Translocation and Metabolism of BAS111 in Apple Seedlings

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Additional index words. growth retardant, gibberellin biosynthesis inhibitor, *Malus domestica*

Abstract. The synthetic triazole derivative ^{14}C -labeled BAS111 (^{14}C -BAS111) was readily translocated throughout 8-week-old apple seedlings (*Malus domestica* Borkh.) within 3 days of application to roots in hydroponic nutrient solution. The majority of labeled BAS111 and total ^{14}C activity was recovered from middle leaves and root tissues. Two metabolites of labeled BAS111 were found. Both ^{14}C -BAS111 and metabolite 1 were recovered predominantly from leaf and root tissue and from nutrient solution. Metabolite 2, however, was found almost exclusively in root tissue. The rapid degradation of ^{14}C -BAS111 accounts for its low efficacy in comparison with other triazole growth retardants. Chemical name used: [1-phenoxy-5,5-dimethyl-3-(1,2,4-triazol-1-yl)-hexan-4-ol] (BAS111).

BAS111 (BASF Corp., Research Triangle Park, N.C.) is a synthetic triazole plant growth regulator capable of reducing the vegetative growth of plants by inhibiting gibberellin biosynthesis (Rademacher et al., 1987). (E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (uniconazole) (Chevron Chemical Co., Richmond, Calif.) (Bassi et al., 1986) and (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol (paclobutrazol) (ICI Americas, Goldsboro, N.C.) (Lever, 1986; Williams et al., 1986) are potent and persistent growth retardants. Curry and Reed (1989) indicated that BAS111 is less active than paclobutrazol or (E)-(RS)-1-cyclohexyl-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)-pent-1-en-3-ol (triapenthenol) (Mobay Corp., Kansas City, Mo.) in repressing shoot growth in apple seedlings. They concluded triazole efficacy could be explained by several factors including differences in: 1) uptake and translocation patterns, 2) relative half-lives, and/or 3) rates of metabolism within the plant. Reed et al. (1989) reported BAS111, paclobutrazol, and triapenthenol to have the same translocation patterns within sunflower plants

and apple trees and seedlings. However, the quantity of parent BAS111 triazole compound detected was consistently lower than paclobutrazol and triapenthenol in all plant tissues and soil samples within a few days after treatment.

Upon injection of ^{14}C -labeled gibberellin biosynthesis inhibitors into 1-year-old apple trees, Sterrett (1985, 1988) and Sterrett and Tworowski (1987) found little degradation of paclobutrazol at 27 days, uniconazole at 28 days, and α -(1-methylethyl)- α -[4-(trifluoromethoxy)phenyl]-5-pyrimidinemethanol (flurprimidol) (Eli Lilly, Indianapolis) at 35 days following injection. In contrast, Early and Martin (1988) found significant degradation of ^{14}C -labeled paclobutrazol in 2-month-old peach seedlings after 9 days in treated nutrient culture.

The objectives of this study were to determine the translocation and metabolism of ^{14}C -BAS111 in young apple seedlings and relate these findings to responses observed in earlier studies.

Received for publication 3 Jan. 1989. This research was supported in part by a grant (1-779-84) from the U.S.-Israel Binational Agricultural Research and Development Fund (BARD). Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable. 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.

Fig. 1. Chemical structure of triazole, BAS111, with labeled (*) carbons indicated.

Fig. 2. Relative distribution of total ^{14}C , ^{14}C -labeled BAS111, and ^{14}C -labeled metabolite activity in tissues of apple seedlings treated with ^{14}C -labeled BAS111 in nutrient solution. TL, top leaves; TS, top stem; ML, middle leaves; MS, middle stem; BL, bottom leaves; BS, bottom stem; RT, root. Data are means of three seedlings $\pm$ SE.

Table 1. Distribution of ^{14}C activity in apple seedlings: top leaves (TL), top stem (TS), mid leaves (ML), mid stem (MS), bottom leaves (BL), bottom stem (BS), root (RT), and nutrient solution (NS). Data are means of three seedlings $\pm$ SE.

Tissue	^{14}C dpm/g fresh wt $\pm$ SE (thousands)		
	Day 3	Day 6	Day 9
<i>^{14}C-BAS111</i>			
TL	26.8 $\pm$ 7.8	4.0 $\pm$ 1.4	6.1 $\pm$ 1.3
TS	365.6 $\pm$ 113.0	6.4 $\pm$ 1.3	7.2 $\pm$ 1.7
ML	7.5 $\pm$ 0.9	1.5 $\pm$ 0.2	2.5 $\pm$ 0.3
MS	34.0 $\pm$ 7.0	7.0 $\pm$ 1.2	5.5 $\pm$ 0.7
BL	5.1 $\pm$ 1.4	2.4 $\pm$ 0.6	2.9 $\pm$ 0.8
BS	11.5 $\pm$ 1.9	4.8 $\pm$ 0.8	3.8 $\pm$ 0.9
RT	24.4 $\pm$ 7.5	10.5 $\pm$ 0.7	5.0 $\pm$ 1.0
NS	1.6 $\pm$ 0.2	0.4 $\pm$ 0.1	0.2 $\pm$ 0.06
<i>^{14}C-Metabolite 1</i>			
TL	15.2 $\pm$ 1.6	22.8 $\pm$ 2.4	23.5 $\pm$ 5.6
TS	17.9 $\pm$ 7.5	6.0 $\pm$ 1.8	5.8 $\pm$ 1.7
ML	9.7 $\pm$ 1.0	10.9 $\pm$ 1.4	16.8 $\pm$ 2.1
MS	3.2 $\pm$ 0.7	2.1 $\pm$ 0.2	1.9 $\pm$ 0.2
BL	8.6 $\pm$ 0.5	16.4 $\pm$ 2.5	22.0 $\pm$ 3.7
BS	1.6 $\pm$ 0.5	2.4 $\pm$ 0.3	2.1 $\pm$ 0.5
RT	66.7 $\pm$ 17.5	38.7 $\pm$ 1.1	21.8 $\pm$ 4.0
NS	0.5 $\pm$ 0.06	1.3 $\pm$ 0.1	1.1 $\pm$ 0.07
<i>^{14}C-Metabolite 2</i>			
TL	0.06 $\pm$ 0.05	0.3 $\pm$ 0.2	0.2 $\pm$ 0.2
TS	4.7 $\pm$ 2.1	0.7 $\pm$ 0.7	1.3 $\pm$ 1.2
ML	0.05 $\pm$ 0.03	0.02 $\pm$ 0.01	0.1 $\pm$ 0.1
MS	0.2 $\pm$ 0.1	1.1 $\pm$ 0.8	0.1 $\pm$ 0.1
BL	0.01 $\pm$ 0.01	0.06 $\pm$ 0.03	0.2 $\pm$ 0.1
BS	0.02 $\pm$ 0.01	0.04 $\pm$ 0.04	0.1 $\pm$ 0.1
RT	0.6 $\pm$ 0.3	4.3 $\pm$ 0.3	2.4 $\pm$ 0.5
NS	0.02 $\pm$ 0.01	0.03 $\pm$ 0.01	0.01 $\pm$ 0.003

Twenty 3-week-old 'Delicious' apple seedlings, which had been grown under continuous light provided by fluorescent lamps (PAR of $210 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$) in a 1 sand : 1 vermiculite (v/v) mixture, were transferred to hydroponic nutrient solutions. There, the seedlings were grown in 900-ml containers that were continuously aerated and set under a metal halide lamp that provided a PAR level of $220 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ for 16 hr/day at 25°C. The nutrient solutions were replenished daily, changed completely each week, and contained the following (in mg-liter $^{-1}$): Ca, 101; Mg, 26; N, 102; K, 104; P, 27; Cl,

27; Fe, 5; S, 16; Zn, 0.25; Cu, 0.06; B, 0.50; Mo, 0.03; Mn, 0.33; and Na, trace. After 5 weeks in nutrient solution, nine seedlings of uniform appearance and height (average 30 to 35 cm) were chosen for ^{14}C -BAS111 treatment. All small axillary leaves were removed before treatment and each seedling was marked at the internode of the most recent fully expanded leaf.

^{14}C -BAS111 (specific activity 0.332 GBq $\cdot\text{mmol}^{-1}$) (Fig. 1) obtained from BASF Corp. was purified by multiple 200- μl injections of ^{14}C -BAS111 in 50% ethanol onto HPLC (6000A pump and model 440 UV de-

tector, Waters and Associates, Milford, Mass.). The HPLC was equipped with a C_{18} - $\mu\text{Bondapak}$ column (25 cm $\times$ 4 mm). Conditions for the purification were an isocratic system of 50 acetonitrile : 50 water (v/v) at 1.0 ml $\cdot\text{min}^{-1}$ for 15 min. Fifteen-second fractions were collected and counted by liquid scintillation (Beckman model 3801, Irvine, Calif.). A 4-min fraction centered at ^{14}C -BAS111 elution time was made for each subsequent injection. The ^{14}C -BAS111 fractions were pooled and concentrated on a C_{18} solid-phase extraction column and then eluted with ethanol. The purified ^{14}C -BAS111 was diluted with ethanol to 10.0 ml and used as the stock treatment solution.

Each nutrient solution received 100 μl of ethanol containing 2.76×10^6 dpm ^{14}C -BAS111 to yield a radioactive solution of 0.05 MBq $\cdot\text{liter}^{-1}$. Nutrient solution (≈ 150 ml) was added daily to each container to replenish transpiration losses. Three seedlings were harvested 3, 6, and 9 days after treatment. At harvest, each seedling was sectioned into root and shoot tissues. Shoot tissue below the internode marked at time 0 was divided into middle and bottom sections, each ≈ 15 cm long. Length of apical tissue above the marked internode averaged 10, 14, and 18 cm on the three sampling dates. Upon sampling, the shoots were divided into leaf (petiole and blades) and stem tissues. Root tissues were rinsed with 50 ml of water and 75 ml of 95% ethanol to remove any residual surface activity. Washings were combined with the nutrient solution, and the total volume was adjusted to 1 liter. A 50-ml aliquot of each nutrient solution was stored at -20°C . All tissues were weighed fresh and stored at -20°C . Following the 9-day study, samples were dried at 60°C for 72 hr, reweighed, and ground in a Wiley mill to pass through a 40-mesh screen.

Individual seedling extractions and HPLC procedures were run for each harvested tissue type and nutrient solution. All sets of collected HPLC fractions were counted twice by liquid scintillation.

Ground root, shoot, and leaf tissues were weighed, extracted separately in 15 ml of 100% methanol at 2°C for 24 hr, centrifuged ($5800 \times g$), and the methanol extract decanted and stored at 2°C . The entire extraction procedure was repeated and the two methanol extracts within individual tissues and individual seedlings were combined. Solvent was removed under reduced pressure in a rotary evaporator. Prewashed boiling flasks used to dry the methanol extracts were reweighed to determine residue weights. The flasks were then rinsed with 1.5 ml of 100% methanol, sonicated, and reweighed. Aliquots (100 μl) of methanol were filtered (0.45 μm), weighed, and injected into an HPLC. Three additional unfiltered aliquots (100 μl) were taken to determine total radioactivity extracted. Conditions for HPLC analysis were as described above with a solvent profile modification of 45 acetonitrile : 55 water (v/v) for the first 16 min, followed by an immediate switch to 100% methanol for an additional 20 min. One-milliliter fractions were col-

lected across the entire HPLC profile and counted by liquid scintillation. Frozen samples of nutrient solution were thawed, filtered (0.45 μ m), and 1.0-ml volumes were injected into HPLC. Fractions were collected and counted as described above.

In addition to the nine treated seedlings, a single container filled with nutrient solution without a seedling was treated with an equal amount of 14 C-BAS111 and aerated as described above. The solution and container were weighed at day 0. On days 3, 6, and 9 following treatment, nutrient solution was added to obtain the previous weight to adjust for evaporation losses, and a 10-ml sample was removed and frozen until HPLC analysis was conducted as described above.

Three areas of activity were detected in the HPLC and liquid scintillation analysis of seedling tissues and nutrient solutions. 14 C-BAS111 eluted between 9 and 13 min. An earlier unidentified peak of activity that eluted between 4 and 8 min was labeled metabolite 1. A later unidentified peak eluted between 20 and 24 min was labeled metabolite 2. Table 1 presents mean values with standard errors of the distribution of total 14 C, 14 C-BAS111, metabolite 1, and metabolite 2 expressed as dpm/g on a fresh-weight basis.

Activity associated with 14 C-BAS111 decreased in all tissues and nutrient solution between days 3 and 6. At day 9, 14 C-BAS111 activity in all leaf and top stem tissues increased in comparison to day 6 levels, but were less than the corresponding levels found on day 3. In contrast, the 14 C activity associated with metabolite 1 increased at each sampling time in all leaf tissues and decreased steadily in root and top and middle stem tissues. The relatively high 14 C activity associated with metabolite 1 in all leaf and root tissues was most pronounced by day 9.

The majority of the total activity found in stem sections was associated with 14 C-BAS111 and was isolated in the middle and bottom stem sections. In the first sampling period, the majority of 14 C-BAS111 activity expressed on a dpm/g fresh-weight basis was associated with stem and root tissues. This trend was still present on day 9, but less pronounced as it had been earlier.

More than 60% of the total activity in nutrient solution with seedlings present on day 3 was associated with BAS111. This percentage dropped sharply, to $\approx$ 20% of the total activity by day 9. Only a trace of activity associated with metabolite 2 was detected in solution. In the container that was only treated with 14 C-BAS111 and did not have a seedling present, the amount of 14 C activity associated with BAS111 was $>$ 98%, 96%, and 95% of the total 14 C activity for days 3, 6, and 9 after treatment, respec-

tively. Metabolite 1 accounted for the remainder of activity.

The majority of radioactivity remained in nutrient solution during the 9 days. The average ratio of total activity in nutrient solution to total activity in each seedling was 6.67, 5.87, and 2.64 for days 3, 6, and 9 after treatment, respectively. The total recovery of 14 C from nutrient solution and seedling tissues averaged 88%, 80%, and 65% for days 3, 6, and 9, respectively.

On a dpm/g fresh-weight basis, stem tissue appears to contain a large portion of the radioactivity recovered (Table 1). However, on a relative percent basis within tissues, stem tissue accounts for only a small percentage of the total activity (Fig. 2). Over the 9 days, total 14 C activity detected in combined leaf and stem tissues and root tissues represented $\approx$ 52%, 10%, and 37%, respectively (Fig. 2A). In leaf tissues, most 14 C activity was translocated to middle and bottom leaves. Activity associated with 14 C-BAS111 (Fig. 2B) was not as compartmentalized to leaf and root tissue as were metabolites 1 and 2. Activity associated with metabolite 1 in apple seedlings was found almost exclusively in leaf and root tissues (Fig. 2C). In general, the percentage of metabolite 1 activity in root tissue decreased as the percentage in leaf tissue increased. 14 C activity associated with metabolite 2 was relatively stable, and the bulk was present in root tissue (Fig. 2D).

The results presented show that the triazole derivative BAS111 is readily translocated throughout apple seedlings and rapidly metabolized following its application via the nutrient solution. Roots and relatively mature leaf tissues act as sinks for the majority of the applied growth regulator. Similar translocation patterns and rapid breakdown were observed by Early and Martin (1988) with 14 C-labeled paclobutrazol-treated peach seedlings.

In contrast to earlier studies of paclobutrazol, uniconazole, and flurprimidol (Sterrett, 1985, 1988; and Sterrett and Tworowski, 1987), BAS111 was significantly metabolized in a much shorter time. Sterrett (1985) found that 5% of 14 C-labeled uniconazole injected into apple trees had been translocated and $<$ 10% had been metabolized 28 days after treatment. Sterrett and Tworowski (1987) found 20% of 14 C-labeled flurprimidol had been translocated and a high percentage of the activity was unmetabolized after 35 days. Similar results of lack of metabolism and translocation were found with 14 C-labeled paclobutrazol 27 days after treatment (Sterrett, 1988). Bausher et al. (1985) also found little translocation of 14 C-labeled paclobutrazol when applied to actively growing citrus root tips.

Two metabolites of BAS111 were produced by apple seedlings. Since the 14 C-BAS111 was labeled at only two carbons, other nonlabeled metabolites could also be produced. Of the two metabolites found, metabolite 1 predominated and appears to be more polar than BAS111, while metabolite 2 appears to be less polar, considering their HPLC elution times. Metabolite 1 was released or exuded at a significant rate by the apple seedling into the nutrient solution. Metabolite 2 appeared to be produced in the roots. With the exception of a small percentage in the middle leaf tissue, metabolite 2 is generally root-bound and is not present in the nutrient solution.

The previously suggested factors concerning triazole efficacy and differences in recovery of parent triazole compounds following treatment could be attributed to different rates of triazole metabolism within the plant. If the desired amount of growth control can be obtained, the rapid breakdown of BAS111 will make it an attractive plant growth regulator in terms of environmental residue, provided the metabolites pose no risk.

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