Omasaiye, O.; Cheryan, M. *Cereal Chem.* **1979**, *56*, 58.
Posternak, T. In "The Cyclitols"; Hermann: Paris, 1965; Chapter 12.
Reddy, N. R.; Sathe, S. K.; Salunkhe, D. K. *Adv. Food Res.* **1982**, *28*, 1.
Tanford, C. In "Electrochemistry in Biology and Medicine"; Shedlovsky, T., Ed.; Wiley: New York, 1955; p 248.
Taussky, H. H.; Shorr, E. *J. Biol. Chem.* **1953**, *202*, 675.

Received for review April 1, 1983. Revised manuscript received September 14, 1983. Accepted December 19, 1983.

Level of Application and Period of Exposure Affecting Accumulation and Distribution of Chromium-51 and Zinc-65 in Hydroponically Grown Kale, Bush Beans, and Soybeans

Heidi A. Schmitt and Connie M. Weaver*

These experiments were conducted to assess the efficiency of various conditions for intrinsic labeling of kale, soybeans, and bush beans with ^{51}Cr and ^{65}Zn. Various harvest times, exposure levels, and exposure periods were employed and nuclide concentration, percent of applied dose accumulated by various plant parts, and nuclide distribution were calculated. ^{51}Cr and ^{65}Zn concentration increased proportionally with increasing level of application. The efficiency of incorporation of ^{51}Cr or ^{65}Zn varied little within each plant species among various treatment levels. Exposure period and level of ^{51}Cr and ^{65}Zn affected the nuclide concentration significantly ($P < 0.05$) but not the distribution of ^{51}Cr or ^{65}Zn. Radionuclide concentration was generally greater when plants were exposed throughout the growth cycle than for shorter periods of time, but the greater efficiency of incorporation into soybean seeds occurred when plants were exposed during the reproductive period of growth.

Chromium and zinc are essential elements for human nutrition. Plant foods are a dietary source of both elements. Biologically labeled plant tissues can be used to conveniently study the form and localization of micronutrients, the bioavailability of micronutrients from specific foods, and the effect of processing on trace element content and chemical associations. Knowledge of accumulation and distribution of chromium and zinc in plants throughout the plant growth cycle is necessary for the efficient production of intrinsically labeled plant material.

Whereas zinc has an intermediate mobility in plants (Epstein, 1972), chromium accumulates in the roots of plants and is poorly translocated to the shoots (Lahouti and Peterson, 1979; Turner and Rust, 1971). Although Skeffington et al. (1976) reported a greater transfer of ^{51}Cr from roots to shoots of barley plants when applied as CrO_4^{2-} rather than Cr^{3+}, others have concluded that accumulation of chromium in plants is independent of the form of administration (Huffman and Allaway, 1973; Blincoe, 1974; Starich and Blincoe, 1982). Cary et al. (1977b) presented evidence to suggest that Cr^{3+} is rapidly converted to Cr^{6+} when added to soils, but the CrO_4^{2-} anion is the form taken up by the plant from the soils. In an attempt to further understand accumulation behavior of chromium and zinc in plants, three experiments were conducted in which a selection of plants was exposed to ^{51}Cr and/or ^{65}Zn via a circulating nutrient solution. Radionuclide concentration and efficiency of accumulation of an applied dose for various plant parts were determined as a function of exposure period, level of radionuclide application, and stage of maturity when plants were harvested.

Department of Foods and Nutrition Purdue University, West Lafayette, Indiana 47907.

MATERIALS AND METHODS

Plants were germinated under conditions described previously (Levine et al., 1982) in a hydroponic system consisting of six compartments holding 85 L each of the circulating nutrient solution culture. The composition of the nutrient solution (pH 5.5) was 0.22 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.25 mM EDTA, 0.26 mM $K_2HPO_4 \cdot 3H_2O$, 0.37 mM NH_4NO_3, 0.30 mM $MgSO_4 \cdot 7H_2O$, 0.67 mM KOH, 16 μM $FeSO_4 \cdot 7H_2O$, 34 μM $ZnSO_4 \cdot 7H_2O$, 35 μM H_3BO_3, 15 μM $NaMoO_4 \cdot 2H_2O$, 2.3 μM $CuSO_4 \cdot 5H_2O$, 0.10 μM $Co(NO_3)_2 \cdot 6H_2O$, and 13.5 μM $MnCl_2 \cdot 4H_2O$. Unlabeled nutrient solution contained no added stable chromium (<0.045 ppb of Cr as determined by atomic absorption spectroscopy). Seeds were supported by using BR-8 grow blocks (Famco, Inc., Medina, OH), and when further support was necessary, as for soybeans, the plants were suspended from a support wire with string. Radionuclides, $^{51}CrCl_3$ (351 mCi/mg Cr) and $^{65}ZnCl_2$ (23 mCi/mg Zn), were administered to the plants via the circulating nutrient solution. The treatments and harvest schedule for all three experiments are given in Tables I and II, respectively.

Experiment I: Trilevel ^{51}Cr and ^{65}Zn Experiment. Soybeans (*Glycine max* L. Merr. var. "Verde"), bush beans (*Phaseolus vulgaris* L. var. "Blue Lake"), and kale (*Brassica oleracea* var. *acephala* D.C. "Dwarf Blue Curled Vates") were germinated, and the plants were continually exposed to one of three levels of ^{51}Cr or ^{65}Zn commencing 1 week from germination throughout maturation. Nutrient solution and nuclides were replaced weekly.

Harvests for both ^{51}Cr and ^{65}Zn were conducted 3 times during the plant growth cycle: preflowering (vegetative), flowering, and maturity for beans and premidmaturity, midmaturity, and maturity for kale (Table II). The harvest was conducted by removing the plants from the hydroponic system and cutting the roots from the stem. Roots were not analyzed. Each plant was put into separate plastic

0021-8561/84/1432-0498$01.50/0

Table I. Nuclide Treatments in Experiments I-III

		cpm/L (μCi/L)		
		1×	5×	10×
expt I (trilevel):	^{51}Cr	2.2×10^4 (0.1)[a]	1.1×10^5 (0.5)	2.2×10^5 (1.0
			2×	6×
	^{65}Zn	4.4×10^5 (1.26)[b]	8.8×10^5 (2.52)	2.6×10^6 (7.56)
expt II (transfer):	^{51}Cr		2.1×10^5 (0.43)	
	^{65}Zn		8.8×10^5 (2.52)	
expt III (dual label):	^{51}Cr	2.1×10^5 (0.41)		10× 2.2×10^6 (4.13)
	^{65}Zn	7.8×10^5 (2.11)		3× 2.3×10^6 (6.22)

[a] Equivalent to 3×10^{-4} μg of Cr/L of nutrient solution. [b] Equivalent to 0.05 μg of Zn/L of nutrient solution.

Table II. Harvest Schedule for Experiments I-III

	weeks from germination for					
	^{51}Cr			^{65}Zn		
	1	2	3	1	2	3
expt I (trilevel)						
soybeans	3	7	10	4	7	11
bush beans	3	5	8	3	5	9
kale	3	6	11	5	8	11
expt II (transfer)						
kale	7	10		7	10	
expt III (dual label)						
soybeans	5	11		5	11	

bags and fresh weights were obtained. Beans and flowers were separated from the vegetative portion of the legumes, and they were bagged and weighted. Each plant portion was macerated by using a Le Chef food processor equipped with a cutting blade. When possible, the whole plant was analyzed; otherwise, an aliquot of the plant portion was taken. Samples were dried in a vacuum oven at 70 °C (<25 mmHg) for at least 12 h and cooled in a desiccator, and the dry weight was determined.

Chromium-51 and ^{65}Zn activity was determined by γ spectroscopy. Corrections in cpm were made for background, decay between harvest date and counting date, and counter stability.

Experiment II: Single-Label ^{51}Cr and ^{65}Zn Transfer Experiment. Kale (*B. oleracea* var. *acephala* D.C. "Dwarf Blue Curled Vates") were exposed to either ^{51}Cr or ^{65}Zn (Table I). There were three periods of exposure: continually (from week 1 to week 10 after germination), midmaturity (from week 1 to week 7), and late maturity (from week 7 to week 10). A portion of the labeled plants were harvested at week 7 to determine the midmature concentration of nuclides. The final harvest occurred at 10 weeks. Sample preparation and analysis were similar to those of experiment I. Plants transferred out of the labeled nutrient solution were adjusted in radioactive decay between the transfer date and harvest date.

Experiment III: Dual-Label ^{51}Cr–^{65}Zn Transfer Experiment. Soybeans (*G. max* L. Merr. var. "Verde") were grown exposed to the dual label of ^{51}Cr plus ^{65}Zn at two different levels (Table I). In this experiment there were three exposure periods: continually (from 1 week after germination to maturity at 11 weeks), reproductive (from 5 weeks at flowering to maturity at 11 weeks), and vegetative (from 1 week to flowering at 5 weeks). Plants exposed to the radionuclide during the vegetative period of growth only were transferred to an unlabeled nutrient solution at 5 weeks from germination, and plants exposed during the reproductive period only were transferred into the labeled nutrient solution. A transfer harvest was made at this time (week 5) of plants that had been exposed from week 1 to determine a midmature accumulation and for comparison to mature harvests.

Sample preparation was identical with that described in experiment I except the beans from each plant were further separated into seeds and pods. Activity of the vegetative exposure was adjusted for decay between date of transfer out of the radionuclide solution and harvest date.

Statistical Analysis. A Cochran's C test was employed to determine homogeneity of variance in each group (n = 6), and the Newman-Kuels sequencial range test was used to determine mean differences in mineral content between various levels of exposure and exposure times by using an α-level of 0.05 (Weiner, 1971).

RESULTS AND DISCUSSION

Experiment I: Trilevel ^{51}Cr and ^{65}Zn Experiment. *Chromium.* Concentrations of ^{51}Cr (cpm/g dry weight) and the percentage accumulation of the applied dose (the amount of radioactivity accumulated by each plant within a treatment with respect to the total applied dose for that treatment) for kale, soybeans, and bush beans exposed to the three levels of ^{51}Cr and harvested at different stages of maturity are presented in Table III. Significant increases ($P < 0.05$) were seen in ^{51}Cr concentration of all three plant species as the level of ^{51}Cr application increased. Within each treatment group, kale had a significant decrease in the radioactive concentration from plants harvested at 3 weeks of age compared to those harvested at maturity. Bush bean stems and leaves showed an increase in ^{51}Cr concentration from vegetative to flowering stages and a decrease at the mature harvest for all exposure levels. The flowers of the bush beans contained a higher concentration of ^{51}Cr than their mature counterparts. Chromium-51 concentration in soybean plants harvested at 3 weeks of age was approximately 2 times greater than at the mature harvest. The midmature harvest had the lowest ^{51}Cr concentration. As with bush beans soybean flowers had a higher concentration of ^{51}Cr than mature seeds and pods. It has been noted in barley seedlings that $^{51}CrO_4^{2-}$ uptake was linear over a 24-h period and uptake did increase with increasing chromate concentration (Shewry and Peterson, 1974). Other investigators have also noticed an increase in tissue chromium with increasing levels of chromium application in a variety of plants (Turner and Rust, 1971; Cary et al., 1977a; Levine et al., 1982).

Few significant differences in percent of applied dose of ^{51}Cr were observed for kale, bush beans, and soybeans harvested at different stages of maturity or exposed to different levels of radionuclides (Table III). Differences that were significant among different levels of ^{51}Cr application included the greater percent accumulation by bush beans harvested at 3 weeks at the 1× level ^{51}Cr dose than either the 5× or 10× levels. Also, the stems and

Table III. Percent Accumulation of Applied Dose and ^{51}Cr Concentration (Mean ± SD) in Kale, Bush Beans, and Soybeans Exposed to Three Levels of Radioactivity

	radionuclide treatment					
	1× ^{51}Cr		5× ^{51}Cr		10× ^{51}Cr	
harvest[a] and plant tissue	concn, cpm × 10^3/g dry wt	% accumulation	concn, cpm × 10^4/g dry wt	% accumulation	concn, cpm × 10^4/g dry wt	% accumulation
kale						
1	15.5 ± 3.4	0.016 ± 0.013	11.2 ± 2.3	0.022 ± 0.012	18.0 ± 2.5	0.041 ± 0.069
3	3.7 ± 0.5	0.034 ± 0.036	2.1 ± 0.2	0.025 ± 0.010	5.6 ± 0.9	0.023 ± 0.009
bush beans						
1	1.2 ± 0.6	0.257 ± 0.096	1.8 ± 0.1	0.031 ± 0.033	2.7 ± 0.8	0.041 ± 0.032
2, stems and leaves	7.8 ± 0.6	0.275 ± 0.127	4.1 ± 0.3	0.344 ± 0.101	8.9 ± 0.8	0.273 ± 0.128
2, flowers[b]	1.2	0.119	0.9	1.161	2.2	0.310
3, stems and leaves	4.3 ± 0.3	0.104 ± 0.050	2.7 ± 0.3	0.090 ± 0.083	6.5 ± 0.8	0.230 ± 0.094
3, beans[b]	0.3	0.041	0.2	0.019	0.5	0.053
soybeans						
1	4.7 ± 0.9	0.051 ± 0.008	2.2 ± 0.2	0.052 ± 0.007	4.0 ± 0.7	0.041 ± 0.016
2, stems and leaves	1.1 ± 0.2	0.040 ± 0.025	0.7 ± 0.1	0.059 ± 0.032	1.6 ± 0.2	0.060 ± 0.031
2, flowers[b]	0.3	0.001	0.2	0.004	0.4	0.003
3, stems and leaves	2.0 ± 0.2	0.083 ± 0.014	1.1 ± 0.1	0.119 ± 0.069	2.4 ± 0.5	0.121 ± 0.060
3, beans[b]	0.2	0.010	0.1	0.042	0.3	0.041

[a] 1 = premidmaturity (3 weeks from germination); 2 = midmaturity or flowering; 3 = mature. [b] The flowers or beans from all of the plants within each treatment were pooled and counted together.

Table IV. Percent Accumulation of Applied Dose and ^{65}Zn Concentration (cpm/g Dry Weight) in Kale, Bush Beans, and Soybeans Exposed to Three Levels of Radioactivity[a]

	radionuclide treatment					
	1× ^{65}Zn		2× ^{65}Zn		6× ^{65}Zn	
harvest[b] and plant tissue	concn, cpm × 10^3/g dry wt	% accumulation	concn, cpm × 10^4/g dry wt	% accumulation	concn, cpm × 10^4/g dry wt	% accumulation
kale						
1	12.8 ± 1.8	0.005 ± 0.002	2.4 ± 0.2	0.004 ± 0.001	8.8 ± 0.8	0.004 ± 0.001
2	11.0 ± 2.2	0.016 ± 0.004	2.9 ± 0.5	0.016 ± 0.010	5.9 ± 0.5	0.011 ± 0.006
3	8.0 ± 1.4	0.016 ± 0.007	1.5 ± 0.3	0.013 ± 0.009	4.5 ± 0.7	0.008 ± 0.002
bush beans						
1	10.0 ± 1.0	0.006 ± 0.001	1.7 ± 0.1	0.006 ± 0.003	4.3 ± 0.5	0.006 ± 0.002
2, stems and leaves	13.0 ± 2.4	0.007 ± 0.003	2.6 ± 0.2	0.008 ± 0.004	6.0 ± 0.5	0.010 ± 0.004
2, flowers[c]	23.3	0.001	5.0	0.006	14.0	0.008
3, stems and leaves	8.5 ± 1.0	0.008 ± 0.002	1.8 ± 0.2	0.008 ± 0.002	4.7 ± 1.1	0.010 ± 0.003
3, beans	12.5 ± 1.5	0.011 ± 0.002	2.4 ± 0.3	0.012 ± 0.005	6.9 ± 1.3	0.013 ± 0.003
soybeans						
1	10.0 ± 1.6	0.015 ± 0.002	1.8 ± 0.1	0.017 ± 0.003	5.2 ± 0.3	0.013 ± 0.002
2, stems and leaves	5.8 ± 1.1	0.017 ± 0.003	1.2 ± 0.2	0.016 ± 0.003	2.7 ± 0.3	0.017 ± 0.004
2, flowers[c]	19.4	0.009	3.9	0.006	9.9	0.007
3, stems and leaves	8.0 ± 1.0	0.033 ± 0.024	2.1 ± 0.1	0.035 ± 0.007	6.1 ± 0.3	0.020 ± 0.007
3, beans	9.6 ± 0.8	0.015 ± 0.002	2.2 ± 0.9	0.015 ± 0.004	3.5 ± 0.5	0.018 ± 0.006

[a] Mean ± SD. [b] 1, premidmaturity (3 weeks from germination); 2 = midmaturity or flowering; 3 = maturity. [c] Flowers from respective plants were pooled as one sample.

leaves of bush beans exposed to the 10× level of ^{51}Cr accumulated significantly more of the ^{51}Cr dose than those exposed to the 5× level of ^{51}Cr. Significant differences in percent accumulation of ^{51}Cr by bush beans among harvest times were between those harvested at the vegetative and mature stage for both the 1× and 10× levels of application, for those harvested at the vegetative and reproductive and stages for both 5× and 10× levels of application, and for those harvested at the reproduction and mature stages of growth for the 5× level of application. For soybeans exposed to ^{51}Cr, significant differences among harvest times were between plants harvested at the vegetative and mature phases of growth for both the 5× and 10× levels of application and for plants harvested at the reproductive phases of growth for both the 5× and 10× levels of application. No other differences within each plant species among different levels of ^{51}Cr application or among different harvest times were significant.

Ratios of ^{51}Cr concentration in reproductive to vegetative plant parts were calculated by using cpm/g dry weight for bush beans and soybeans harvested at flowering and at maturity. In all cases, the ratios were less than 0.45, indicating a concentration of ^{51}Cr in the vegetative portions of the plants. Thus, mobility of ^{51}Cr to reproductive plant parts is slight. These findings are in agreement with other investigators who have reported chromium to be relatively immobile and to reside mostly on the roots (Cary et al., 1977a; Lyon et al., 1969; Lahouti and Peterson, 1979). Shewry and Peterson (1974) postulated that chromium may not be easily transported due to spacial localization in subcelluar compartments such as vacuoles of root cells. In bean (*P. vulgaris*) leaves, Huffman and Allaway (1973) found the translocated ^{51}Cr to be associated with a single low molecular weight anionic complex and not associated with any subcellular organelles.

Zinc. Radioactive concentrations of ^{65}Zn for kale, bush beans, and soybeans for each harvest increased as the exposure level increased and, in many cases, proportionally to the level of application (Table IV). Carroll and Loneragan (1969) found linear uptakes of zinc in a variety of

Table V. Concentration of Radioactivity (Mean ± SD) by Kale Exposed to ^{51}Cr or ^{65}Zn throughout the Growth Cycle vs. during Early or Late Stages of Growth[a]

exposure period	radionuclide treatment	
	^{51}Cr cpm/g dry wt	^{65}Zn cpm/g dry wt
week 1-10	2618 ± 836[a,b]	13061 ± 2794[a]
week 7-10	3258 ± 386[a]	9508 ± 2140[b]
week 1-7	667 ± 179[b]	4769 ± 727[c]

[a] The transfer harvest (plants continually exposed from week 1 to week 7 and harvested at week 7) had 978 ± 354 cpm of ^{51}Cr/g of dry weight and 6813 ± 656 cpm of ^{65}Zn/g of dry weight. [b] The numbers within a column having different letters are significantly different at the 5% level according to the Newman-Kuels sequencial range test.

plants over a broad range of zinc concentrations. Also, Singh and Steenberg (1974) noted that an increase in stable zinc application resulted in a greater uptake of both ^{65}Zn and stable zinc in maize and barley.

Among the three harvest times, the concentration of ^{65}Zn decreased with maturity for kale exposed to the 1× and 6× but not for the 2× level of application. All comparisons were significantly different except the first two harvests of kale exposed to levels 1× and 2×. Bush beans harvested at flowering accumulated the most ^{65}Zn as was true for ^{51}Cr. Also, flowers at this harvest had approximately 2 times greater concentration of ^{65}Zn than the mature beans. Within each exposure level, bush bean stems and leaves from the first and mature harvest had significantly different concentrations of ^{65}Zn. Soybean ^{65}Zn concentrations were significantly lower for plants harvested at flowering than vegetative or mature harvests for all levels of comparisons.

Percent accumulation of applied dose of ^{65}Zn in all plants was, in general, insignificantly different with a few exceptions. Percent accumulation of applied ^{65}Zn in kale was significantly greater for plants exposed to the 1× level of ^{65}Zn and harvested at 7 weeks of maturity than for plants harvested at 3 weeks. Percent of applied ^{65}Zn accumulated by kale was significantly greater for plants exposed to the 6× level than for those exposed to the 1× level of ^{65}Zn at the mature harvest. For bush beans, efficiency of accumulation of applied dose varied insignificantly except for a significantly greater percent accumulation of applied dose by stems and leaves of mature plants harvested at 3 weeks for plants exposed to the 1× level of ^{65}Zn. Percent accumulation of applied dose in soybeans was only significantly different in the case of the first harvest between levels 2× and 6× and for plants harvested at flowering and maturity within exposure level 2×.

The high concentrations of ^{65}Zn concentration in reproductive compared to vegetative plant parts indicate that ^{65}Zn is transported to and remains in high concentration in the reproductive plant parts. Zinc is considered to have intermediate mobility (Epstein, 1972) and functions as a cofactor in a variety of enzymatic reactions (Luttge and Higinbotham, 1979); thus it is reasonable to expect a higher concentration of ^{65}Zn in regions of high metabolic activity such as reproductive tissues.

Experiment II: Single-Label ^{51}Cr and ^{65}Zn Transfer Experiment. Kale was used to determine the stage of growth in which maximum labeling of ^{51}Cr and ^{65}Zn occurs. The concentration of ^{51}Cr in kale exposed to the nuclide throughout the growth cycle was not significantly different from kale exposed only during the last weeks of growth (weeks 7–10) (Table V). Plants exposed during weeks 1–7 accumulated significantly less of the radionuclide. The ^{65}Zn concentration was significantly greater when plants

Table VI. Percent Distribution[a] and Radioactive Concentration (cpm/g dry wt) of ^{65}Zn and ^{51}Cr in Soybeans Exposed to Different Levels of Radioactivity and for Different Periods of the Plant Growth Cycle[b]

plant tissue/exposure	radionuclide treatment							
	^{65}Zn				^{51}Cr			
	in 1× ^{51}Cr/1× ^{65}Zn		in 10× ^{51}Cr/3× ^{65}Zn		in 1× ^{51}Cr/1× ^{65}Zn		in 10× ^{51}Cr/3× ^{65}Zn	
	cpm/g dry wt	% nuclide	cpm/g dry wt	% nuclide	cpm/g dry wt	% nuclide	cpm/g dry wt	% nuclide
seed/continual	3323 ± 53	21.6 ± 1.4	8751 ± 265	27.5 ± 9.9	729 ± 38	17.2 ± 0.9	2237 ± 193	18.2 ± 13.1
seed/reproductive	1996 ± 93	28.3 ± 18.4	5463 ± 213	24.4 ± 9.6	533 ± 15	22.6 ± 15.7	1519 ± 94	15.6 ± 7.2
seed/vegetative	745 ± 54	16.9 ± 2.3	1838 ± 117	13.6 ± 7.1	499 ± 16	17.0 ± 10.2	1149 ± 73	12.5 ± 7.4
pod/continual	1228 ± 137	4.4 ± 0.7	4667 ± 156	9.4 ± 3.8	265 ± 11	3.5 ± 0.7	1395 ± 35	7.1 ± 4.9
pod/reproductive	885 ± 42	5.0 ± 3.2	2664 ± 290	4.2 ± 2.1	281 ± 4	4.6 ± 3.2	760 ± 33	2.7 ± 1.5
pod/vegetative	396 ± 19	3.6 ± 0.6	736 ± 250	3.3 ± 1.7	496 ± 116	6.6 ± 2.7	512 ± 176	3.4 ± 1.9
stems and leaves/continual	3565 ± 1171	74.0 ± 1.7	8531 ± 2192	63.1 ± 13.6	1030 ± 270	79.3 ± 0.2	4319 ± 431	74.7 ± 18.0
stems and leaves/reproductive	1796 ± 297	66.7 ± 21.5	7239 ± 2585	71.4 ± 11.7	777 ± 148	72.7 ± 18.8	4140 ± 926	81.6 ± 8.5
stems and leaves/vegetative	1276 ± 416	79.4 ± 3.0	4282 ± 1503	83.1 ± 8.8	968 ± 164	76.7 ± 13.0	3010 ± 985	84.1 ± 9.4
transfer harvest flowers/continual	11559 ± 2218	10.1 ± 6.6	42148 ± 10944	5.4 ± 2.9	794 ± 228	4.6 ± 5.1	2122 ± 1349	1.7 ± 1.0
transfer harvest stems and leaves/continual	5148 ± 970	89.9 ± 6.6	14384 ± 3419	94.6 ± 2.9	1122 ± 375	95.4 ± 5.1	2107 ± 562	98.3 ± 1.0

[a] The percent distribution of the nuclide is based on the percentage cpm in the particular plant tissue out of the total cpm in all the above-ground portions of the plant within each exposure period. [b] Means ± SD.

Table VII. Percent of Applied Dose Present at Time of Harvest in Soybean Seeds Dually Labeled with ^{51}Cr and ^{65}Zn

	% of dose[a] for radionuclide treatment			
	^{65}Zn		^{51}Cr	
exposure period	in 1× ^{51}Cr/1× ^{65}Zn	in 10× ^{51}Cr/3× ^{65}Zn	in 1× ^{51}Cr/1× ^{65}Zn	in 10× ^{51}Cr/3× ^{65}Zn
continual	0.073 ± 0.006	0.047 ± 0.012	0.057 ± 0.006	0.013 ± 0.006
reproductive	0.10 ± 0.006	0.107 ± 0.006	0.06 ± 0	0.033 ± 0.006
vegetative	0.04 ± 0	0.037 ± 0.006	0.037 ± 0.006	0.010 ± 0
adjusted[b] vegetative	0.04 ± 0	0.040 ± 0	0.10 ± 0.012	0.027 ± 0.006

[a] Mean ± SD of the ratio (as percent) of the total seed yield per plant to the total radioactivity in the culture media. [b] The cpm of the vegetative harvest were not adjusted for decay between the time of removal from radioactive solution and the harvest date of mature plants, whereas adjusted vegetative cpm were adjusted.

were exposed throughout the growing period than for shorter periods of exposure. In comparing concentrations of ^{51}Cr or ^{65}Zn in plants dosed from week 1 to week 7 and harvested at week 7 with those exposed for the same time period grown to maturity, there was a decrease in concentration of both radionuclides for the kale grown to maturity due to dilution of the radionuclide by an increase in plant tissue.

Experiment III. Dual-Label ^{51}Cr–^{65}Zn Transfer Experiment. Although the effects of the level of application on accumulation of ^{65}Zn and ^{51}Cr in soybeans harvested at different stages of the plant growth cycle were described in experiment I, this information does not indicate the interrelationships between level of application and exposure period on accumulation of minerals. Intrinsic labeling with radionuclides may be more efficiently accomplished by exposing plants to a high level of radioactivity for a short period during the plant growth cycle or by exposing plants to a lower level of radioactivity throughout the growth cycle. To answer this question, soybeans were exposed to two levels of ^{65}Zn and ^{51}Cr either throughout the growth cycle, only during the vegetative period (first 5 weeks), or only during the reproductive phase of growth (last 6 weeks).

Chromium. All plant tissues analyzed except the pods were significantly higher in concentration of ^{51}Cr when exposed to the higher level of radioactivity (Table VI). Concentration of ^{51}Cr by seeds of plants exposed during the reproductive vs. vegetative periods did not significantly differ from seeds of plants exposed continually for the 1× level of application but the difference was significant for the 10× level. The concentration of ^{51}Cr in the stems and leaves and in the pods did not significantly differ among various exposure periods for either level of application.

Within the 1× ^{51}Cr treatment, percent distribution of ^{51}Cr was not significantly different among the three exposure periods for any plant part. However, the percent distribution of ^{51}Cr was significantly different among the different plant parts within each exposure period. The percent distribution of the ^{51}Cr accumulated by the different plant parts of plants exposed to the 10× level of ^{51}Cr was similar to that for plants exposed to the 1× level.

Zinc. All plant tissue analyzed had significantly higher ^{65}Zn concentrations when exposed to the higher level of radioactivity except pods of plants exposed during the vegetative period (Table VI). However, no significant differences in percent distribution were observed between the two levels of application.

Concentration of ^{65}Zn in seeds was the greatest when plants were continually exposed. Accumulation was greater for plants exposed during the reproductive than the vegetative period of growth. Similarly, accumulation of a single dose of ^{65}Zn by "Century" soybean seeds was greater if administered during flowering than during the vegetative period of plant growth (Janghorbani et al., 1983).

Among the three exposure periods for both 1× and 3× ^{65}Zn treatments, accumulation of ^{65}Zn was significantly different for both seeds and pods. Concentration of ^{65}Zn in all plant parts was significantly different for all exposure periods in the 1× ^{65}Zn treatment. Concentrations of ^{65}Zn in stems and leaves of plants continually and vegetatively exposed to the 3× level of ^{65}Zn compared to those reproductively exposed were not significantly different, yet concentrations of ^{65}Zn in stems and leaves of plants exposed continually and vegetatively were significantly different. There was no difference in the percent distribution of ^{65}Zn in the seeds among exposure times at the 1× level, but for the 3× ^{65}Zn treatment, percent distribution between reproductive vs. vegetative exposure periods was significantly different. The same was true for the stems and leaves.

The percent of accumulated dose present in the seeds at harvest is represented in Table VII. For both ^{65}Zn and ^{51}Cr, a considerably higher percent of the dose was accumulated when exposure to the nuclide occurred only during the reproductive phase of growth. For ^{51}Cr, the percent of applied dose present at harvest was lower than the percent of applied dose actually taken up by the seeds due to the short half-life (28 days) of this radionuclide.

A greater percent of applied ^{51}Cr was accumulated in seeds when exposed to the 1× than the 10× level except for plants exposed only during the vegetative period. This decrease in accumulation of ^{51}Cr at the higher level could indicate a slight toxicity. A decrease in whole tissue dry weight was seen at the 10× level (3.22 ± 1.94 g of seed/plant) compared to the 1× level (5.13 ± 1.92 g of seed/plant) of experiment III. Chromium toxicity has been correlated with a decrease in soybean yield (Ham and Dowdy, 1978; Turner and Rust, 1971) and decreased uptake of ^{65}Zn in bush beans (Schmitt and Weaver, 1980).

CONCLUSIONS

When ^{51}Cr and ^{65}Zn were compared, many similar accumulation patterns were observed. An increase in nuclide deposition in the plant tissue with increasing applied dose was found for both nuclides. Both ^{65}Zn and ^{51}Cr accumulated in a proportional manner with respect to the increased applied dose in experiment I but not for ^{51}Cr in experiment III, which employed higher levels of ^{51}Cr, creating possible toxicity as indicated by reduced yields.

Little variation in percent distribution of applied dose among above-ground plant parts was observed in soybeans exposed to increasing levels of ^{51}Cr or ^{65}Zn. For both nuclides, maximal concentration in bush beans occurred at flowering but at preflowering or maturity in soybeans.

When an approach for labeling plants with ^{65}Zn or ^{51}Cr is selected, the optimum exposure period and level of application depend on the specific tissue concentration and efficiency of incorporation of the applied dose desired. Some investigations such as micronutrient subcellular distribution studies, separating and identifying mineral

complexes, and bioavailability studies might necessitate seeds or other plant parts with very high specific activities, whereas other investigations such as general overall uptake and distribution studies and those determining the effect of processing on mineral concentrations would not warrant such expense. For labeling plants with either ^{51}Cr or ^{65}Zn, the higher the dose, the higher the nuclide concentration. For soybean plant parts, the highest nuclide concentration occurred when plants were continually exposed to the nuclide. For kale, plants exposed continually also accumulated the most ^{65}Zn, but nuclide concentration of ^{51}Cr was not significantly different between plants exposed only during the last 3 weeks of growth and those exposed continually. However, the exposure period and level of application that resulted in higher nuclide concentration may not result in the highest percent accumulation of an applied dose since a lower level of application may be taken up more efficiently.

The most efficient accumulation of applied dose, e.g., the most economical, for soybean seeds occurred when exposures only encompassed the reproductive phase of growth in all levels of application (Table VII). Dosing only during the reproductive period would be even more economical for ^{51}Cr than for ^{65}Zn given the short half-life of ^{51}Cr (28 days). Chromium-51 accumulation was less efficient when yields were reduced at higher levels of ^{51}Cr exposure, whereas efficiency of ^{65}Zn accumulation was similar for each level of application.

ACKNOWLEDGMENT

We gratefully acknowledge the statistical assistance so generously given by L. Weaver.

Registry No. Chromium-51, 14392-02-0; zinc-65, 13982-39-3.

LITERATURE CITED

Blincoe, C. *J. Sci. Food Agric.* **1974**, *25*, 973.
Carroll, M. D.; Loneragan, J. F. *Aust. J. Agric. Res.* **1969**, *20*, 457.
Cary, E. E.; Allaway, W. H.; Olson, O. E. *J. Agric. Food Chem.* **1977a**, *25*, 300.
Cary, E. E.; Allaway, W. H.; Olson, O. E. *J. Agric. Food Chem.* **1977b**, *25*, 305.
Epstein, E. "Mineral Nutrition of Plants: Principles and Perspectives"; Wiley: New York, 1972.
Ham, G. E.; Dowdy, R. H. *Agron. J.* **1978**, *70*, 326.
Huffman, E. W. D., Jr.; Allaway, W. H. *J. Agric. Food Chem.* **1973**, *21*, 982.
Janghorbani, M.; Weaver, C. M.; Ting, B. T. G.; Young, V. R. *J. Nutr.* **1983**, *113*, 973.
Lahouti, M.; Peterson, P. J. *J. Sci. Food Agric.* **1979**, *30*, 136.
Levine, S. E.; Weaver, C. M.; Kirleis, A. W. *J. Food Sci.* **1982**, *47*, 1283.
Luttge, U.; Higinbotham, N. "Transport in Plants"; Springer-Verlag: New York, 1979; p 17.
Lyon, G. L.; Peterson, P. J.; Brooks, R. R. *Planta* **1969**, *88*, 282.
Schmitt, H. A.; Weaver, C. M. *Proc. Indiana Acad. Sci.* **1980**, *90*, 125.
Shewry, P. R.; Peterson, P. J. *J. Exp. Bot.* **1974**, *25*, 785.
Singh, B. R.; Steenberg, K. *Plant Soil.* **1974**, *40*, 637.
Skeffington, R. A.; Shewry, P. R.; Peterson, P. J. *Planta* **1976**, *132*, 209.
Starich, G. H.; Blincoe, C. *J. Agric. Food Chem.* **1982**, *30*, 458.
Turner, M. A.; Rust, R. H. *Soil Sci. Soc. Am. Proc.* **1971**, *35*, 755.
Weiner, B. J. "Statistical Principles in Experimental Design", 2nd ed.; McGraw-Hill: New York, 1971; p 399.

Received for review April 13, 1983. Revised manuscript received November 15, 1983. Accepted Janurary 17, 1984. Journal Paper No. 9718, Indiana Agriculatural Experiment Station, West Lafayette, IN 47907.

Isolation and Identification of Volatile Constituents of Sunflowers (*Helianthus annuus* L.)

Patrick X. Etievant,* Mona Azar, Min H. Pham-Delegue, and Claudine J. Masson

Headspace and solvent extraction techniques were used to isolate volatile components of sunflowers of different varieties. Gas chromatographic and mass spectrometric analysis of the extracts and of polar fractions isolated after chromatography on silica gel led to the detection of 84 components among which 20 terpene hydrocarbons, 9 alcohols, 3 phenols, 6 esters, and 19 oxygenated compounds were identified. Forty-seven of these volatile constituents have not been reported previously in sunflowers.

Most plants are pollinated by insects. The series of new hybrids is therefore closely linked to pollen transfer from male to female lines between two plants. The discovery of cytoplasmic male sterility (Leclercq, 1969) has provided more control over the creation of new sunflower hybrids, making it possible to improve oil yield and disease resistance. Nevertheless, field observations have shown that hybrids are difficult to produce despite apparently good parentage. These low yields could be associated with a lack of pollination due to a selective visitation of insects, in particular honeybees (Cirnu and Dumitrache, 1976; Pham-Delegue et al., 1982). As the scent of a flower is one of the prime factors attracting honeybees (von Frisch, 1967), the present work was undertaken to determine the importance of individual flower volatiles in attracting insects toward both male and female parents. While a number of workers have examined sunflower oil volatiles, only one author has studied the aroma constituents of the sunflower itself (Popescu, 1979, 1982; Popescu and Fagarasan, 1979). The aim of this work was thus to complete our knowledge on volatile components emitted by sunflower heads.

Ministere de l'Agriculture, INRA, Laboratoire de Recherches sur les Aromes, 21034 Dijon Cedex, France (P.X.E. and M.A.), and Ministere de l'agriculture, INRA, Station de Recherches sur l'Abeille et les Insectes Sociaux, La Guillonerie, 91440 Bures sur Yvette, France (M.H.P-D. and C.J.M.).

EXPERIMENTAL SECTION

Material. Flower heads were removed from the stems and stored at −25 °C. All samples were analyzed within 6 months of harvest. Two batches of flower heads were examined. A bulk sample (batch A) was composed of

0021-8561/84/1432-0503$01.50/0