Using Fruit-bearing Explants for Physiological Experiments

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Abstract. One- and three-node nectarine explants were compared with intact potted units of similar dimension. The explants and intact plants performed similarly as judged by rate of leaf photosynthesis, leaf and fruit respiration, and changes in fresh and dry weights. Water loss and transpiration were less in explants than intact plants after 24 h. Explants with fruit of nectarine, olive, and prune were used to evaluate uptake and distribution of ^{14}C -labeled paclobutrazol (PBZ), daminozide, and sucrose in plant parts. These comparisons reveal that the explant system is useful for primary testing of hypotheses, screening of chemicals, and evaluating species response for later testing of selected parameters in the field. Three-node explants containing fruit are reliable for experiments lasting up to 4 days. Chemical names used: succinic acid 2,2 dimethylhydrazide [daminozide (SADH)]; β -(4-chlorophenyl)methyl- α -(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol [paclobutrazol (PBZ)].

Physiological experiments with perennial plants in the field are made difficult by variable climatic conditions and orchard practices. Partial climate control is possible in the laboratory, but mature bearing trees cannot be accommodated in such a setting. A laboratory model system is needed using excised plant parts that will resemble plant metabolism when attached to a tree in the field. Such a model system affords primary testing of hypotheses concerning physiological response to horticultural or chemical treatment. An excised plant model system, as compared to large orchard trees, would be useful and less expensive in studying the complex interaction and multifactorial controls necessary for difficult interpretations (Zucconi and Bukovac, 1989).

Greenhouse containerized seedlings or grafted fruit trees grown on solid substrates or liquid media are useful for different applications of unlabelled or labelled chemicals and for checking their mobility and stability (Intrieri and Ryugo, 1974; Sterret, 1985; Richardson and Quinlan, 1986; Early and Martin, 1988a, 1988b). The presence of fruit adds further complexity to the model plant; still, small, potted, bearing apple trees have been used to evaluate growth substance movement and activity (Biasi, 1990; Samaraweera and Hill-Cottingham, 1980). The purpose of our work was to 1) achieve a high level of simplicity and reliability using a fruiting explant model system to carry out short-term physiological studies; 2) verify the reliability and usefulness of the excised model plant system using ^{14}C forms of daminozide and paclobutrazol (PBZ) and 3) compare daminozide and PBZ transport and accumulation with that of ^{14}C -labeled sucrose, a compound distributed all over the plant. The hypothesis we tested was that the fruiting excised model plant system could be used as a primary screening test to determine the ultimate accumulation and distribution of candidate chemicals to be tested later in the field.

Materials and Methods

Physiological parameters. Excised nectarine shoots ('Spring Red') were chosen when average fruit diameter was 25 to 30 mm. One-year-old shoots cut from adult trees were used to test two models: 1) a one-node model with one leaf and one fruit and 2) a three-node model with one leaf and one fruit per node. Representative, similar one-node units were also tagged on potted adult nectarine trees to compare results using the excised model. Excised model plants were kept in beakers containing 10 ml water. Evaporation was prevented by wrapping the stem with a plastic film and covering the top of the beaker. Both excised and intact model plants were kept in the laboratory at 45% relative humidity and 23 to 24°C. Experiments were conducted with four replicates per treatment and repeated twice when physiological measurements were made.

Total water use, leaf C assimilation, transpiration, and respiration of leaves and fruits were measured to evaluate the performance and longevity of each system. Total water use was determined by measuring the water volume needed to replace the water used each day. Carbon dioxide assimilation (A) and transpiration were measured with a portable gas-exchange system (model LCA1; Analytical Development Co., Hoddesdon, U.K.). The measurements were carried out on both excised and intact models. For one-node model plants, the gas exchange was measured at 0, 2, 6, 24, and 48 h after excision. For three-node model plants, measurements were taken at 0, 1, 2, 4, and 7 days after excision. Each time an excised model was tested for the above parameters, the one-node models on intact plants were tested. Explants were exposed to stepwise increases in photosynthetic photon flux density (PPFD) from 0 to 900 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Respiration rates of leaves and fruits of the model plant were measured at 0, 1, and 2 days for the one-node model and 0, 1, 2, 4, and 7 days for the three-node and field models by using the polarographic O_2 electrode (model 5300; YSI, Yellow Spring, Ohio) technique (Mottley, 1985). For this measurement, 100 mg leaf or fruit mesocarp was isolated and maintained in ice immediately after cutting. The sample was minced with a razor blade and was incubated in 0.5 M phosphate

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buffer for 3 min at room temperature. After equilibration, O_2 consumption was monitored for 5 min and expressed as $\mu\text{mol } O_2/\text{g}$ fresh weight per sec. Two or three runs were carried out for each sample. To estimate the tissue respiration rate of intact models, nectarine shoots were taken from potted trees, and leaf and fruit respiration was measured immediately after excision. The fresh and dry weights of stems, leaves, and fruit for each model plant were determined at the end of the experiment.

Chemical distribution. Experiments were carried out using excised shoots of 'Stark Red Gold' nectarine, 'Manzanillo' olive, and 'French' prune. Explants were formed by excising shoots with one to three nodes and at least one leaf and one fruit per node. 'Stark Red Gold' nectarine shoots were taken at two different times—30 and 42 days after full bloom (AFB), corresponding to 11 and 23 mm in fruit diameter. 'Manzanillo' olive shoots were excised 42 days AFB and 'French' prune shoots were excised 20 days before harvest.

Compounds used and plant tissue extraction. The following compounds were used: ^{14}C -labeled PBZ ($0.044 \text{ Ci}\cdot\text{mmol}^{-1}$ specific activity, 92.7% purity, molecular weight (MW) 293.6), ^{14}C -labeled daminozide ($0.0251 \text{ Ci}\cdot\text{mmol}^{-1}$ specific activity, 99% purity, MW 160), and ^{14}C -labeled sucrose ($0.56 \text{ Ci}\cdot\text{mmol}^{-1}$ specific activity, 99% purity, MW 360). Plant tissues treated with ^{14}C -labeled PBZ were extracted with the procedure used in previous studies (Early and Martin, 1988a, 1988b), while those treated with ^{14}C -labeled daminozide were homogenized and extracted in 80% methanol overnight and centrifuged. The supernatant was collected and dried down to the water fraction and adjusted to pH 3 with 1 N HCl. The supernatant was then partitioned against ethyl acetate, and water fractions were saved, dried down, solubilized in high performance liquid chromatography (HPLC) solvent system (9 0.05 M NaH_2PO_4 water : 1 methanol), and injected into an HPLC (LDC, Milton Roy HPLC, Riviera Beach, Fla.) under the following conditions: $10 \times 150 \text{ mm}$ stainless steel, ODS 18 semi-preparative column; 90% 0.05 M NaH_2PO_4 water : 10% methanol isocratic solvent system; $2 \text{ ml}\cdot\text{min}^{-1}$ flow rate and an ultraviolet detector wavelength of 220 nm. Based on these conditions, daminozide has a retention time of $\approx 7 \text{ min}$. To determine ^{14}C -labeled daminozide breakdown products, 1-min fractions were collected and radioactivity was checked.

Time of uptake. ^{14}C -Carbon-labeled PBZ at 0.06 Ci was applied to 'Manzanillo' olive excised shoots via the stem placed in 1-ml solutions in vials and left for 8, 16, and 24 h. After the established uptake time, shoots were divided into stem, leaf, fruit, and peduncle. The treated part was rinsed with water and methanol, homogenized, and extracted in 80% methanol for 24 h. After centrifugation, the supernatant was dried down, the residue was redissolved in a known amount of methanol, and an aliquot was taken to determine ^{14}C distribution in plant parts. The radioactivity in the beaker was also determined.

Species response. ^{14}C -Carbon-labeled PBZ at 0.06 Ci was applied to prune, olive, and nectarine excised shoots with the stem end placed in 1-ml solutions in beakers and left for 24 h. The solution volume was replenished as needed. Subsequently, handling procedures for sampling, extraction, and counting conditions were as listed above.

Compounds tested and application methods. ^{14}C -Carbon-labeled PBZ and ^{14}C -labeled daminozide at 0.06 Ci and ^{14}C -labeled sucrose at 0.11 Ci were applied to 'Stark Red Gold' nectarine one-node excised shoots. Compounds were applied either directly to the fruit surface, the leaf surface, or the cut stem in the vial as in the two previous experiments. Uptake was allowed for 24 h.

Breakdown. ^{14}C -Carbon-labeled PBZ and ^{14}C -labeled daminozide

at 0.06 Ci were applied to 'Stark Red Gold' nectarine at the cut stem in the vial and uptake was allowed for 5 days. Extraction of plant tissue and identification procedures for ^{14}C -labeled metabolites were as listed above.

Results

Water use. Under laboratory conditions, one- and three-node models used the most water during the 24 h after excision (Fig. 1), and the three-node model used more water than the one-node model. Both models showed a marked decrease in water use from 1 to 4 days. The one-node model here and in subsequent comparisons was not used beyond 2 days because of deteriorating leaf appearance.

Fresh and dry weight. At the end of the experiments, i.e., 2 days after the explant for the one-node model and 7 days for the three-node model, no significant differences were found among leaves or fruit fresh weight : dry weight ratios when compared to intact plants. Less water loss occurred from stems of the intact plants and one-node model than from the three-node model, resulting in fresh weight : dry weight ratios of 29.3, 35.8, and $37.4 \text{ mg}\cdot\text{g}^{-1}$, respectively.

Respiration. For the first 2 days after taking the explant, leaf and fruit respiration of the one-node model were similar to the values measured in the intact model (Table 1). Values for the three-node model varied slightly through day 4 but declined precipitously by day 7. Respiration in the intact model remained steady for leaf or fruit at each comparison period.

Gas exchange. One-node models responded to increasing light intensity (Fig. 2A). Positive A values were measured at $\approx 100 \text{ PPFD}$ for the intact plants and for the excised model 2 h after the explant was made, and at $\approx 200 \text{ PPFD}$ for subsequent measurements. The highest value for A was found for the intact plants and excised model plants immediately after the explant was made, while it decreased over 48 h of model aging (Fig. 2A). The three-node model also responded to increased light intensity (Fig. 2B). Positive A values were measured for intact plants and excised plants at 0, 1, 2, and 4 days after the explant was made at $\approx 100 \text{ PPFD}$, whereas 7 days later $\approx 400 \text{ PPFD}$ was required for the positive value. As light increased, similar values for PPFD were obtained for the intact plant and 1- and 2-day-old model plants. By 4 days, the model plants showed a reduction in the A values starting from 200 PPFD, with the lowest values in the experiment occur-

Fig. 1. Total water consumption of model plants after excision. Average water loss by evaporation was $0.1 \text{ ml}\cdot\text{day}^{-1}$. Bars indicate SE.

Table 1. Oxygen consumption of nectarine model and intact plants ($\mu\text{mol O}_2/\text{g}$ fresh weight per h).^z

Days from explant	One-node model		Three-node model		Intact plant	
	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit
0	18.5 ± 1.7	10.7 ± 0.9	18.3 ± 0.8	12.5 ± 1.5	19.0 ± 1.3	11.6 ± 1.7
1	15.5 ± 1.3	10.3 ± 0.2	16.3 ± 0.4	12.3 ± 0.1	18.3 ± 0.6	12.1 ± 0.9
2	19.6 ± 4.2	14.2 ± 2.4	14.2 ± 3.5	9.4 ± 0.1	19.0 ± 1.2	11.4 ± 1.5
4	---	---	16.2 ± 1.2	10.0 ± 1.4	19.5 ± 2.1	10.9 ± 1.1
7	---	---	4.2 ± 1.0	---	19.5 ± 1.9	11.1 ± 1.2

^zMeans with SE.

Fig. 2. Relationship between assimilation rate and photosynthetic photon flux density (PPFD) of the intact model shown with a "C" on the graphs and the one-node model plants (A) and intact and three-node model plants (B) during time-course measurement. Intact model and 0-h values for one- and three-node models were similar and only one curve is presented.

ring at 7 days (Fig. 2B). Transpiration rate values show the one-node model losing more water than the intact plant 2 h after excision. Thereafter, transpiration was less than in the intact plant (Table 2). In the three-node model, the transpiration values were higher than the intact plant 1 day after the explant was made. Thereafter, the transpiration rate was lower than in the intact plant (Table 2).

Time of uptake. The distribution of ^{14}C -labeled PBZ over an increasing period of uptake time in excised olive shoots led to the greatest accumulation in the stem and leaf (Table 3). After 8 h, although total radioactivity increased, there was little change in the distribution of recovered radioactivity among plant parts analyzed. The stem and leaf contained 97.1% of the recovered radioactivity at 8 h and 96.0% by 24 h. Very little radioactivity was found in the fruit at 8 h, and by 24 h only 1.3% was recovered from olive fruit.

Species response. Recovered radioactivity for ^{14}C -labeled PBZ treatment ranged from the highest content in the stem to the lowest in the fruit in all three species (Table 4). From 90% to 98% of the recovered radioactivity was found in the combined stem and leaf in all three species with radioactivity in fruit ranging from 0.9% to 3.4%. 'French' prune had the highest percentage of radioactivity in the leaf and lowest in the fruit, whereas 'Stark Red Gold' nectarine had the lowest percentage of radioactivity in the leaf and highest in the fruit.

Compounds tested and application method. The two ^{14}C -labeled growth retardants and ^{14}C -labeled sucrose showed different distribution patterns in nectarine (Table 5). When applied to stems via the solution in the vials, the radioactivity was observed in all plant parts. When applied to leaves or fruit, the uptake and distribution of the ^{14}C compounds were restricted. When applied to fruit, greater distribution of radioactivity was found from ^{14}C -labeled daminozide treatment than from ^{14}C -labeled PBZ. When applied to leaf or stem, greater distribution of radioactivity was found from ^{14}C -labeled PBZ than from ^{14}C -labeled daminozide application (Table 5).

In these comparisons, the applied radioactivity was most prominent at the place of application. ^{14}C -Carbon-labeled daminozide penetrated into treated fruit to a greater extent than ^{14}C -labeled PBZ, as judged by radioactivity in fruit and rinse comparisons. In all comparisons, less ^{14}C -labeled PBZ was found in fruits than was

Table 2. Transpiration rate ($\text{mmol H}_2\text{O}/\text{m}^2$ per sec) of one- and three-node models and intact plants.^z

Time (h)	One-node model	Intact plant	Time (days)	Three-node model	Intact plant
0	7.02 ± 1.0	7.05 ± 2.3	0	5.85 ± 2.2	7.93 ± 1.3
2	8.23 ± 1.11	6.40 ± 1.4	1	8.01 ± 0.56	6.70 ± 2.4
6	5.15 ± 1.17	6.75 ± 0.6	2	5.19 ± 0.30	6.59 ± 1.4
24	3.63 ± 1.20	6.70 ± 2.4	4	4.23 ± 0.03	7.48 ± 0.7
48	4.21 ± 0.13	6.59 ± 1.4	7	5.77 ± 0.31	5.95 ± 0.8

^zMeans with SE.

Table 3. Distribution of the radioactivity in a one-node excised shoot of olive as related to different periods of ¹⁴C-labeled paclobutrazol uptake when applied to stems.^z

Plant part	Time of uptake (h)					
	8		16		24	
	nCi	(%) ^y	nCi	(%)	nCi	(%)
stem	33.44	74.6	31.73	70.8	32.04	71.7
leaf	9.65	21.6	12.11	27.0	11.04	24.7
fruit	0.27	0.6	0.27	0.6	0.58	1.3
peduncle	0.22	0.5	0.22	0.5	0.36	0.8
vial	1.21	2.7	0.47	1.1	0.67	1.5

^zThe phenological stage of the ‘Manzanillo’ olive at the time of the application of the ¹⁴C-labeled paclobutrazol was 6 weeks after full bloom.

^yOf the radioactivity recovered, the percent found in each plant part. Of the total radioactivity applied, the average percent recovered was 74.4%.

Table 4. Distribution of the recovered radioactivity in one-node excised shoots of prune, olive, and nectarine after 24 h of ¹⁴C-labeled paclobutrazol stem application^z.

Plant part	‘French’ prune		‘Manzanillo’ olive		‘Stark Red Gold’ nectarine	
	nCi	(%) ^y	nCi	(%)	nCi	(%)
Stem	25.89	57.6	32.04	71.7	38.55	85.6
Leaf	17.68	39.4	11.04	24.6	4.93	11.0
Fruit	0.40	0.9	0.58	1.3	1.52	3.4
Peduncle	0.85	1.9	0.36	0.8	---	---
Vial	0.09	0.2	0.67	1.5	---	---

^zPhenological stages of the fruit at the time of application: ‘Stark Red Gold’ nectarine, 30-days after full bloom (AFB) when the fruit diameter was 11 mm; ‘Manzanillo’ olive, 6 weeks AFB, which is 7 months before physiological maturity; ‘French’ prune, 20 days before harvest.

^yOf the radioactivity recovered, the percent found in each plant part. Of the total radioactivity applied, the average percent recovered was 76.7%.

¹⁴C-labeled sucrose or ¹⁴C-labeled daminozide.

Breakdown. Breakdown compounds from ¹⁴C-labeled PBZ application were found at 8 and 9 min in nectarine fruit, with the major peak shown at the same retention time as PBZ (Fig. 3A). In contrast, no recovered radioactive breakdown compounds from ¹⁴C-daminozide application were found in the plant parts tested (Fig. 3B).

Discussion

The model systems examined in this study provide short-term vitality for physiological experiments. As judged by C assimilation, respiration, and fresh or dry weights, the one- and three-node models would be reliable for 2 and 4 days, respectively. After that, explants began to wilt and measured physiological parameters declined. Water use and transpiration in both models rapidly declined after 24 h. In part, this may be offset by using 8-hydroxyquinoline citrate. This additive in the uptake solution lowers bacterial populations that can occlude xylar pathways that decrease water uptake (Glandon and Staby, 1973).

Model plant convenience was revealed in the uptake and distribution of ¹⁴C compounds. This feature makes the explant system useful for short-term screening of candidate chemicals or treatments to be used later on field-grown trees. The tested responses of daminozide and PBZ in the explants compare well with published research on intact plants in the orchard or greenhouse

Table 5. Distribution of the radioactivity in one node excised shoots of nectarine as related to three ¹⁴C-labeled compounds [paclobutrazol, daminozide, sucrose] and application (leaf, fruit, vial)^z.

Plant party ^y	Sucrose		Daminozide		PBZ	
	nCi	(%) ^x	nCi	(%)	nCi	(%)
Stem	3.54	5.4	0.11	0.3	0.04	0.1
[Fruit]	11.69	17.8	12.36	32.6	3.81	8.5
Leaf	0.13	0.2	0.11	0.3	0.04	0.1
Rinse	50.17	76.5	25.22	66.5	40.93	91.2
Vial	0.06	0.1	0.11	0.3	0.04	0.1
Stem	0.39	0.6	0.19	0.5	0.31	0.7
Fruit	0.33	0.5	0.72	1.9	0.13	0.3
[Leaf]	4.14	6.3	0.91	2.4	4.57	10.2
Rinse	60.74	92.5	35.91	94.7	39.72	88.5
Vial	0.06	0.1	0.19	0.5	0.1	0.3
Stem	27.97	41.8	7.42	17.8	34.06	75.9
Fruit	17.47	26.1	14.44	34.6	1.52	3.4
Leaf	6.69	10.0	16.80	40.3	4.17	9.3
Rinse	0.0	0.0	0.0	0.0	0.0	0.0
[Vial]	14.84	22.1	3.03	7.3	0.51	11.4

^zThe phenological stage of the ‘Stark Red Gold’ nectarine at the moment of the application of the ¹⁴C-labeled compounds was 62 days after full bloom when fruit diameter was 23 mm.

^yThe bracket indicates where the ¹⁴C-radioactive compound was applied.

^xOf the radioactivity recovered, the percent found in each plant part. Of the total radioactivity applied, the average percent recovered was 59.7% for sucrose, 63.2% for daminozide, and 74.7% for PBZ.

Fig. 3. Chromatogram of ¹⁴C-labeled paclobutrazol (PBZ) (A) and ¹⁴C-labeled daminozide (SADH) (B) extracted from nectarine leaf and fruit tissues.

(Early and Martin 1988a,1988b; Martin and Williams, 1966; Martin et al., 1964; Steffens et al., 1983). The uptake and distribution of ^{14}C -labeled daminozide and ^{14}C -labeled sucrose were similar, while both compounds behaved differently than ^{14}C -labeled PBZ. When ^{14}C -labeled PBZ was applied via the nectarine stem, only 3.4% of the recovered radioactivity was found in the fruit and up to 76% was found in the stem. In contrast, >34% ^{14}C -labeled daminozide was recovered from fruit. The PBZ results obtained with excised model plant system agree with those previously reported with intact plants, confirming PBZ movement in the xylem (Early and Martin, 1988a) and reduced translocation to other parts of the plant when applied to the leaf (Xang et al., 1986) or even to the fruit.

Our results showed that daminozide and sucrose achieved greater overall distribution in the excised plants than PBZ. The daminozide results are consistent with previous findings on whole plant distribution (Martin and Williams, 1966; Undurraga and Ryugo, 1970). Further, after foliar sprays, presence of daminozide in fruits is high for both apples (Looney et al., 1972; Samaraweera and Hill-Cottingham, 1980) and cherries (Peisker and Sternkoff, 1976; Ryugo, 1966).

For all three compounds tested, stem treatment via solution in the vials was the most effective for uptake and allowed greater distribution of the ^{14}C -labeled growth retardants and ^{14}C -labeled sucrose. The preliminary observation on the breakdown of the two growth retardants showed that the excised model plant system may also be used for such investigations. We found PBZ breakdown in these short-term experiments similar to that reported by others with whole plants (Early and Martin, 1988b). We did not find daminozide breakdown in 24 h, whereas other authors detected daminozide residues in fruit several weeks after treatment (Edgerton and Greenhalgh, 1967; Martin et al., 1964; Martin and Williams, 1966; Winter et al., 1983). In some investigations, further decomposition of daminozide in fruits was indicated after the fruit had reached full size (Samaraweera and Hill-Cottingham, 1980).

The explant system confirms previous results, demonstrating that, to minimize accumulation of compounds in the fruit and achieve the best results, PBZ and daminozide should be applied in different ways. PBZ is effective when applied to leaves or as a soil-drench, whereas daminozide must be applied to foliage (Costa and Biasi, 1986; Curry, 1988; Martin et al., 1987; Quinlan, 1981). Thus, PBZ should be applied to roots, as less PBZ accumulated in fruit when applied via stem treatment than when applied to fruit surface. In addition, PBZ effectively controlled peach seedling vegetative growth when applied at doses as low as $3.4 \times 10^{-1} \mu\text{g}$ (Early and Martin, 1988b).

A similar amount of recovered radioactivity after ^{14}C -labeled daminozide treatment was found with both fruit or stem applications. With daminozide, the only practical way to reduce accumulation in fruit is to use the lowest chemical amount needed to obtain the desired effect or to apply the compound as a fall treatment after the fruits have been harvested, which reduces carryover effects on the next year's crop (Costa et al., 1975).

In conclusion, the explant system can be used as a primary, short-term duration screen for physiological questions to be later tested in the field after treatment number has been reduced. We offer three useful features of the explant system: 1) the use of labelled chemicals in the laboratory setting where contamination is more easily contained than in field plots, 2) rapid screening of hypotheses, and 3) opportunity for initial description of specific species and chemical response interactions.

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