

100-N Area Strontium-90 Treatability
Demonstration Project:
WBS-08: Phytoremediation Along the 100-
N Columbia River Riparian Zone
- Food Chain Transfer Study

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Potential for Food-Chain Interactions/Off-Site Transfer

- Engineered barriers and management practices will prevent intrusion of large/small animal herbivores and off-site transfer of plant detritus but will have limited effect on mobile phytophagous insects.
- Three major means by which resident and transitory insects may accumulate ^{90}Sr :
 - consumption of pollen - **obviated by harvesting prior to flowering**
 - **consumption of sap** - aphids
 - **consumption of the foliage and tender shoots** - Lepidoptera and Orthoptera
- The objective of this Task is to evaluate the potential for contamination of insects that may consume plant material grown in soils contaminated with ^{90}Sr .
- Will employ growth chamber experiments using plants grown in 100-N contaminated sediment and exposed to insects.

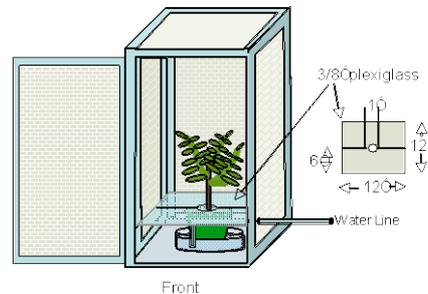
Task Structure and Progress

- Sub Task 1 - Management - *Ongoing*
- Sub Task 2 - General Soil/Plant Preparation - *Completed*
 - Sediment was obtained through cores taken along the shoreline at the 100-N Area at the 20004100NC4473 borehole collection. Sediment collections from different depths and well locations were bulked and sub-sampled for ^{90}Sr -activity
 - Coyote willow (*Salix exigua*) cuttings were obtained from Wildlands, Inc. of Richland, Washington. These originate from alongside the Yakima River in Benton County. The proximal (closer to the trunk) end was dipped into a commercial rooting media (Rootone[®], Garden Tech Inc., Lexington, Kentucky) and kept in distilled water for 14 days until roots and shoots have been initiated from the stem.
 - Plants were then potted in the 100-N sediment and maintained in the growth chamber for a minimum of 60 days to provide sufficient vegetation for the experiment.

Task Structure and Progress

- Sub-Task 3 - Aphid Feeding Study - *Completed*

- Twelve plants were grown in the contaminated 100-N sediment for 70- to 90-days. They were transferred to prepared cages which separated root (pot) from shoot.



- Aphids (*Pterocomma salicis*, Black Willow Aphid?) were obtained from infested Coyote willow plants growing in the PNNL greenhouse. Insects (≥ 1000 per plant) were introduced onto the foliage of six plants. The insects were allowed to feed for periods of seven and seventeen days. At the conclusion of this, the plant was flushed with CO_2 to euthanize the insects. The insects were collected from the plant, counted, and analyzed for ^{90}Sr .
- The plastic bottom of the cage as well as leaf surfaces and the surfaces of the younger green stems) were flushed with a 10% (v/v) ethanol solution to solubilize aphid exudates (honeydew). The liquid was collected, freeze-dried, weighed, and analyzed.
- The shoots (above ground portions) of the plants were removed from the pots, weighed, frozen, freeze-dried, weighed, ground, and analyzed for ^{90}Sr to determine the tissue activity (pCi/g).

Aphid Experiment - Results (Cont.)

- Aphid number and weight.

Plant Number	Days Exposed	Tot. Aphid Dry Wt. per Plant at Conclusion of Exposure	Estimated ¹ Number of Aphids per Plant at conclusion of Exposure
		<i>g</i>	
1	7	0.0696	3247
2	7	0.0643	3000
3	7	0.0276	1288
	Avg	0.0538	2512
	SD	0.0229	1067
4	17	0.0683	3187
5	17	0.0213	994
6	17	0.0294	1372
	Avg	0.04675	1851
	SD	0.02285	1172

Aphids on plant. Note honeydew exudate and various life stages in population

¹Based on average weight of $2.55 \times 10^{-5} \pm 1.02 \times 10^{-5}$ g per aphid from 3600 counted insects (all life stages).

Aphid Experiment - Results

- The initial activity of the 100-N Sediment in which the willow cuttings were grown was 217 ± 4 pCi $^{90}\text{Sr}/\text{g}$ dry wt. as determined by HNO_3 extraction.
- At the end of 80 days the pot sediment ^{90}Sr activity had decreased to 119 ± 4 pCi/g dry wt. also determined by HNO_3 extraction.
- The soil porewater activity at the conclusion of the experiment was determined by bulk soil centrifugation to be 6.8 pCi $^{90}\text{Sr}/\text{mL}$. The soil porewater ^{90}Sr content is the actual activity present in the rhizosphere of the plant root and available for plant uptake.
- The plant concentration ratio defined as (pCi $^{90}\text{Sr}/\text{g}$ dry wt. of new growth tissue)/(pCi $^{90}\text{Sr}/\text{g}$ soil porewater) was 61 ± 10 .

Aphid Experiment - Results (Cont.)

- Total new growth tissue (stems and leaves) which emerged from the original cutting averaged 2.98 ± 0.68 g dry wt. after 60 days of growth.
- Average tissue ^{90}Sr specific activity (pCi ^{90}Sr /g dry wt.) extracted by the plant from the 100-N sediment over the 60-day growth period was 415 ± 77 pCi ^{90}Sr /g dry wt.
- Individually, the stem activity was 364 ± 76 pCi ^{90}Sr /g dry wt. and the leaf activity was 437 ± 89 pCi ^{90}Sr /g dry wt.
- Of the total phytoextracted ^{90}Sr from the 100-N sediment during this experiment, $98.7 \pm 0.3\%$ was found in the plant itself. Approximately 67% of this was contained in the leaves of the plant while the new stems contained over 31%.

Plant No. ¹	^{90}Sr Activity in Collected Honeydew	^{90}Sr Activity in Collected Aphids	^{90}Sr Activity in New Growth Stem and Leaves	Sum of ^{90}Sr Activity per Plant	Percent of Plant Extracted ^{90}Sr in Honeydew	Percent of Plant Extracted ^{90}Sr in Aphids	Percent of Plant Extracted ^{90}Sr in Stem and Leaves
	pCi	pCi	pCi	pCi	%	%	%
1	14.3	1.9	1291.9	1308.1	1.09	0.15	98.76
3	9.1	0.7	1032.1	1041.8	0.87	0.06	99.06
4	24.5	2.9	1733.8	1761.2	1.39	0.17	98.44
5	12.7	1.9	818.5	833.1	1.53	0.23	98.25
6	12.7	1.7	1287.4	1301.8	0.98	0.13	98.89
Avg	14.7	1.8	1232.7	1249.2	1.17	0.15	98.68
SD	5.8	0.8	342.6	348.1	0.28	0.06	0.33

¹. Portions of plant #2 were lost in sample preparation and are not included in this table.

Aphid Experiment - Results (Cont.)

- Total average production of honeydew collected from the plant and cage surfaces averaged 0.9 ± 0.1 g after 7 days and 1.6 ± 0.3 g after 17 days.
- The honeydew specific activity was not significantly different over time with 23.5 ± 18 pCi ^{90}Sr /g dry wt. at 7 days and 11.0 ± 5.6 pCi ^{90}Sr /g dry wt. at 17 days.
- The total ^{90}Sr activity in the honeydew consistently averaged $1.17 \pm 0.28\%$ of that ^{90}Sr extracted from the sediment by the plant.

Plant No. ¹	Days of Honeydew Exudation	Tot Dry Wt. of Honeydew per Plant	Honeydew Dry Wt/day per Plant	Total ^{90}Sr Recovered in Honeydew per Plant	Activity of ^{90}Sr -Honeydew Produced per Day per Plant	Projected Total ^{90}Sr Release per Plant Over Growing Season (Mar 15 - Oct 15) or 213 days
		<i>g</i>	<i>g</i>	<i>pCi</i>	<i>pCi</i>	<i>pCi</i>
1	7	0.906	0.129	14.3	2.0	435.7
3	7	0.869	0.124	9.1	1.3	276.6
4	17	1.4109	0.083	24.5	1.4	307.5
5	17	1.4356	0.084	12.7	0.7	159.5
6	17	1.8655	0.110	12.7	0.7	159.5
		Avg	0.106	14.7	1.3	267.8
		SD	0.022	5.8	0.5	115.4

¹: Portions of plant #2 were lost in sample preparation and are not included in this table.

Aphid Experiment - Conclusions

- At the heavy level of aphid infestation applied to the plants here there was an average of 0.1 ± 0.02 g dry wt. of honeydew deposited on the aphids, plant surfaces, and floor of the exposure cages per day containing $1.3 \pm .5$ pCi of ^{90}Sr .
- In a worst case scenario over an entire growing season of 213 days (March 15 to October 15) this could total an estimated 268 ± 115 pCi of ^{90}Sr per infected plant. This would be distributed over an area of soil/shoreline of from one to several square meters and would be below detection levels.
- To illustrate this point some rough calculations on an extreme case were performed:
 - 1) The current plan is to place the trees on a grid along the shoreline at 100-N with a 1 m spacing. This gives a 1 m^2 area per tree.
 - 2) Given a tree with a size and leaf area ten times that used in this experiment and with a corresponding ten times infestation of aphids, and stipulating that all of the honeydew was washed off the plant into the soil, the resulting deposition of ^{90}Sr to the shoreline sediment would be about 2700 pCi per growing season based on our experimental results.
 - 3) Taking a possible soil bulk density of 1.5 g/cm^3 (Hanford soil ranges from 1 to 2.5+ based on location and the shoreline bulk density would be higher because of the cobble).
 - 4) Using the 1 m^2 to a depth of 15 cm (a standard 6 in. in agriculture) we have $15 \times 100 \times 100 \text{ cm}$ or $150,000 \text{ cm}^3$ of possible area below the plant.
 - 5) This means the weight of the soil would be $\sim 225,000 \text{ g}$ ($\sim 225 \text{ kg}$) and if the activity of the area is 2700 pCi then $2700/225,000$ would give 0.012 pCi/g . This is below detection in a soil profile that already ranges from 100 to 300 pCi/g. (Source: Well N-122)

Insect Larvae Feeding Study - Subtask 4

- Problems associated with growth of the plants including seasonal availability of suitable cuttings and mechanical problems with the growth chamber have delayed the start of this experiment. We anticipate starting the exposure itself next week.
- Twelve 90-day-growth plants have been grown in the contaminated 100-N Area sediment that has a specific activity of 25 ± 2 pCi/g dry wt. (HNO_3 Extraction). Preliminary analysis of leaf tissue from plants has a specific activity of 171 ± 11 pCi/g dry wt. (stems and leaves).
- Several moth species indigenous to the Hanford area. Their larval (caterpillar) stages may be voracious consumers of foliage. Further, many of these species may have several generations over the growing season.

- Two choices may be used depending on availability and maintenance of the colonies.



Western Yellow-Striped Armyworm



Alfalfa Looper

- The Western Yellow-Striped Army Worm (*Spodoptera praefica*), and
- The Alfalfa Looper (*Autographa californica*).
- Both species are generalists and will feed on a number of plant types, including willow. The moths are amenable to culturing in the laboratory or greenhouse, and have life-cycles of 30 to 40 days. Cultures of both or either will be provided by the Yakima Agricultural Research Laboratory (USDA-ARS) courtesy of Dr. Peter J Landolt.

Insect Larvae Feeding Study - Subtask 4

- A minimum of fifteen larvae (late fourth to fifth instar stage) will be placed on each of the six plants in the insect tents.
 - They will be allowed to feed for seven days on the foliage while still in the larval stage.
- At that time one-third of the insects will be removed, euthanized, and processed for weight and per insect ^{90}Sr content (weight normalized).
- A second one-third of the insects will be transferred to another cage where they will be allowed to “clear” their digestive tracts on control plant tissue for 48 hours and then processed for ^{90}Sr content.
- The remaining insects will be allowed to pupate on the plants, the adults collected and processed.
- Both the larval and the adult stages are considered prey by larger animals.
- Plant tissue will also be processed for ^{90}Sr content.

Earned Value Report

Phytoremediation Field Treatability Study	FY-08
BCWS	\$300,000
BCWP	\$225,870
ACWP	\$182,184
SV	-\$74,130
CV	\$43,686

- Values reflect work delays and accompanying lower expenditures caused by plant availability.
- Schedule has been re-baselined