustion of plant parts. For root treatments, plants were harvested 3 and 7 days after treatment. The roots were rinsed twice in 25 mL of distilled water and blotted dry. A 2-mL aliquot of the root wash solution was added to 15 mL of scintillation solution and assayed for radioactivity with a Beckman LS 5800 liquid scintillation counter. The ^{14}C remaining in the Hoagland and Arnon solution was also measured by assaying 2 mL from each jar. The plants were divided to youngest leaf and shoot. The plant parts were dried at 50 °C for 48 h, weighed, and ground in a mill. Each 50-mg sample of the ground tissue was taken and combusted in a sample oxidizer (OX-300; P. J. Harvey Instrument Corp., Hillsdale, NJ). The CO$_2$ was trapped in 15 mL of CO$_2$ absorber and scintillation fluid, and the radioactivity was quantified as described above. Radioactivity determinations were expressed as percent of applied or calculated as dpm of [^{14}C]thuringiensin/50 mg of dry plant tissue. For foliar-applied treatments, corn plants were harvested at 3 and 7 days after treatment. The treated leaves of each plant were washed in 25 mL of distilled water. The ^{14}C in the leaf wash was assayed by a liquid scintillation counter from a 0.5 mL of aliquot. The plants were separated into shoots and roots. The ^{14}C in each plant part was quantified as described above.

Metabolism of Leaf-Applied [^{14}C]Thuringiensin by Corn Shoot. Ground shoot tissue saved from the absorption and translocation study was used for metabolism. The shoot tissue was placed in 100 mL of distilled water and homogenized with a Polytron homogenizer (Brinkmann Instrument Co., Westbury, NY) at high speed for a total of 5 min. The pellet was washed with four 20-mL portions of distilled water, and the wash solution was poured through a Buchner funnel. The resulting filtrate was transferred to a 250-mL beaker, and the pH was adjusted to 9.5 with 1 N NaOH. The filtrate was concentrated to approximately 3 mL by rotary evaporation under vacuum in a water bath at 40 °C. A 10-μL sample of the concentrated extract was spotted on 5 × 20 cm, 250-μm, thin-layer chromatography plates (Analtech, Inc., Newark, DE). All spotted plates were developed in the solvent system containing 1-butanol–acetone–acetic acid–5% NH$_4$OH–distilled water (30:15:10:10:35) for 15 cm. On

Table II. [^{14}C]Thuringiensin Found in Leaf Washings and Absorbed by Corn after Foliar Application[a]

harvesting time, days	^{14}C in leaf wash[b]		^{14}C absorbed[b]		recovered,[b]
	dpm	% appl	dpm	% appl	% appl
3	66519	82.3	9776	12.1	94.4
7	69422	85.9	10359	12.8	98.7

[a] Data are averages of two experiments (six replications). [b] Means within a column were not significantly different as determined by the LSD test at the 0.05 level of probability.

separate TLC plates, standard [^{14}C]thuringiensin was spotted and developed alongside plates with extract concentrates. The distribution of radioactivity on plates was assayed by scintillation spectrometry after scraping 15 1-cm^2 bands from the origin to the solvent front into scintillation vials containing 10 mL of scintillation cocktail. Quench corrections were made by an external standard method, and counts were converted to dpm/cm^2 band. Two plates were used for each treatment, and the average of the two is presented.

Statistical Analysis. Experiments were repeated with at least three replications each time. An analysis of variance was performed for the combined experiments. Where a significant F value was found, treatment means were separated by the LSD test at the 0.05 probability level.

RESULTS AND DISCUSSION

Thuringiensin Uptake. For the root application, the ^{14}C recovered in the nutrient solution, root washing, and whole plant was 93 and 96% of the applied amount at 3 and 7 days of exposure, respectively (Table I). Corn roots absorbed more [^{14}C]thuringiensin at 7 days compared to 3 days after treatment. Root systems, mainly fibrous roots, were larger (210 mg/plant) at 7 days than at 3 days (173 mg/plant) after treatment, and this might have increased the absorption of the insecticide. About 73 and 71% of the applied ^{14}C was found in the nutrient solution 3 and 7 days after application, respectively. The root wash solution contained about 16% of the applied radioactivity. On the whole, less than 10% of the [^{14}C]thuringiensin was absorbed even 7 days after treatment.

Similarly, only 12% of the foliar-applied insecticide was detected in the whole plant, and corn absorbed similar amounts of [^{14}C]thuringiensin 3 and 7 days after treatment (Table II). More than 80% of the applied radioactivity was found in the leaf wash at both times of harvest, indicating that a large proportion of the compound remained on the treated leaf surface without penetrating the leaf tissue. Unlike the root treatment, there was no significant difference in the amount of leaf-absorbed thuringiensin between 3 and 7 days of exposure. In addition, corn absorbed more leaf-applied than root-applied thuringiensin.

It is apparent that thuringiensin is not readily absorbed by roots and leaves. Because this insecticide is primarily targeted for leaf-eating insects, foliar application would be of major interest. Work with ^{14}C-labeled compounds indicates that there are two routes by which molecules may

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Table III. Distribution of ^{14}C in Various Parts of Corn Harvested 3 and 7 Days after Root Application of [^{14}C]Thuringiensin[a]

plant part	3 days		7 days	
	% of abs radioact[b]	dpm/g[b]	% of abs radioact[b]	dpm/g[b]
youngest leaf	1.7 b	558 b	0.7 b	282 b
shoot	1.8 b	576 b	0.9 b	362 b
root	96.8 a	30840 a	98.4 a	41800 a

[a] Data are averages of two experiments (six replications). [b] Values in a column followed by the same letter are not significantly different as determined by the LSD test at the 0.05 level of probability.

Table IV. Distribution of ^{14}C in Various Parts of Corn Harvested 3 and 7 Days after Foliar Application of [^{14}C]Thuringiensin[a]

plant part	3 days		7 days	
	% of abs radioact[b]	dpm/g[b]	% of abs radioact[b]	dpm/g[b]
treated leaf	84.8 a	365340 a	79.8 a	360598 a
shoot	10.3 b	3300 b	16.4 b	3346 b
root	4.8 b	2420 b	3.8 b	2050 b

[a] Data are averages of two experiments (six replications). [b] Values in a column followed by the same letter are not significantly different as determined by the LSD test at the 0.05 level of probability.

traverse the distance from the leaf cuticle surface into the living inner cells: a lipoid route and an aqueous pathway (Crafts, 1961). Compounds that enter via the aqueous pathway penetrate slowly, and their penetration is greatly affected by environmental factors, especially relative humidity. Thuringiensin is a nucleotide derivative, with high water solubility and molecular weight of 700. From its physicochemical properties, thuringiensin would be expected to enter the leaf through the aqueous phase.

Thuringiensin is not a contact poison, and it should be ingested to be effective against leaf-eating insects. For this, it has to be absorbed by the leaf or remain stable on the leaf surface. However, as our results showed, it is not readily absorbed and the data for the leaf wash indicated it could easily be removed from the surface by water, i.e. rain and perhaps dew droplets in the morning.

Thuringiensin Translocation. Quantitative measurements (Table III) showed that more than 95% of the absorbed radioactivity remained in the root. Higher concentrations of the insecticide were detected in roots at 7 days (2090 dpm/50 mg) than at 3 days (1542 dpm/50 mg). This could be attributed to more actively growing roots being in contact with the insecticide in the solution. However, time of exposure did not alter the distribution pattern of [^{14}C]thuringiensin in the plant as evidenced by the lack of significant difference in the concentration of ^{14}C between the youngest leaf and the rest of the shoot (Table III).

For foliar-applied thuringiensin, about 80% of the absorbed radioactivity remained in the treated leaf (Table IV). Data indicated that time of exposure did not have a significant effect on the amount of ^{14}C translocated out of the shoot. Less than 5% of the absorbed ^{14}C moved from treated leaf to root. The amount of ^{14}C detected in the root is not significantly different from that of the untreated shoot. In addition, time of exposure did not significantly influence the relative distribution of ^{14}C in shoots and roots.

Maximum effective control of insects can be gained if a compound is translocated to plant parts that did not have direct contact at the time of spraying. A lack of systemic

Table V. R_f Values of [^{14}C]Thuringiensin and Corn Shoot Extracts[a]

R_f	^{14}C distribution (% rec)		
	3 days	7 days	std[b]
0.2	19	24	6
0.5	60	54	89
0.8	21	22	5

[a] Values are means of three chromatograms. [b] Standard authentic [^{14}C]thuringiensin.

action by thuringiensin means that the insecticide should be applied in sufficient water to achieve adequate coverage or multiple application may be required.

Metabolism of Leaf-Applied [^{14}C]Thuringiensin by Corn Shoot. Thin-layer chromatography was found to be a sensitive detection method for thuringiensin with detection limits as low at 1 μg. The migration of corn shoot extract on TLC plates was similar at 3- and 7-day exposures (Table V). There was no evidence for the metabolism of thuringiensin for either treatment time. Of the total ^{14}C recovered from the chromatograms of corn shoot extracts, 60 and 54% for 3 and 7 days, respectively, were chromatographed with [^{14}C]thuringiensin with R_f 0.5. The ^{14}C retained at R_f 0.2 was 19 and 24% for 3- and 7-day exposure, respectively. It is possible that the ^{14}C-labeled compound remaining at 0.2 may have been intact parent insecticide bound to minute shoot residues that did not migrate from the origin. ^{14}C recovered at R_f 0.8 was 21 and 22% for 3 and 7 days harvest, respectively. There was no significant difference in the amount of radioactivity observed at R_f 0.2 and 0.8. Similarly, about 5% of the authentic thuringiensin was observed at R_f 0.8 and was similar to what was observed at R_f 0.2.

Thuringiensin is deactivated by dephosphorylation. In mammalian systems, the combination of acidic pH and phosphatase is effective in breaking this compound; however, in plants the compound is not readily absorbed and metabolized. Wolfenbarger et al. (1972) reported that thuringiensin persisted in cotton leaves 6 to 12 days without degradation.

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