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Translocation and Degradation of Injected Uniconazole in Apple During a 4-Month Growing Period

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Abstract One-year-old 'Golden Delicious' apple trees grafted onto MM 106 rootstocks were injected in the rootstock stem (shank) with ^{14}C-uniconazole to determine the extent to which uniconazole is translocated and degraded over the length of an average growing season. In 4 months, 16% of recovered ^{14}C-activity was translocated to the new shoots. Most of the ^{14}C-activity remained in the rootstock. Chromatographic evaluation of shoot extracts demonstrated that the ^{14}C-activity associated with uniconazole decreased 49% in 4 months. However, shoot growth was still inhibited which suggests that the amount of uniconazole that was degraded did not interfere with the inhibition of gibberellin biosynthesis, probably due to the continuous translocation of uniconazole that occurred.

There is much interest in the use of growth inhibitors to retard the growth of fruit and amenity trees for at least an entire growing season. Mechanical pruning is very expensive. Uniconazole [(E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol] is one of a group of new plant growth regulators that inhibits gibberellin biosynthesis (Izumi et al. 1985, Sterrett 1985, 1988). In earlier research, growth of 1-year-old trees of 'Golden Delicious' apple was inhibited 28 days after injecting the stem with 2 mg of ^{14}C-labeled uniconazole; 2% of the ^{14}C-activity translocated into the new growth and 92% remained undegraded (Sterrett 1988).

The objective of this study was to determine the extent to which uniconazole is translocated and degraded in apple over a 4-month period—the approximate length of a growing season.

Materials and Methods

Translocation

Dormant apple trees were transferred from cold storage to 28-cm diameter pots filled with a mixture of loam:sand:peatmoss:perlite (3:1:1:1 by volume) in a controlled environment chamber at 25 ± 1°C, 65 ± 10% relative humidity, and 158 μmol s^{-1} m^{-2} photosynthetically active radiation (400–700 nm) (14-h photoperiod). Before new shoot growth occurred, the rootstock was injected 5 cm above the root/stem transition zone of the rootstock with 1.0 mg ^{14}C-uniconazole (phenyl-ring labeled) (2.5 μCi/tree; specific activity, 74.6 mCi/mmol) in 0.5 ml methanol (Sterrett 1979). The top was pruned to 80 cm above the injection site and all buds on the scion were removed except those located in the top 20-cm section. To insure inhibition, 1.5 months later a second dose of unlabeled uniconazole (1.0 mg) was injected into the rootstock 4 cm above and opposite the first injection site. One sample of three trees (three replications) was harvested and assayed immediately after injection. Four trees/time period (four replications) were harvested and assayed for ^{14}C-activity 2, 3, and 4 months after injection. A 1-month assay was conducted in an earlier study (Sterrett 1988). Trees were arranged in a randomized block design. Harvesting consisted of separating each tree into two parts: (1) a 25-cm long rootstock and (2) a 60-cm long scion containing new shoot growth (leaves and stems) located in the top 20 cm. The wood was separated from the bark of the 1-year-old scion and new shoots and assayed individually for activity. Harvested plant parts were immediately freeze-dried, ground to 0.5 mm in a Wiley mill, and oxidized (Petersen 1969). Ground tissue samples from three trees/time period (nine trees total) were retained for extraction and chromatography. The amount of $^{14}CO_2$ evolved was quantitated by liquid scintillation spectrometry using Reich solution (Petersen 1969). A group of three control trees/time period (nine trees total) was used for growth inhibition determinations and to provide extracts of untreated new growth material for reference samples in the metabolism research.

Degradation

Samples of the dried, ground new-growth tissue (0.5–1.0 g, leaves and stems) from the scion of 1-year-old apple trees 2, 3, or 4 months after ^{14}C-uniconazole injection were extracted with

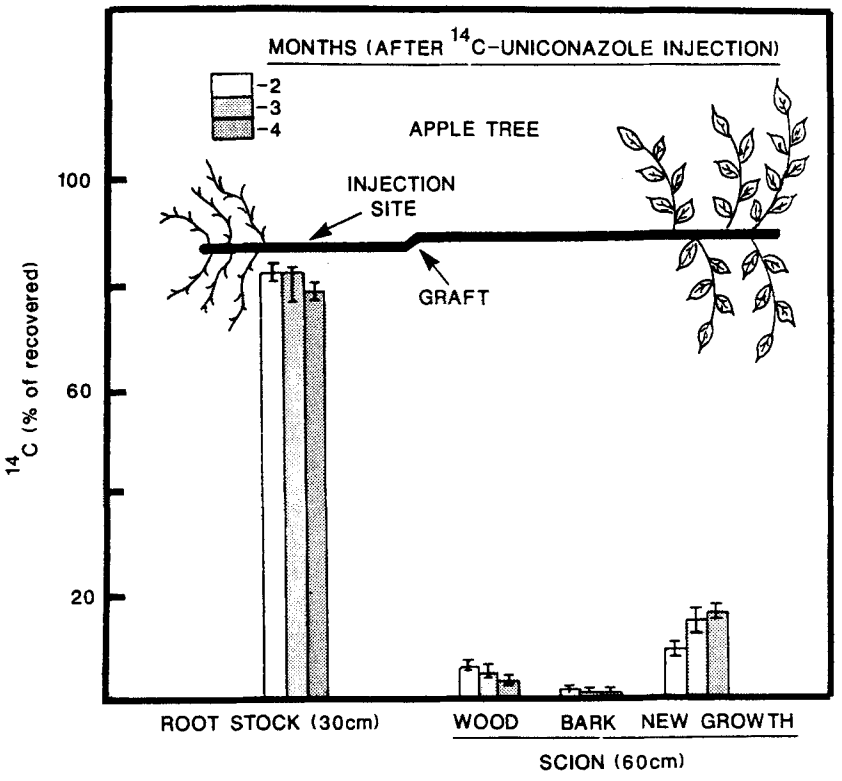

Fig. 1. Location of ^{14}C-activity recovered from 1-year-old 'Golden Delicious' apple trees 2, 3, and 4 months after injection with ^{14}C-uniconazole. Immediately after injection, 96% of ^{14}C-activity recovered was detected in the rootstock and 4% in the scion. Vertical lines indicate SEM.

Table 1. Growth of apple tree shoots and translocation of ^{14}C-uniconazole into new shoot growth.

Time after injection (months)	Growth in length/new shoot (cm)		Dry weight (g)[a]	^{14}C-uniconazole detected in new shoots[a]	
	Treated	Control	Treated	dpm/mg	% recovered
2	10 ± 1.1[b]	14 ± 0.7	16 ± 1.3	33.6 ± 4.4	9.8 ± 0.5
3	13 ± 1.2	16 ± 1.1	22 ± 2.3	32.4 ± 5.3	14.2 ± 2.8
4	11 ± 0.4	21 ± 1.3	26 ± 1.8	33.2 ± 2.7	16.2 ± 0.5

[a] Includes total leaves and stems of new shoot growth/apple tree.
[b] SEM used to determine variation (four replications for treated trees; three replications for control trees).

80% methanol. The methanol extract contained an average of 84% of the ^{14}C-activity, which was detected in the translocation experiment. Water was added and the extract evaporated to an aqueous solution (pH 7.0) followed by partitioning with methylene chloride. Acetone extracts, which contained an average of 45% ^{14}C-uniconazole detected in the methanol extraction, were applied to thin-layer chromatography (TLC) plates [Preadsorbent Silica Gel 7010-Si250F.PA (19c), J. T. Baker Chemical Co.], and the chromatograms were developed for 15 cm in chloroform:ethyl acetate:acetic acid (3:6:1, vol:vol:vol). In addition, extracts of new growth tissue obtained from untreated plants and fortified with ^{14}C-uniconazole were chromatographed as references. Developed chromatograms were quantified by an imaging scanner (Bioscan Inc.).

The chromatographic results of extracts from three trees/time period (three replications) were analyzed using the pooled standard error of the mean (SEM).

Results and Discussion

Translocation of ^{14}C-uniconazole occurred throughout the 2- to 4-month period (Fig. 1). Ten percent of the total ^{14}C-activity recovered was translocated into the new growth 2 months after treatment and 16% after 4 months. Also, shoot growth elongation remained inhibited for the 4-month period probably due, in part, to the continuous translocation that occurred (Table 1). Apparently, growth inhibition from uniconazole did not stop the production of assimilates found in the new shoots. Even though shoot growth was inhibited, disintegrations per minute (dpm)/mg of shoot tissue were the same at 2, 3, or 4 months because dry weight increased from

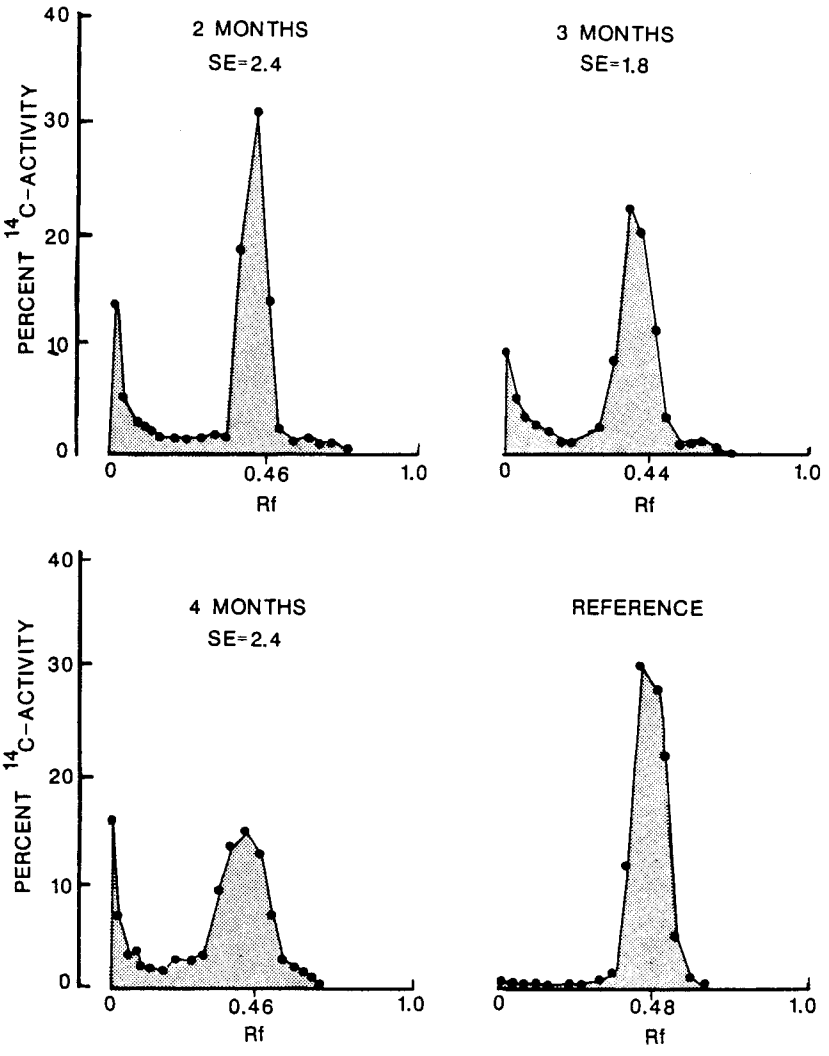

Fig. 2. Thin-layer chromatograms of methanolic extracts of new leaf and stem tissue from 1-year-old apple trees 2, 3, and 4 months after injection with ^{14}C-uniconazole. Reference chromatogram is of an extract from new tissue of untreated trees fortified with ^{14}C-uniconazole. Note the increase in polar degradation product(s) located at the Rf just above the origin. SE is the pooled standard error of Rf percentage means (three replications/time period).

16–26 g (Table 1). Immediately after injection, 96% of the ^{14}C-activity was detected in the rootstock, within 20 cm of the injection site and 4% was forced into the scion wood (Fig. 1). Translocation of ^{14}C-uniconazole was determined to be acropetal in earlier research by Oshio and Izumi (1986) and Sterrett (1988). Since no apparent basipetal movement of ^{14}C-uniconazole occurred, the most plausible explanation for the presence of ^{14}C-activity in the bark (phloem) is ^{14}C-uniconazole was translocated via the xylem whence it was translocated into the phloem. Most of the ^{14}C-activity remained in the rootstock during the 4-month period (Fig. 1).

Chromatographic evaluation of shoots extracted in 80% methanol demonstrated that the ^{14}C-activity associated with uniconazole decreased to 60% in 2 months, 55% in 3 months, and 51% in 4 months (Fig. 2). One apparent polar degradation product which increased throughout the 2- to 4-month period was located at an Rf just above the origin (Fig. 2).

Even though the inhibitory activity of uniconazole may have been reduced when it was degraded to the polar form, inhibition of shoot growth was not affected during the 2- to 4-month period after injection, probably because of the continuous translocation of uniconazole from both injections. Inhibition of new shoot growth occurred for the approximate length of a growing season, and the only visible symptoms of uniconazole throughout the 4-month period were smaller dark green foliage and shortened shoots. Therefore, uniconazole should be an effective inhibitor of woody plants for the control of both fruit and amenity trees.

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