A Simple, Room-temperature Extraction Method for ^{14}C -labeled Red Raspberry Leaf Tissues

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Abstract. Labeled (^{14}C) compounds were recovered from tissue disks taken from $^{14}\text{CO}_2$ -fed leaves of 1-year-old greenhouse-grown plants and 1-month-old ex vitro transplants of red raspberry (*Rubus idaeus* L.) by hot (boiling in 80% ethanol immediately after ^{14}C exposure), delayed-hot (boiling in 80% ethanol after a 2- to 3-day ethanol soak), and room-temperature (RT) (2- to 3-day soak in 80% ethanol) extraction methods. The RT extraction method was simple but as effective for extracting ^{14}C -labeled compounds from red raspberry leaf tissues as hot and delayed-hot extraction methods.

Radioisotope labeling methods are used widely for measuring CO_2 uptake, C translocation, and metabolism. Labeling begins by introducing the radioactive isotope ^{14}C into the plant tissues via photosynthetic uptake of $^{14}\text{CO}_2$; absorption; or injection of ^{14}C -labeled sugars, amino acids, or other compounds. Radioactivity in plant tissues then is determined by liquid scintillation (LS) spectrometry, for which sample preparation is critical (Peng, 1981).

The most commonly used method for extracting ^{14}C -labeled compounds from plant tissues is by using heat-extraction procedures, because of the perceived need to achieve com-

plete extraction or to rush the extraction process. Immediately after exposure, labeled plant tissues are heated or boiled in test tubes containing 70% to 80% ethanol in a water bath for various time intervals, depending on tissue type (Bender et al., 1987; Donnelly et al., 1984; Koch and Schrader, 1984; Lingle and Chevalier, 1984; Shelp and Shattuck, 1986). Hot extraction generally gives accurate and reproducible results. However, logistical and safety difficulties are presented when many specimens must be extracted in boiling ethanol. Although concern for translocation and respiration losses makes it imperative that samples be submerged immediately in a fixative solution, hot extraction may not be essential.

We compared the recovery of ^{14}C -labeled compounds from labeled red raspberry plant tissues by hot (boiling in 80% ethanol immediately after ^{14}C exposure), delayed-hot (boiling in 80% ethanol after a 2- to 3-day ethanol soak), and room-temperature (RT) (2- to 3-day soak in 80% ethanol) extraction methods. Our objective was to develop a sample prepa-

ration method for LS counting that would enable us to label tissues and extract labeled compounds from hundreds of specimens quickly and accurately.

The labeling apparatus consisted of a closed gas circuit system with a plexiglass sample chamber (15 cm³) to which light was supplied at 330 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ [400 to 700 nm measured by a photometer (model LI-1000; LI-COR, Lincoln, Neb.) equipped with a pH 3778 quantum sensor] by a 400-W high-pressure sodium lamp. The circuit included a gas generator, gas mixing chamber, and vacuum pump. A 0.50-ml aliquot of $\text{Na}_2^{14}\text{CO}_3$ (ICN Biomedicals, Costa Mesa, Calif.) solution (370 kBq) was placed in the gas generator receptacle, and 6 ml 1 N HCl was injected through the rubber stopper into the receptacle using a syringe to release the $^{14}\text{CO}_2$ into the gas circuit, excluding the sample chamber. To equilibrate, the $^{14}\text{CO}_2$ gas was circulated through the gas circuit by a vacuum pump (MasterFlex; Cole Parmer Instrument, Chicago) at 300 $\text{ml}\cdot\text{min}^{-1}$ for 10 min. Each leaf was exposed to the $^{14}\text{CO}_2$ gas mixture for 5 min. Excess $^{14}\text{CO}_2$ was absorbed in 200 ml 2 N NaOH at the end of each exposure.

Samples included 15 intact, mature-red raspberry leaves from healthy 1-year-old greenhouse-grown plants and 15 intact leaves from 1-month-old ex vitro transplants. After exposure, sample leaves were removed rapidly from the chamber and six interveinal leaf disks (29.50 mm²) per leaf were taken using a paper punch. Two leaf disks from each sampled leaf were used for each of the three extraction methods. For hot extraction, the leaf disks were placed in centrifuge tubes containing 3 ml hot 80% ethanol ($\approx 80^\circ\text{C}$) and the tubes were placed immediately in a hot water bath at the same temperature for 10 min. For delayed-hot extraction, the leaf disks were soaked in 3 ml 80% ethanol at ambient temperature ($\approx 20^\circ\text{C}$) for 2 to 3 days preceding hot extraction. For RT extraction, the leaf disks were soaked in 2 ml 80% ethanol at ambient temperature for 2 to 3 days. Extracts from hot and delayed-hot extractions were cooled down rapidly on ice and the extract volume was adjusted to 2 ml.

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Table 1. Relative effectiveness of hot and room-temperature (RT) extractions on total ^{14}C activity ($\text{Bq}\cdot\text{cm}^{-2}$) in leaves (after 1 to 7 days of soaking in ethanol) of greenhouse-grown (G) plants and ex vitro transplants of red raspberry. Values represent the mean of 15 leaves.

Leaf source	^{14}C extraction method					
	RT					
	Extraction period (days)					
	Hot	1	2	3	4	7
Total ^{14}C activity ($\text{Bq}\cdot\text{cm}^{-2}$)						
G plants	1.87 ± 0.09	1.76 ± 0.09 (94) ^z	1.84 ± 0.08 (98)	1.80 ± 0.09 (96)	1.83 ± 0.09 (98)	1.87 ± 0.11 (100)
Transplants	0.51 ± 0.02	0.49 ± 0.03 (96)	0.51 ± 0.04 (100)	0.52 ± 0.03 (101)	0.50 ± 0.02 (98)	0.51 ± 0.04 (100)

^zPercentage of hot extraction.

The ^{14}C activity was determined for three 0.10-ml subsamples in 5 ml LS cocktail (Universol, ICN Biomedicals) with an LS spectrometer (model LS-5801; Beckman Instrument, Irvine, Calif.). The ^{14}C activity was determined by the differential counting method (Peng, 1981); counting efficiency of 90% to 95% was indicated by the H number, which varied from 60 to 80. The total ^{14}C activity levels were corrected for background and adjusted according to the volume of the ethanol extract. The experiment was repeated with one modification to the RT extraction-sampling occurred at 1-day intervals for 1 week.

Hot, delayed-hot, and RT extraction methods were effective for extracting ^{14}C -labeled compounds in labeled red raspberry leaf tissues of greenhouse-grown plants and ex vitro transplants. Differences between hot and delayed-hot extraction periods of 2 and 3 days were not significant (data not shown). Heating apparently is not necessary for extraction if

tissues are soaked for at least 2 days. No apparent loss of activity occurred after an extended RT extraction period of up to 1 week (Table 1).

Lee (1980) recommended chlorophyll bleaching in the ethanol extract using chlorine gas, benzoyl peroxide, or light, since the ethanol extract is green. Delayed-hot and RT extractions yielded paler extracts than the hot extraction method, probably due to chlorophyll degradation during soaking. Bleaching chlorophyll was not necessary for our red raspberry samples, as mixtures of 0.10 ml ethanol extract in 5 ml LS cocktail were almost colorless. Color quench was negligible, as indicated by low H number (60 to 80) and high counting efficiency (90% to 95%).

The RT extraction method has several advantages over the hot and delayed-hot extraction methods. The RT extraction method is simpler, as it can be completed by soaking the labeled tissues in 80% ethanol at ambient

temperature for 2 to 3 days. More plant samples can be labeled and more specimen disks can be transferred to ethanol than with the hot extraction method and without the danger of maintaining and handling boiling ethanol solutions after each chase period. RT extraction increases experimental flexibility without losing reproducibility and is more economical with respect to equipment and time when many samples are tested. We conclude that RT extraction can be used during sample preparation for LS counting to effectively extract ^{14}C -labeled compounds in leaves of greenhouse-grown plants and ex vitro transplants.

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