J. AMER. SOC. HORT. SCI. 112(2):341–345. 1987.

Flurprimidol: Plant Response, Translocation, and Metabolism

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Additional index words. *Liriodendron tulipifera, Platanus occidentalis, Ligustrium ovalifolium, Phaseolus vulgaris, Malus domestica,* gibberellin, injection

Abstract. **Flurprimidol was injected into several species to evaluate effects on growth. Height growth was inhibited 85% in bean (*Phaseolus vulgaris* L. 'Black Valentine') and 90% in California privet (*Ligustrium ovalifolium* Hassk.) by the lowest flurprimidol doses (125 and 625 μg/plant, respectively). Shoot growth was further suppressed as doses increased. Gibberellic acid reversed the inhibitory effect of flurprimidol on privet. In June, height growth of field-grown yellow-poplar (*Liriodendron tulipifera* L.) and American sycamore (*Platanus occidentalis* L.) was uniformly reduced 35% by all flurprimidol doses. By late July, height growth increment decreased linearly as flurprimidol increased from 5 to 40 mg/tree. Thirty-five days after injection of 2.5 mg ^{14}C-labeled flurprimidol in 1-year-old apple (*Malus domestica* Borkh.), 10% had moved into the new shoots, 1.5% into the scion phloem, and 80% remained near the injection site. A high percentage of the ^{14}C activity was unmetabolized flurprimidol; 95% of the ^{14}C activity in the xylem, 86% in the phloem, and 75% in the shoot. Although it is not highly mobile, flurprimidol effectively inhibits shoot growth, apparently inhibiting gibberellin synthesis. Chemical names used: α-(1-methylethyl)-α-[4-(trifluoromethoxy)phenyl]-5-pyrimidinemethanol (flurprimidol).**

Utility company foresters and fruit growers are actively investigating chemical growth inhibitors for reducing the high cost of mechanical trimming (14). Trunk injection (via the vascular system) of growth inhibitors is a popular technique used to prevent chemical contact with nontarget plants and reduce environmental residues. This approach has met with limited success in a variety of woody species, necessitating a search for new compounds (1, 4, 8, 12, 13). Flurprimidol is a plant growth regulator that suppresses shoot internode elongation without causing overt injury. In this respect it is similar to α-cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidinemethanol (ancymidol) and β-[(4-chlorophenyl)methyl]-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol (paclobutrazol) and, like these compounds, flurprimidol appears to inhibit gibberellin biosynthesis (2, 10). Most research shows flurprimidol to be foliar- and root-active (3, 5, 6). Also, woody plants respond to injections of flurprimidol (S.D. Cockreham, personal communication). The objectives of this study were to determine if stem-injected flurprimidol would inhibit the growth of bean and several species of woody plants, and if this effect could be counteracted by gibberellin. Our 3rd objective was to determine the extent to which flurprimidol is translocated and metabolized in woody species.

Materials and Methods

Treatments in each of the following experiments were replicated five times in a randomized complete block design and each experiment repeated unless otherwise noted.

Growth responses. Bean seeds were sown in 10-cm-diameter pots filled with a mixture of 3 loam : 1 sand : 1 peatmoss : 1 perlite (by volume) in a controlled environment chamber at 25° ± 1°C, 60% ± 10% RH, and 158 $\mu mol \cdot s^{-1} \cdot m^{-2}$ photosynthetically active radiation (PAR) (400 to 700 nm) (16-hr photoperiod). At 10 days from emergence, beans were injected (11) in the hypocotyl cavity with 100 μl of flurprimidol in methanol at doses ranging from 0 to 2000 μg/plant. Stem height was measured immediately before treatment and 7 days later. Shoot weight and trifoliolate leaf area were measured 7 days after treatment. Leaf area was determined with a photoelectric leaf-area meter (Lambda Instruments).

California privet seedlings (2-years-old), with single 1-cm-diameter stems, were grown in the greenhouse in 1-liter pots filled with 1 loam : 1 sand : 1 peatmoss (by volume). The stems were injected (11) with 625 μg to 10 mg of flurprimidol in 0.5 ml methanol 6 cm above the soil line. Immediately following injection, part of the main shoot was excised, leaving a 10-cm stump. The number and length of new stump sprouts were determined periodically after injection. A previous study (12) with azosulfamide dye indicated that injected solutions were forced throughout the entire stump both above and below the injection site. Since the solution was forced throughout the entire stem by the pressure of injection, it is assumed that the total dose may not have been retained within the stem tissue after excision. However, the pruned stumps were perfused with the solutions.

Six-year-old yellow poplar and 4-year-old American sycamore field-planted saplings (Frederick, Md.) were treated with 5 to 40 mg flurprimidol in 4 ml methanol by pressure injection (11) on 31 May 1984. All branches were pruned to a single lateral of 2-year-old wood at budbreak in 1984. Shoot growth of three tagged branches per sapling was measured throughout the growing season.

To determine the effect of flurprimidol on various woody plant growth systems and the injection dose for the translocation study with apple, 1-year-old dormant 'Golden Delicious' apple (*Malus domestica* Borkh.) trees were placed in half-strength aerated nutrient solution (7) in 7.3-liter containers and maintained in a controlled environment at 25° ± 1°C, 65% ± 10% RH, and 158 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR (400 to 700 nm) (16-hr photoperiod). Each tree (except control) was injected 15 cm below the graft with 1.25, 2.5, or 5.0 mg of flurprimidol in 0.5 ml of methanol. In a previous study (12), 0.5 ml was found to remain below the graft after injection. Trees were pruned to 80 cm above the injection site immediately after injection. Growth of

Received for publication 4 June 1986. We thank C.R. Baker, F. Meyers, and N. Monath for technical assistance and Lilly Research Laboartories for furnishing samples of flurprimidol (Cutless, EL500). We also thank J.G. Phillips for statistical assistance. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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Table 1. Response of 2-year-old 'Golden Delicious' apple trees 34 days after injection of flurprimidol in 0.5 ml methanol.

Flurprimidol (mg/tree)	Shoot growth[z] (cm)	No. nodes[y]	No. Leaves[y]	Leaf area[y] (cm^2)		Weight/leaf[y] (g)	
				Total	Per leaf	Fresh	Dry
0	80 ± 1	36 ± 1	43 ± 0	2512 ± 8	58 ± 1	0.84 ± 0.02	0.27 ± 0.0
1.25	38 ± 8	26 ± 4	34 ± 4	1581 ± 183	46 ± 1	0.59 ± 0.02	0.21 ± 0.01
2.5	30 ± 4	28 ± 2	34 ± 1	1559 ± 283	46 ± 7	0.63 ± 0.12	0.21 ± 0.04
5.0	33 ± 12	26 ± 1	32 ± 2	1543 ± 1	48 ± 2	0.62 ± 0.08	0.20 ± 0.02

[z]Based on top 10 shoots; ± SE of the mean.
[y]Based on top three shoots; ± SE of the mean.

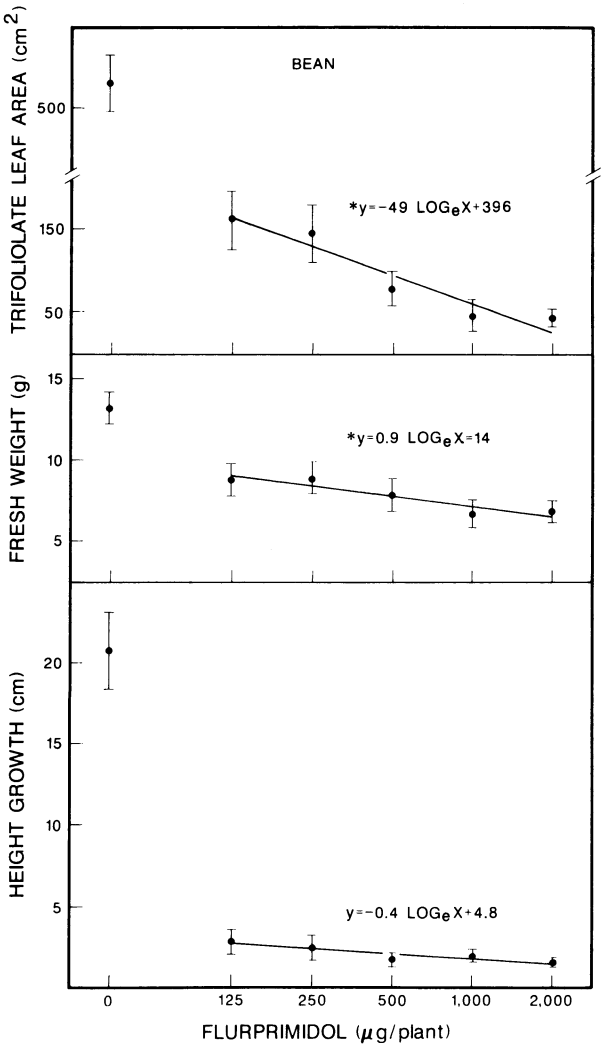

Fig. 1. Response of 10-day-old bean plants to stem injection of 100 μl of flurprimidol in methanol 7 days after injection as derived from regression analysis. Vertical lines indicate SE of the mean. Asterisk indicates significant linear regression coefficient, 5% level.

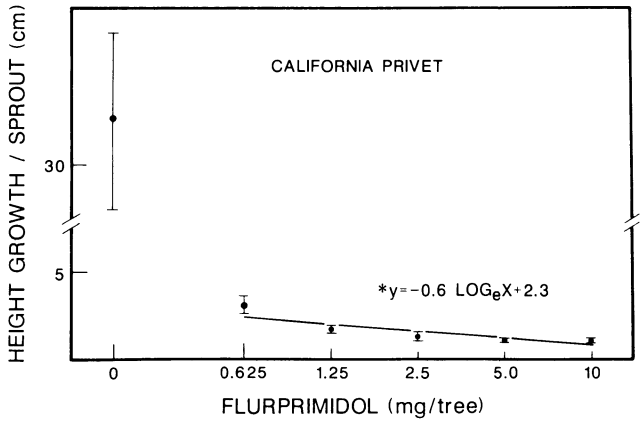

Fig. 2. Sprout growth from California privet stumps 5 months after injection of 0.5 ml of flurprimidol as derived from regression analysis. Vertical lines indicate SE of the mean. Asterisk indicates significant linear regression coefficient, 5% level.

the upper 10 shoots was measured weekly. At the end of 34 days, the number of nodes, leaf area, and weight of leaves were measured from the top three shoots. Treatments were replicated two times in a randomized complete block design.

Gibberellin effect. Nine-month-old single-shoot California privet plants were grown in half-strength aerated nutrient solution in 10-cm-diameter pots under controlled environmental conditions similar to the apple experiment above. Privet roots were either immersed in nutrient solution only or in nutrient solution containing 0.5 ppm flurprimidol (day 1). The shoots of these privet were left untreated or were dipped in 25 ppm gibberellic acid (GA_3) on day 1 or day 7. Shoot growth was determined at about 3-day intervals for 27 days. Treatments were replicated five times in a randomized complete block design. The experiment was not repeated.

Translocation. One-year-old apple trees were placed in half-strength aerated nutrient solution in 7.3-liter containers and maintained in a controlled environment similar to the environment used for apple trees in the growth experiment above (7). Dormant trees were transferred from cold storage to nutrient solution and injected immediately with 2.5 mg of ^{14}C-labeled flurprimidol (1-C labeled) [0.3 μCi/tree (1 Ci = 37 GBq)] in 0.5 ml of methanol. The stem was injected 5 cm above the root–stem transition zone and the top pruned to 80 cm above the injection site. One group of four trees was harvested and assayed. Thirty-five days after injection, when growth inhibition of the top 10 shoots was obvious, a 2nd group of four trees was harvested and assayed. Harvesting consisted of separating each tree into two parts: a) 25-cm-long rootstock containing the roots and the injection site; and b) 60-cm-long scion containing buds or new shoot growth. The wood (xylem), bark (phloem), buds, and new shoots were separated and assayed individually for activity. No buds or new shoots were obtained from section 1. Fibrous and tap roots were assayed together. Harvested plants were immediately freeze-dried, ground to 0.5 mm in a Wiley mill, and oxidized (9). The amount of $^{14}CO_2$ evolved was quantitated by liquid scintillation spectrometry using Reich solution.

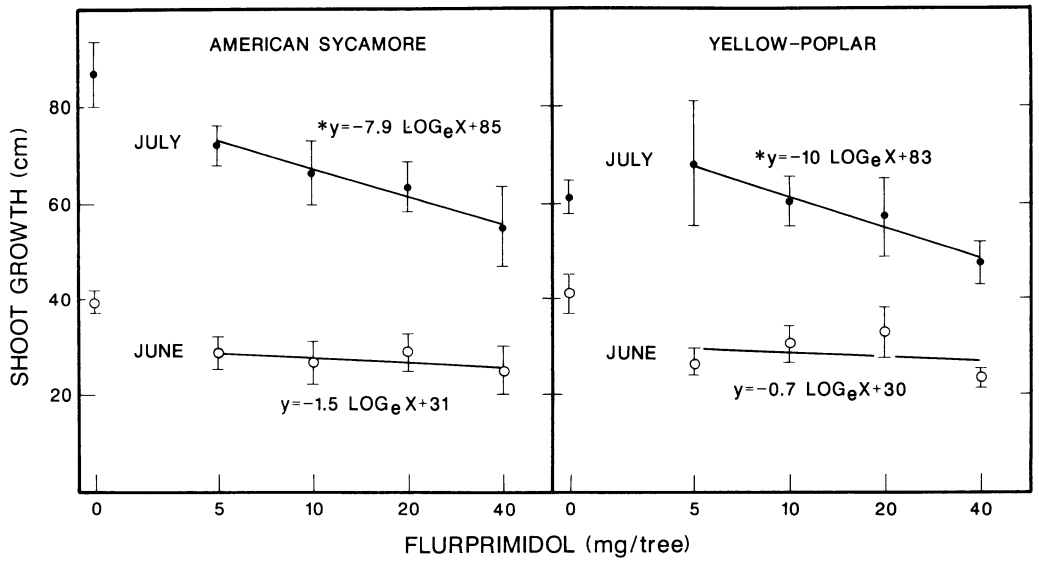

Fig. 3. Shoot growth of woody saplings in June and July 1984, after injections of flurprimidol in May. Vertical lines represent SE of the mean. Asterisk indicates significant linear regression coefficient, 5% level.

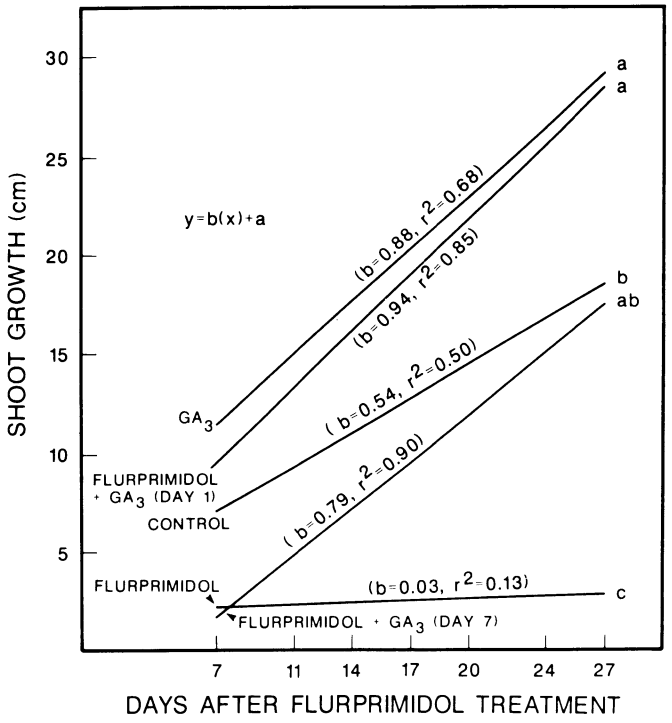

Fig. 4. Response of 9-month-old hydroponically grown California privet to root treatments of 0.5 ppm flurprimidol. Shoots were dipped in 25 ppm GA_3 immediately after root treatment or 7 days later. The slopes of linear regressions followed by a different letter are significantly different at the 1% level as determined by *t* test (N = 35).

Metabolism. Samples of the dried, ground xylem (2 g) and phloem (3 g) from the rootstock and complete shoots (3 g) from the scion of 1-year-old apple trees 35 days after injection with ^{14}C-labeled flurprimidol were extracted with methanol for 18 hr at 25°C. Water was then added, and the extract was evaporated to an aqueous solution followed by partitioning with methylene chloride. Extracts were then applied to the TLC plates (Redi-Plate Silica Gel GF, 250 μM, Fisher) and the chromatograms were developed for 15 cm in 2 chloroform : 3 ethyl acetate (by volume). A known aliquot of the reference sample was chromatographed beside all unknown samples. Developed chromatograms were quantified by an imaging scanner (Bioscan). The experiment was repeated three times and the data pooled for analyses.

Results and Discussion

Growth responses. The height growth, weight, or leaf area of bean plants treated with any applied dose of flurprimidol was less than untreated plants (Fig. 1). Weight and leaf area increment of bean plants decreased after 7 days as the dose of flurprimidol increased from 125 to 2000 μg/plant. No injury was evident except for slight chlorosis of the primary leaf veins, which was more strongly evident at doses of 1000 and 2000 μg/plant. Apparently, chlorosis of the veins was due to the interaction of methanol with flurprimidol in the formulation because plants treated with formulations containing little or no methanol in another experiment did not exhibit chlorosis.

After 5 months, sprout growth of California privet was reduced with increasing amounts of flurprimidol ranging from 0.625 to 10 mg/plant (Fig. 2). Foliage of seedlings treated with flurprimidol was smaller and darker green than that of untreated seedlings. This apparent increase in chlorophyll content was also observed by Sterrett (12) when California privet was injected with paclobutrazol. There was no apparent injury to California privet, and, unlike beans, no chlorosis occurred.

One month after flurprimidol injection (June), shoot growth of American sycamore and yellow poplar was inhibited (Fig. 3). However, in July, shoot height increments of both American sycamore and yellow poplar decreased as the dose of fluorprimidol increased from 5 to 40 mg/tree. There is a strong possibility that doses of flurprimidol higher than 40 mg/tree may inhibit yellow poplar growth for longer periods. No injury was evident on either woody species, although there were smaller dark green leaves and shortened internodal growth similar to that observed by Sterrett (12) on woody saplings injected with paclobutrazol. Also, there was no detectable injury or chlorosis to the foliage as occurred from injections of methanol solutions in bean plants.

The predictive value of the bean data was excellent. The bean injection experiment not only required a shorter time period to determine the activity of flurprimidol than the woody plant injection experiments (7 days for bean vs. 1 month for woody species), it provided a dose-response effect that could be easily extrapolated to the seedling and sapling-sized woody plants.

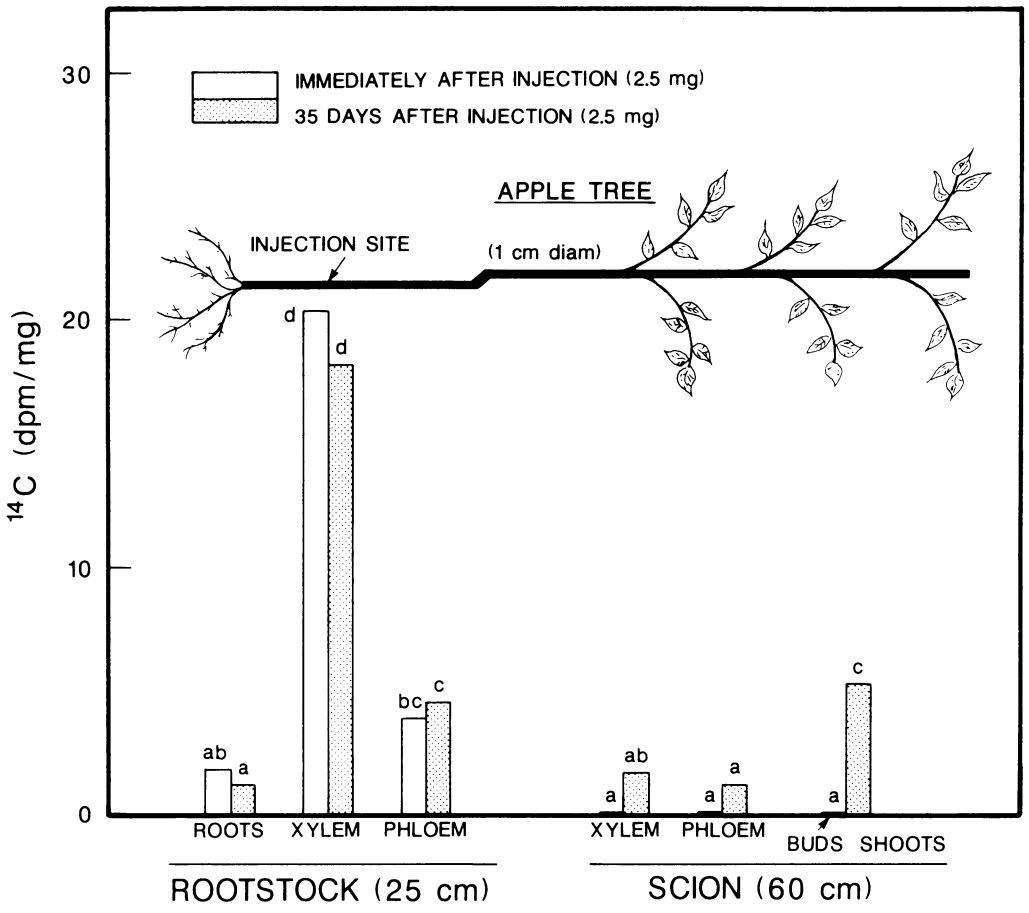

Fig. 5. Location of ^{14}C activity in young 'Golden Delicious' apple trees immediately and 35 days after injection with ^{14}C-labeled flurprimidol. Means are separated by Duncan's multiple range test, 5% level.

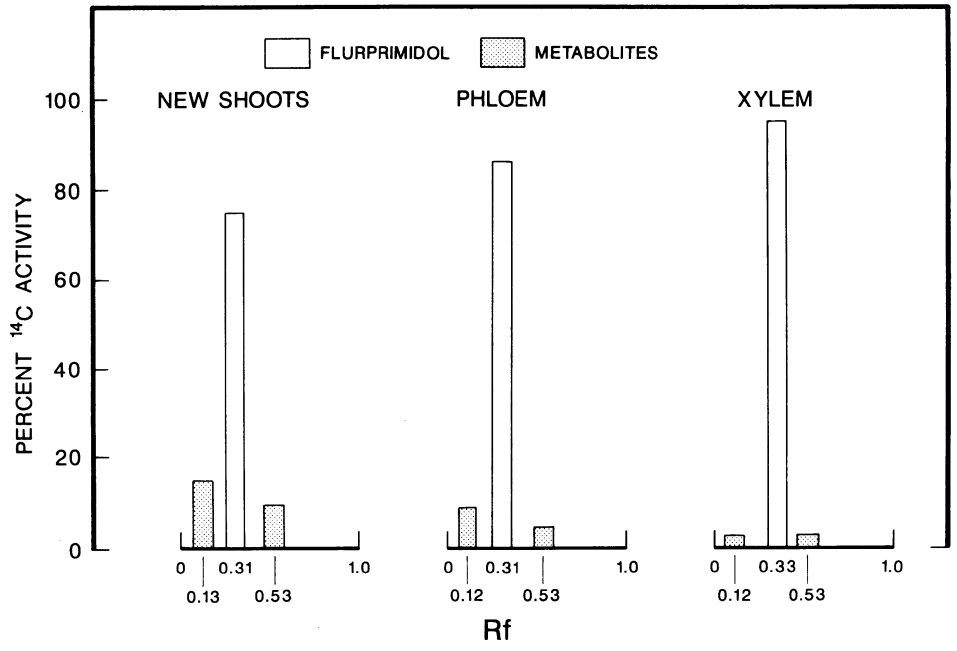

Fig. 6. Distribution of ^{14}C in extracts of xylem and phloem from rootstock and new shoot tissue from scion of apple trees 35 days after injection of ^{14}C-labeled flurprimidol (1-C labeled). Methanolic extracts were thin layer chromatrographed for 15 cm in a 2 chloroform : 3 ethyl acetate solution (v/v).

Shoot growth of apple trees was inhibited by all doses of flurprimidol (Table 1). Numbers of nodes and leaves were less on trees treated with flurprimidol than on untreated controls; also, leaf area and weight were lower on treated trees.

Gibberellin effect. Gibberellin reversed the inhibitory effect of flurprimidol on shoot growth when GA_3 was applied to privet shoots 7 days after flurprimidol treatment (Fig. 4). These results confirm that flurprimidol is a likely gibberellin biosynthesis inhibitor since GA_3 reversed the inhibitory effect of flurprimidol similar to paclobutrazol in apple (10) and ancymidol in Alaska peas (*Pisum sativum*) (2).

Translocation. Immediately after injection of 2.5 mg of ^{14}C-

labeled flurprimidol into young apple trees, a high concentration of the ^{14}C activity was found in the xylem of the rootstock within 20 cm of the injection site (Fig. 5). This concentration represented 99% of the activity. Most of the ^{14}C-labeled flurprimidol was in the xylem following injection because the xylem tissue was more receptive to the injected solution than phloem tissue. After 35 days, when obvious growth inhibition of the injected trees had occurred, 10% of the ^{14}C activity was detected in the new shoots and 1.5% moved into the phloem of the scion, but more than 80% remained in the rootstock near the injection site. The overall recovery of the ^{14}C was 94% immediately after injection and 81% 35 days after injection. As occurred with paclobutrazol in apple (12), virtually all of the ^{14}C activity found in the roots with flurprimidol was forced there by the pressure injector.

Metabolism. A high percentage of ^{14}C activity detected was flurprimidol; 95% of the ^{14}C activity in the xylem, 86% in the phloem, and 75% in the shoot tissue chromatographed with flurprimidol (Fig. 6). Two apparent metabolites of flurprimidol were evident in all tissues. These results indicate that flurprimidol was translocated into the xylem and phloem of the scion and into the new shoots. Translocation to the roots did not occur.

The site and rate of metabolism are unknown. It is possible that flurprimidol translocation occurred in an unmetabolized form and subsequently converted to two products. On the other hand, metabolism could have taken place immediately following injection with the resulting metabolites translocating differentially. The most polar form (R_f 0.13) of flurprimidol was the predominant metabolite in new shoots while the least predominant was the least polar (R_f 0.53), possibly the result of differential movement in the xylem. Functional importance of these metabolites must also be explored.

Flurprimidol is not phytotoxic to woody plants at high concentrations. Even when applied in methanol, woody plants showed no damage. The only visible signs of flurprimidol in woody plants were small, dark-green foliage and shortened internodes. All species investigated were inhibited by flurprimidol. Significant decreases in height increment associated with increasing doses indicate that higher levels of flurprimidol can be used to inhibit shoot growth. Flurprimidol is stable and is not highly mobile in plants, indicating that it is active in low amounts. Since there was no detectable basipetal movement of flurprimidol, it appears to be more readily transported via the xylem than the phloem, similar to paclobutrazol (12). Further, this research supports the hypothesis that flurprimidol is a GA biosynthesis inhibitor. As was recommended for paclobutrazol (12), more research should be performed with flurprimidol to improve uniformity of uptake into large woody plants by improving the formulation and techniques for injection.

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