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Translocation of Uniconazole After Trunk Injection of Silver Maple Saplings

Geoffrey P. Arron

Biological Research Section, Building KD120, Ontario Hydro Research Division, 800 Kipling Avenue, Toronto, Ontario, Canada M8Z 5S4

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Abstract. Four-year-old silver maple (*Acer saccharinum L.*) saplings were trimmed and trunk injected with ^{14}C-labeled uniconazole [(E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-l-yl)-1-penten-3-ol] in May of 1987. Inhibition of shoot growth was observed in 1988 but not in 1987. The ^{14}C-activity detected in the foliage during 1987 and 1988 increased in a linear fashion, and the maximum concentration reached in 1988 was about one third that reached in 1987. Approximately 7.1 and 2.3% of the total ^{14}C-activity injected into the saplings was present in senescing foliage harvested in the fall of 1987 and 1988, respectively. Most of the ^{14}C-activity in the saplings 17, 134, or 500 days after injection remained around the injection site. Between 29 and 49% of the ^{14}C-activity found in the foliage collected in the fall was associated with metabolites rather than uniconazole, and there was no increase in the proportion in metabolites from 1987 to 1988, suggesting that metabolism occurred in the foliage and not the stem.

Trees under electrical distribution lines are trimmed regularly. A reduction in the rate of regrowth after trimming would reduce the frequency of trimming and, as a consequence, costs.

Paclobutrazol [(2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1,2,4-triazol-l-yl) pentan-3-ol)], flurprimidol [(α-(1-methylethyl)-α-(4-trifluromethoxy) phenyl)-5-pyrimidine-methanol)], and uniconazole [(E)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-l-yl)-1-penten-3-ol)], inhibit gibberellin biosynthesis which results in suppression of shoot internodal growth (Dalziel and Lawrence 1984, Izumi et al. 1985, Sterrett and Tworkoski 1987). All have been reported to inhibit tree growth (Aron et al. 1985, Arron 1985, 1986, Greene 1986, Hare 1984, Marini 1986, Miller 1982, Steffens 1988, Sterrett 1985, 1988, Sterrett and Tworkoski 1987). Trunk injection offers advantages over other application methods in that the amount applied is precise, chemical contact with nontarget plants is prevented, and environmental residues are reduced (Sterrett 1988).

Previous studies have shown paclobutrazol, flurprimidol, and uniconazole are translocated via the xylem to the shoots and leaves (Early and Martin 1988, Sterrett 1985, Sterrett and Tworkoski 1987, Wang et al. 1986). Both Richardson and Quinlan (1986) and Early and Martin (1988) reported little or no movement of paclobutrazol out of foliage. In these translocation studies, young plant material was used and the studies were short-term, terminating 42 days or less after initiation. This study was designed to determine the effect of trunk-injected uniconazole on the growth of field-grown silver maple (*Acer saccharinum L.*) saplings 18 months after application, movement of uniconazole in the saplings, the proportion of applied uniconazole that was present in foliage in the first and second fall after application, and the amount of unmetabolized uniconazole in that foliage.

Materials and Methods

Trunk-Injection Procedure

Four-year-old silver maple saplings (Wesleyville, Ontario) were trimmed (April 29, 1987) prior to trunk injection with uniconazole (May 25). Approximately one third of the canopy was removed. Two principal experiments were conducted. In the "foliage" experiment, the translocation of uniconazole into foliage was determined over two growing seasons after application. In the "harvest" experiment, the distribution of uniconazole within saplings was determined when whole saplings (foliage, stem, and roots) were harvested at various times after application. In a secondary experiment, the concentration of uniconazole in foliage was determined as the foliage changed color in the

fall. Saplings in half the plot were used in the foliage experiment and those in the other half in the harvest experiment. In both experiments, saplings were selected at random for injection with uniconazole. Saplings were injected using the miniature pressure injector of Sterrett and Creager (1977) with 18 mg ^{14}C-uniconazole (phenyl ring-labeled) (0.128 MBq/sapling; specific activity, 7.096 MBq/g) in 0.9 ml isopropyl alcohol, and then with 0.4 ml isopropyl alcohol. The sampling was injected approximately 40 cm above the soil surface. Control saplings were injected with 1.4 ml isopropyl alcohol. Injection holes were filled with silicone sealant to prevent pathogen entry. In the foliage experiment, five saplings were injected with uniconazole and five with alcohol. One of the five experimental saplings was not injected successfully in that very little ^{14}C-activity appeared in the foliage with time (compared to the four remaining saplings). The data presented in the results section are for the four remaining saplings only. In the harvest experiment, 12 saplings were injected with uniconazole and six with alcohol. A further single sapling injected with uniconazole, and situated among the harvest experiment saplings, was used in a secondary experiment in which foliage color was correlated with uniconazole content.

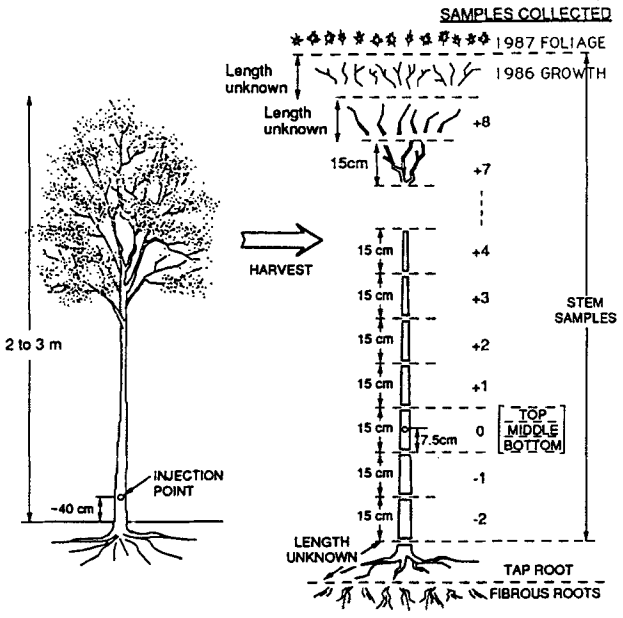

Fig. 1. Foliage, stem, and root samples collected from silver maple saplings harvested immediately after injection with ^{14}C-uniconazole (day 0), 17, 134, and 500 days later. On day 134, a 1987 shoot growth sample was collected in addition to those shown above, and on day 500 1987 and 1988 shoot growth samples were collected. Details in Fig. 2.

Measurement of Growth

In the fall of 1987 and 1988, extension growth of all shoots and sprouts (originating at the root collar) on the saplings in both the foliage and harvest experiments (those harvested 134 and 500 days postinjection) was measured to the nearest 0.5 cm. Those with a length of 1 cm or less were recorded as being 1 cm. Median shoot lengths were compared using a Mann-Whitney nonparametric test.

Harvesting of Foliage, Stem, and Root Samples

In the foliage experiment, 30 leaves were picked at random from each sapling immediately after injection (day 0) and then 7, 17, 25, 35, 45, 65, 85, 101, 122, 133, 375, 416, 444, 472, and 497 days later. On day 133 and 497, all remaining foliage was removed. In the harvest experiment, three saplings injected with uniconazole were dug up and cut into sections as outlined (Fig. 1) on day 0, 17, 134, and 500. Saplings harvested on day 134 included additional shoot samples (1987 growth) to those shown in Fig 1, similarly on day 500 (additional shoot samples of 1987 and 1988 growth). All foliage, stem, and root tissue was frozen and stored (−16°C) prior to ^{14}C-content determination.

Determination of ^{14}C-Content

Foliage was thawed and homogenized with distilled water. Homogenates were frozen overnight (−16°C), freeze-dried, and aliquots (about 0.5 g dry weight) were oxidized (Peterson 1969). The amount of $^{14}CO_2$ evolved after oxidation was determined by liquid scintillation spectrometry using a Permafluor V. Stem samples (−2 through +7, 15 cm in length, see Fig 1), with the exception of stem sample 0, were cut into three 5-cm sections. The ^{14}C-content of the middle section was determined after it was chopped, freeze-dried, ground, and approximately 0.4 g mixed with 0.1 g cellulose (to ensure an even burn) and oxidized. From the dry weight of the three sections the ^{14}C-content of the whole 15-cm stem sample was estimated and expressed on a per gram basis or as the total number of Bq. Stem samples of varied length (+8, see Fig. 1) were cut into 15-cm sections and treated as above. The remaining section and complete samples of less than 15 cm were cut into three equal lengths and the middle part treated as above. Shoots (1986, 1987, and 1988 growth) of less than 15 cm in length were chopped up and the ^{14}C-content determined, and those 15 cm or longer were treated in the same way as the +8 stem samples. Stem sample 0 (containing the injection point) was cut into three 5-cm sections (top, middle, and bottom) and the ^{14}C-content of each section determined. Fibrous roots were separated from the rest of the root system and their ^{14}C-content determined. With the tap root, a section between 5 and 10 cm below the cut surface of the stump was removed and the ^{14}C-content determined. The dry weight of the complete tap root was used to calculate the ^{14}C-content.

Metabolism

Samples of the freeze-dried foliage homogenates (2.5 g) were extracted with methanol followed by methylene chloride (Sterrett 1988). Extracts were applied to TLC plates (Redi-Plate Silica Gel GF, 250 μm, Fisher Scientific Co., Unionville, Ontario, Canada) and the chromatograms developed to 15 cm in chloroform: ethyl acetate:acetic acid (3:6:1 by volume) (Sterrett 1988). An aliquot of the stock ^{14}C-uniconazole solution was run on each plate as a reference. Developed chromatograms were divided into 10 sections, the gel scraped off, and the ^{14}C-content quantified by liquid scintillator spectrometry using a Picofluor 40. With 1988 foliage samples, duplicate 5 g aliquots of freeze-dried homogenates were extracted as above, and then pooled prior to

TLC, because the ^{14}C-content of the tissue was lower than in 1987. The results for extracts from the three saplings in the harvest experiment or the four saplings in the foliage experiment were pooled for data analysis.

Determination of Foliage Color

From the single sapling in the secondary foliage color experiment, three 15 leaf samples were collected at random 498 and 512 days after injection with ^{14}C-uniconazole. The color of each leaf was determined (Munsell Colour Chart for Plant Tissues, Kollmorgen Corp., Baltimore, MD, USA) and the number of red, yellow/red, yellow, and green/yellow recorded. A mean value was calculated from the three samples. The ^{14}C-content of the foliage samples was determined as described above.

Table 1. Effect of uniconazole on median shoot length in silver maple saplings during the 1987 and 1988 growing seasons (foliage experiment).

		Median shoot length (cm)	
Treatment	Sapling	1987	1988
Control	K1	2.00	2.00
	J1	1.50	1.75
	J7	2.75	1.00
	H2	2.75	2.00
	I5	2.00	1.50
Uniconazole	K4	2.50	1.00
	J3	1.50	1.00
	I6	2.00	1.00
	I3	1.00	1.00

Results and Discussion

Growth Inhibition

Treatment of saplings with uniconazole in both the foliage and harvest experiments had no effect on the number of shoots or sprouts per sapling in either the 1987 or 1988 growing seasons (data not shown). A few sprouts were observed at the root collar, below the injection point.

Uniconazole had no significant effect on mean shoot length per sapling in 1987 or 1988 in the foliage experiment; however, inhibition of growth was observed in the harvest experiment (data not shown). Sterrett (1988) reported inhibition of shoot growth by uniconazole in several woody species. In previous studies with growth regulators (Camacho and Arron, submitted for publication), median shoot length proved to be a more sensitive measure of growth than mean shoot length. In 1988 there was a significant reduction of median shoot length in the foliage experiment (Table 1); in the harvest experiment, all three control saplings had a median shoot length of 1.5 cm as compared with 1.0 cm in the experimental. Insufficient uniconazole may have been injected into the saplings to produce a large inhibition, since Sterrett (1988) observed inhibition by uniconazole of shoot growth in 5-year-old American sycamore (*Platanus occidentalis L.*) and 7-year-old yellow-poplar (*Liriodendron tulipifera L.*) which were injected with 160 mg/tree and 240 mg/tree (respectively) in the first year postinjection but not in the second.

Translocation

During the 1987 and 1988 growing seasons, the ^{14}C-activity detected in the foliage collected from saplings in the foliage experiment increased in a linear fashion from late May to early October (data not shown). The highest concentrations of ^{14}C-activity found were 82 ± 26 Bq/g dry weight in 1987 and 27 ± 15 Bq/g in 1988 (mean ± SD). Since leaf dry weight changed little during this time, there was also a linear increase in ^{14}C-activity expressed on a per leaf basis (data not shown).

The increase in the concentration of ^{14}C-activity found in the foliage during the growing season reflects a continuous supply of uniconazole, with little or no movement out of the foliage. Over a 42-day period, Wang et al. (1986) found a continuous increase in the amount of paclobutrazol found in apple ('York Imperial' *Malus domestica* Borkh.) foliage. Early and Martin (1988) and Richardson and Quinlan (1986) reported no movement of paclobutrazol out of foliage. The lower concentrations of ^{14}C-activity found in the maple foliage in 1988 may have been the result of lower rainfall than in the previous year, which would have led to less water being lost via transpiration. The size of the foliage sink (total leaf number or dry weight) did not increase (data not shown). There was a slight decline in the reservoir of ^{14}C-activity in the saplings, as foliage containing label was lost each fall. In 1987 and 1988, 7.2 ± 1.5 and 2.3 ± 0.6%, respectively, of the total ^{14}C-activity injected was present in all the foliage removed.

In saplings harvested 0, 17, 134, and 500 days after trunk injection, ^{14}C-activity, expressed as the percent of the total ^{14}C-activity injected, was confined to stem samples around the injection site (i.e., +1, 0, −1) (Fig. 2). The combined length of these stem samples was 45 cm, with the injection point in the middle (see Fig. 1). Label was forced into these sections at injection, and there was little change in distribution with time. By 17, 134, and 500 days, a little ^{14}C-activity was detected higher in the stem and foliage. Little ^{14}C-activity was detected in the

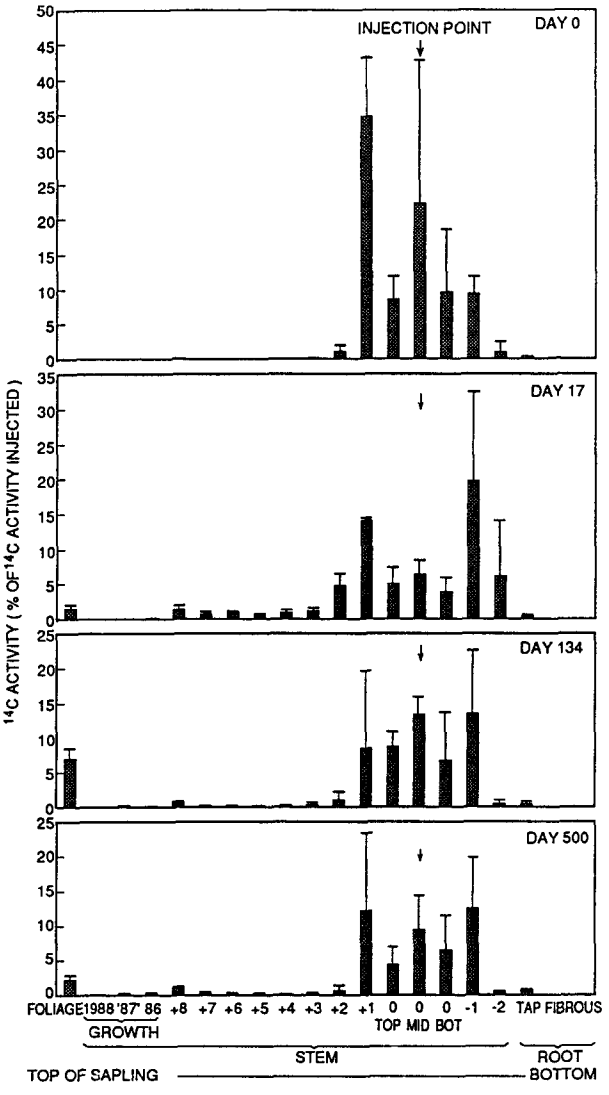

Fig. 2. Location of ^{14}C-activity in silver maple saplings harvested immediately (day 0), 17, 134, and 500 days after injection with ^{14}C-uniconazole. The bar represents the mean value, and the vertical line the standard deviation.

tap root, suggesting little or no downward movement. Sterrett (1988) reported no downward movement of ^{14}C-uniconazole in apple. In previous more short-term experiments, labeled flurprimidol, paclobutrazol, and uniconazole remained predominantly around the injection site (Sterrett 1985, 1988, Sterrett and Tworkoski 1987). Retrieval of ^{14}C-activity in whole saplings declined from 86.7 ± 7.1% at day 0 to 52.7 ± 17.8% at day 500 (Table 2). A similar decline in ^{14}C-activity recovered was reported for paclobutrazol and flurprimidol (Sterrett 1985, Sterrett and Tworkoski 1987). While there was a marked decline in ^{14}C-activity (expressed as a function of ^{14}C-activity injected) in the combined stem samples, +1, 0, and −1 with time, the decline was much less apparent when the data were expressed as a function of ^{14}C-activity retrieved (Table 2).

In the foliage experiment, there was an increase in ^{14}C-activity (expressed in either way) from 0 to 134 days, with lower activity at day 500 (Table 2). The foliage data (expressed as a function of ^{14}C-activity injected) are very similar to that from the foliage experiment, suggesting that periodic removal of leaves in the latter experiment had little if any effect on translocation of labeled material into the foliage.

The ^{14}C-activity distribution data discussed above are in the form of a mass balance and provide no information on the concentration of ^{14}C-activity. The ^{14}C-activity data for the 500-day saplings (Fig. 2) were expressed on a Bq/g dry weight basis (Fig. 3). Around the injection site, ^{14}C-activity was extremely high (about 725 Bq/g dry weight in the middle of stem sample 0), while in the rest of the sapling activity was extremely low (20 Bq/g or less). Data for days 17 and 134 were similar. From these data, in saplings harvested at 134 and 500 days, the concentrations of uniconazole in shoots formed in 1987 were calculated to be 1.0 and 1.3 μg/g dry weight, respectively. In 1988 shoots (saplings harvested at 500 days), the concentration was 0.8 μg/g dry weight. In these calculations it was assumed that all ^{14}C-activity detected was in uniconazole and not the metabolites. A higher value, 5 μg/g dry weight, was reported for uniconazole in apple shoot tissue that exhibited growth inhibition (Sterrett 1988).

Foliage, which was removed from saplings in both experiments in the fall of 1987 and 1988, had begun to turn. Waiting longer might have resulted in loss of foliage, making the determination of total ^{14}C-activity present in all the foliage impossible. To determine if uniconazole was withdrawn from the foliage as chlorophyll was being broken down, an experiment was conducted in which foliage was removed from a sapling 498 and 512 days after injection. While leaf color had changed from green/yellow to yellow or yellow/red (data not shown), the concentration of ^{14}C-activity detected in the foliage was 2.2 ± 0.6 Bq/leaf at 498 days and 2.4 ± 0.5 Bq/leaf at 512, suggesting no withdrawal of label from the foliage.

Metabolism

To determine what proportion of the ^{14}C-activity detected in foliage was ^{14}C-uniconazole versus metabolites, extracts were prepared from samples, collected on days 134 and 497 of the foliage experi-

Table 2. ^{14}C-Activity in tissue of silver maple saplings harvested 0, 17, 134, and 500 days after trunk injection with ^{14}C-uniconazole.

Days after injection	^{14}C-activity retrieved: Whole saplings	% of total ^{14}C-activity injected (as % of total ^{14}C-activity retrieved): Stem samples (+1, 0, −1 combined)[b]		Foliage	
0	86.7 ± 7.1[a]	84.1 ± 8.8	(97.0 ± 3.5)	0.0 ± 0.0	(0.0 ± 0.0)
17	67.6 ± 25.6	49.1 ± 18.0	(72.9 ± 4.8)	1.5 ± 0.7	(2.5 ± 1.6)
134	62.6 ± 5.3	51.1 ± 4.5	(81.2 ± 4.2)	7.1 ± 1.7	(11.3 ± 2.5)
500	52.7 ± 17.8	45.0 ± 18.7	(84.2 ± 8.0)	2.3 ± 0.6	(4.6 ± 1.8)

[a] Values are given as mean ± SD (N = 3).
[b] See Fig. 1.

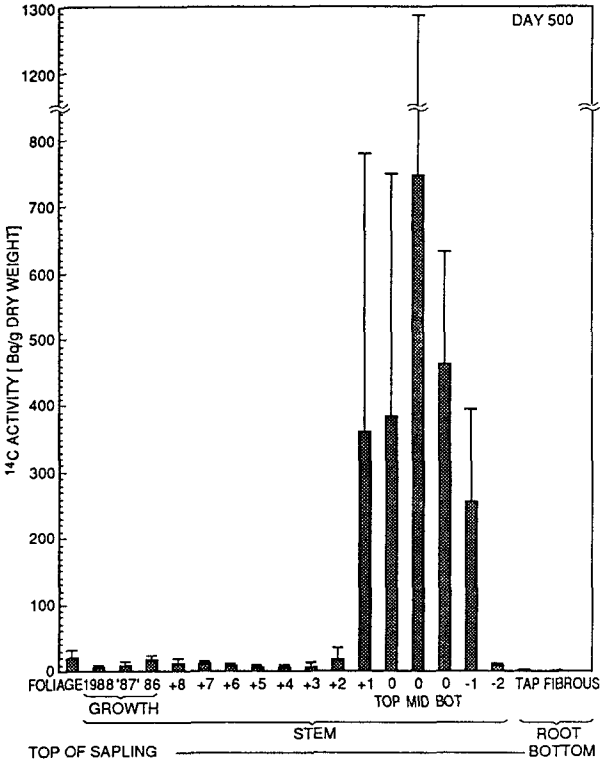

Fig. 3. Location of ^{14}C-activity (expressed as Bq/g dry weight) in silver maple saplings harvested 500 days after injection with ^{14}C-uniconazole. Details as in Fig. 2. Note the break in the vertical scale.

ment, and their components separated. Of the ^{14}C-activity detected, 53 and 71%, respectively, was uniconazole (R_f = 0.7–0.9) (Table 3). Similar results were obtained with extracts from foliage collected on days 134 and 500 in the harvest experiment, where 51 and 55%, respectively, of the activity detected was uniconazole. The lack of an increase in the percentage of ^{14}C-activity in metabolites from 1987 to 1988 suggests that metabolism of uniconazole occurs in the foliage and not the stem. If metabolites were formed in the stem and translocated to the foliage, then the proportion of metabolites found in the foliage would increase from year to year. In the studies of Sterrett and co-workers, chromatography revealed the following: over 85% of the ^{14}C-activity found in young shoot tissue after 27 days was paclobutrazol; 75% of the ^{14}C-activity found after 35 days was flurprimidol; and 92% of the ^{14}C-activity found after 28 days was uniconazole (Sterrett 1985, 1988, Sterrett and Tworkoski 1987). In another 4-month experiment (Sterrett, personal communication), chromatography showed that approximately 50% of the ^{14}C-activity found in young apple shoots (including foliage) was uniconazole, suggesting a correlation between shoot age and the proportion of metabolites formed. However, Early and Martin (1988) reported that 9 days after treatment of peach seedlings, between 82 and 92% of the ^{14}C-activity found in foliage was not associated with labeled paclobutrazol.

Table 3. Distribution of ^{14}C-activity in extracts from foliage collected in the fall of 1987 and 1988 from silver maple saplings trunk injected with ^{14}C-uniconazole in spring 1987 (foliage experiment).

Fraction on chromatography plate counted (R_f values)	1987[a] Extract	1987[a] Uniconazole reference	1988[a] Extract	1988[a] Uniconazole reference
	% ^{14}C-activity on plate			
0.0–0.1	11.0	0.1	5.5	0.1
0.1–0.2	13.2	0.0	8.7	0.0
0.2–0.3	5.9	0.1	2.9	0.0
0.3–0.4	3.3	0.1	0.7	0.0
0.4–0.5	3.5	0.1	3.0	0.0
0.5–0.6	3.6	0.1	3.8	0.0
0.6–0.7	3.1	0.1	3.3	0.1
0.7–0.8	20.9	10.7	17.7	8.5
0.8–0.9	32.2	87.8	53.1	90.1
0.9–1.0	3.5	1.1	1.9	1.2

[a] Foliage collected 133 and 497 days after injection of ^{14}C-uniconazole.

Of the uniconazole injected into silver maple saplings, only a small amount was translocated from the injection site over the 18-month period. A small proportion was found in senescing foliage, when saplings were stripped in the first and second fall after application. An equal ratio of uniconazole to metabolites was found in foliage collected in the fall.

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