BNWL-SA-5132
Conf-740921--17

SUSPENDED PARTICLE INTERACTIONS AND UPTAKE IN TERRESTRIAL PLANTS[1]

Burton E. Vaughan
Ecosystems Department
Battelle, Pacific Northwest Laboratories
Richland, Washington 99352
August 21, 1974

---

[1] Work performed for the United States Atomic Energy Commission under Contract AT(45-1)-1830

DISCLAIMER

Background

The problem of how green plants respond when contaminated by airborne residues is not simple. Whether one considers a radioelement or any of the stable elements, there is evidence that the green leaf, itself, plays an active role in promoting the incorporation and subsequent metabolism of foliar deposits. First, several extrinsic factors operating at the leaf can significantly modify the sorption/translocation process; namely, humidity, light cycle, mass loading of the leaf, possibly absolute size of the aerosol particle, and possibly the nature of the vector (host) particle. Second, it is now more widely recognized that some of the radioelements, and certainly most of the metal elements, do not behave as simple ions in aqueous solution. Once deposited on the green plant, they are likely to be converted to molecular complexes, whose movement is governed by the chemistry of the organic anion to which they are attached. The idea that molecular complexes are involved rests on a number of inferential studies of sorption and desorption kinetics in seaweeds, soil/plant systems, and the soil clay particle, and on chelation studies in the animal blood stream (1 - 4). Recently, however, there has been progress in identifying such molecular complexes by combined electrophoretic and chromatographic separation (5) Third, problems have arisen as to the generality of the presently limited data base concerned with foliar entry of radioelements into plants. The principal data available concern aqueous spray application of radiocesium and radiostrontium (6). These and data for a few other radioelements comprise the data base for conservative estimation in the Hermes ("Year 2000") Model widely used for nuclear energy applications

In assessing health consequences with such models, we find that the food-chain step involving foliar sorption is only a secondary route of entry to man, with respiratory inhalation the dominant route (0.3 μm AMAD particle, for reference purposes). However, in assessing the potential for biological dispersal and interception, by ecological processes acting over very long time intervals, we find that foliar sorption/translocation rates are important to establish quantitatively. Experience in the fuels reprocessing operations of the nuclear industry has sometimes ~~repeatedly~~ led to radioactivity levels in vegetation which exceeded estimates based on standard calculation (4). Even when proximate hazard to man seems remote, public acceptance of these operations will require a better basis for prediction. Besides research on the transport factor, resuspension, there is need for precise quantitative data on several foliar interactions involving, particularly, the transuranium elements other than plutonium-239.

Our ecological program at PNL is aimed at four major aspects of behavior of the transuranium elements in or on environmental surfaces. Although today we will be presenting studies in the last area only, it is useful to keep in mind the coordinated program. These are: 1) ecological pattern of distribution in 200-Area ponds, 2) plant availability under arid weathering/aging conditions, 3) complexation reactions in the soil/plant system, and 4) aerosol particle/plant interaction.

In the foliar studies, a great deal of background effort was needed because of the complexity in handling plants under good physiological conditions. Also, because of the potential for serious accident in circulating plutonium aerosols at 1 mph, much of the plant handling technic was worked out using arbitrary tracer aerosols that were, however, adequate to establish the plant responses. This work has not been widely reported. As it is still useful in guiding current developments, I would like to review several points today. Drs. Betty Klepper, Doug Craig, and Dom Cataldo will speak separately on our current exposure system and on the initial aerosol plutonium depositions.

Foliar Uptake Rates

In the plant physiological literature, uptake has been estimated in fairly unrealistic ways; e.g., using cuticle only (8 - 11), isolated leaf cells (12), and excised leaves (13 - 15). These data do not seem to be too useful for radioecological purposes. Entire plants have been exposed, also, using rather different applications technics; e.g., leaf immersion (16), a drop of known composition applied to the leaf (17) and sprays (18, 6). Depending on method of application, either all or a fraction of the material placed on the leaf remains in the plant. In the radioecological literature, air to plant correlations of fallout deposited, Sr, Cs, and I_2, have led to the value of 0.25 representing the fraction of each month's surface deposition from air and from sprinkler irrigation that is initially retained by the vegetation. This fraction is considered to be applicable to all radionuclides on all crops; and, to remain on the plant with a biological half-life of 14 days, the average value for published data concerning fallout nuclides (7). Obviously, these values represent operational definitions subject to considerable variation due to climatic, chemical and plant characteristics.

Ten-day old plants of the common string bean, Phaseolus vulgaris, were used for the studies below, with culture and handling conditions described in detail elsewhere (19, 20). The following deposition parameters now have been established for 0.8 μm particles under flow rates varying from 0.2 to 20 cm/sec.

TABLE 1

Deposition of ^{198}Au-Labelled Aerosol (0.8 µm) On Bean Plants In Low Velocity Wind Tunnel (Data taken from Craig and Klepper, Ref. 20)

Distribution Characteristics	AMAD, µm	0.78 ± 0.04
	GSD	1.65 ± 0.04
Mean Aerosol Concentration	pg/cm^3	
Deposition Rate Onto Leaves	$pg/cm^2/sec$	
Leaf Area	cm^2	
Mean Deposition Velocity	cm/sec	$(4.1 \pm 1.1) \times 10^{-3}$
Radioactivity, Fraction of Total Generated		$(1.0 \pm 0.3) \times 10^{-3}$

Averages of 12 runs, 4 plants per run; ± 1 S.D. as indicated.

Since deposition velocity will be strongly dependent on particle size, one should note that the average reported here will be particular to the distribution described. We now expect to extend these determinations to larger particle sizes by way of establishing a systematic data base.

Mass Loading

An important feature to note in the data shown above is the very low mass loading on the leaves. The duration of each of these runs was 20 min, leading to a value for loading in the order of 4 pg per cm^2 of leafy surface. This is a highly desirable feature. In earlier work, where we had less control over the rate of generation, it was noticed that mass loadings in excess of about 1 $\mu g/cm^2$ led to grossly impaired function in the plant. For these earlier studies, vector aerosols of anhydrous glycerol were generated, containing ^{22}Na seed nuclei. The system was adapted from the Sinclair La Mer approach (19, 36). Two lines of evidence indicated impaired function at loading in excess of 1 $\mu g/cm^2$.

First, polarographic studies of gas exchange at the leaf surface showed impaired leaf function at high aerosol loadings; e.g., 6-17 mg/cm^2 (Table 2) oxygen evolution, and hence photosynthesis was reduced one-half or more at these loadings. At the highest loading, even plant respiration (O_2 uptake) was inhibited.

TABLE 2

Polarographic[1] Studies of Bean Leaves Unexposed and Exposed to Glycerol Aerosols (AMAD = 0.6 μm; GSD = 1.1). Showing Inhibitory Effects at High Mass Loading. (Data taken from Dehnel and Vaughan, Ref. 19)

	N (pairs)	Light[2], O_2 Evolution ($\mu\ell$ O_2/min) STPS	Dark, O_2 Uptake ($\mu\ell$ O_2/min) STPS
Control (no aerosol)			
26-30 hrs after sectioning	12	5.2 ± 0.6	1.4 ± 0.4
72-168 hrs after sectioning	16	5.3 ± 1.0	2.1 ± 0.6
Aerosol[3]			
6 $\mu g/cm^2$	14	2.8 ± 0.5	1.2 ± 0.2
17 $\mu g/cm^2$	22	1.0 ± 0.4	0.5 ± 0.2

---

[1] Polarographic chamber solution had a CO_2 content of 70 $\mu\ell$.

[2] Irradiance, 3.5 m watts (375-750 nanometers).

[3] Plants were aerosol exposed 24 hours prior to sectioning and polarographically assayed 26-30 hours after sectioning; leaf sections, 20 mm diameter.

The second line of evidence was more sensitive. It depended on measurement of translocated radioactivity from leaf to root with active photosynthesis, more ^{22}Na was transported out of the leaf to the root. Hence, the ratio of root: leaf radioactivity showed increasing values at lower particle loadings, with the highest ratio at a loading of 1 $\mu g/cm^2$ (= 0.07 mg ÷ 70) (Figure 1).

These demonstrations of inhibited transport by high mass loading suggest caution in attempting to extrapolate data from the early publications on test site experiments to the low level, long-term situation. In those cases, mass loadings undoubtedly were very much higher than those reported here. Continuous exposure with low mass loading should be the most effective way of translocating the more mobile radioelements from leaf to root.

Metabolic Transport

Translocation of ^{22}Na from the leaf to other plant parts is probably mediated metabolically by bulk transport of metabolites exported from the leaf. Absorption by the leaf (21 - 24), translocation by roots (25), and protoplasmic permeability (26, see also, 27) are all known to be enhanced by light. Biddulph and Markle (28) have shown for cotton plants that translocation out of the leaf is essentially restricted to the phloem. Also, large accumulations of tracer generally may be considered as evidence of unloading into sinks at the end of a translocation channel (29).

✓ In the glycerol aerosol experiments, light effects on stem and root translocation of ^{22}Na-ion were pronounced (Table 3). Under dark conditions (Group 3), one can see a highly significant reduction in root radioactivity, as compared to roots under standard lighting conditions (Group 1). About 98 percent of the nonelutable radioactivity remained in the dark leaves compared to about 71 percent in lighted leaves. Translocated fractions of radioactivity might be expected to vary widely depending on parts of plant eaten, growth habit of the plant, and type of radioelement. However, the ^{22}Na data should provide an upper limit on the rate of translocation for substances undergoing bulk transport in the main vascular channels of growing plants. The literature gives scarce guidance on this point. In the HERMES Model, translocation fractions are tabulated for grain including

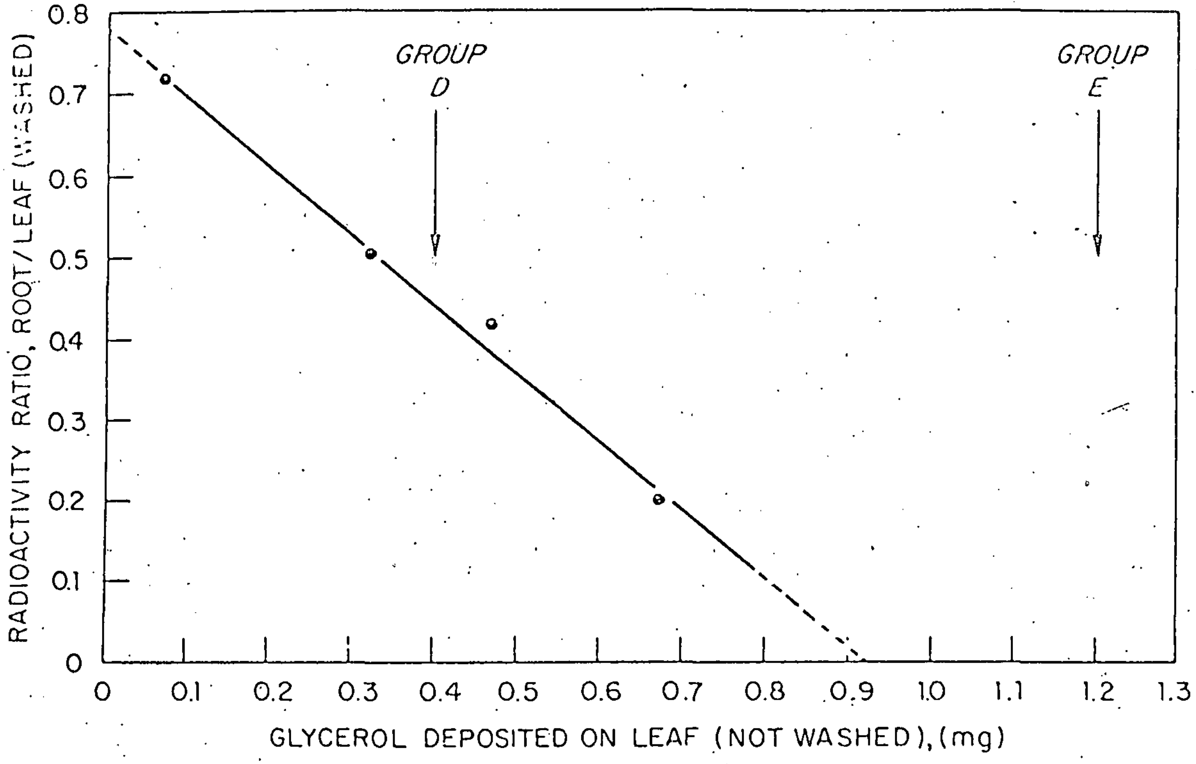

FIGURE 1. Relative transport to root in relation to initial mass of glycerol deposited on leaf. N = 4 to 5 for each point. A similar relation exists for root:stem radioactivity ratios. The two arrows denote glycerol deposition for experiments, reported in Table 2. Leaf area is approximately 70 cm^2 (two sides).

TABLE 3

Comparison of Initially Deposited Radioactivity with Radioactivity Remaining after 6 Second Rinse in 0.36 M Mannitol Solution; ^{22}Na-labelled Glycerol Aerosol Particles (AMAD = 0.6 μm; GSD = 1.1) (Date based on Dehnel, Vaughan and Vaughan, ref. 17)

	Non Eluted	Eluted Segments			
			Fraction of Total Fixed		
	Radioactivity in One Leaf (pC$_i$)	Radioactivity Total from Segments at Right (pC$_i$)	One Leaf (%)	1/2 Stem (%)	1/2 Root (%)
Group 1 Standard Conditions					
	16 ± 2	2	56	22	22
	26 ± 4	5	83	10	8
	29 ± 2	6	69	17	15
	38 ± 4	10	78	10	12
Group 2 High Humidity					
	40 ± 8	9	72	11	17
	46 ± 2	20	72	18	10
Group 3 Continuous Dark					
	47 ± 10	7	97	3*	<1*
Group 4 Continuous Dark					
	75 ± 14	21	99	<1*	<1*
Group 5 Continuous Light					
	20 ± 2	6	60	11	29
	26 ± 5	11	71	11	18
	70 ± 6	12	74	11	15

Two bean plants were exposed to aerosol for one hour in light; then transferred to growth chamber for 24 hours under the conditions shown above. N = 9 plants for each exposure run shown.

*P < .01

leafy vegetables, and for potatoes including root vegetables (7). Typically, these estimated fractions vary from 0.01 to 0.05 for elements as diverse as Na, Sr, Cs, and Ce. The values have been inferred principally from field measurements of Sr, Cs, and I_2. There are no firmly established data for the transuranium elements.

In the experiments shown above, aerosol had access to only two leaves of each plant; stem and root system were protected by a plastic bag. Also, one of the two identically exposed leaves was rinsed, as were the stem and root. Therefore, to facilitate comparisons, among the eluted segments, root radioactivity was multiplied by one-half (during the exposure any radioactivity appearing in stem and roots would have entered the plant by two leaves rather than one).

Leaching

A second important point, from Table 3, is the attempt to estimate radioactivity "fixed" in or on the leaf. Twenty-four hours after the glycerol particle exposure, one of the two identically exposed leafs was rinsed and compared to the other. About 1/4 to 1/3 of the radioactivity impinging on one leaf seemed to be fixed--predominantly in the eluted leaf and, to a smaller extent, root and stem. After this rinse, such radioactivity may still be present in more than one location. It might be associated with irregularities of the epidermal cell cuticular wax (~~9~~30). Here, its removal by an aqueous rinse medium would be restricted by surface tension phenomena. It might also be located in various internal leaf compartments. In the later case, stomatal dimensions are small enough (ref. ~~27~~15 and refs. cited therein) that water surface tension would again restrict mass flow of fluid, hence removal of ^{22}Na-ion by elution from the intercellular spaces. The 0.6 μm anhydrous particle is small enough to penetrate pores* or interstices in the wax surface of the leaf without exclusion due to water surface tension effects. Such conditions are not readily obtainable with water sprays. Thus, it seems likely that for ^{22}Na to enter the plant transport channels, fluid movement from the leaf outward was necessary to hydrate the glycerol particle, with subsequent diffusion of dissolved ^{22}Na-Cl solution to the interior compartments of the leaf.

Humidity effects were not clear-cut in the study discussed above (Table 3). Average values were 82% relative humidity (group 2) compared to 39% (group 1). However, the effect of humidity here may not be simple. With the drop technique (~~22~~31), radioactivity uptake was observed to increase to a plateau as the concentration of solution changed; uptake

---

*Stomata sizes were between 8 x 3 μm (upper surface) and 7 x 3 μm (lower surface) was greatly reduced when the droplets became dessicated. Ambler and Menzel (~~4~~32) also have found that absorption increased with increasing relative humidity, using dried ^{85}Sr droplets. The anhydro~~m~~us glycerol particles used here were found to rehydrate slowly, over many hours, as estimated by particle size growth in saturated atmospheres. Thus, it is likely that the rate of release of ^{22}Na was governed by the slow glycerol hydration.

While highly provisionally, the data above are among the very few concerning aerosol behavior at plant leaf surfaces. Considering the data, it seems unlikely that normally metabolizing plant leaves will behave passively as regards radioelement fixation, and the vector may have dominant influence on its mobility. For example, the hygroscopicity of many aerosol particles can cause movement of water and, undoubtedly, other solutes to the leaf surface. In turn, the presence of organic anions, mucopolysaccharides and other solutes may promote the removal of Pu from the vector and subsequent its translocation once in the plant tissue. For this reason, we intend to look systematically at leaching and leaf fixation as they vary in time, for both primary and vector aerosol deposits.

Vector Type and Particle Size

At this stage of development, it is not clear what kind of vector (host) particle merits most immediate attention. Some further evaluation of data from this conference, and other work in progress, will be needed before priorities are set. The range of particle size of concern falls between 5 and 50 µm. This range includes the median size found for siliceous fallout particles in the NTS series (Romney; 1963, 1970) and the median size thought to be characteristic of Pu-dust particles at Rocky Flats (Nathan, 1971; Volchok, 1971). As regards green plants, differences in uptake, in the NTS experiments, seemed to be small except for wheat, which showed disproportionate uptake of particles 5 µm or less in size. Rocky Flats data indicate that most of the primary Pu particles were submicronic, smaller than 0.2 µm diameter. However, their vector appeared to be dust particles of 10 µm to 20 µm median diameter.

In certain aquatic situations, algal floc drying along the edges of ponds may provide a wind resuspended vector for radioactivity transport. In these cases, we do not yet know what sizes are likely, nor do we know the leaf chemistry for possible interactions with such aerosols.

Agricultural and Other Plant Types

Because of the truly messy problems attaching to the experimental procedures, we are lucky to have any Pu data at all for green plants, during the first year of this program (see Craig & Klepper paper following). To date, we have had to use bean plants, as a concession to practicality; but in the long run, and for the location of this laboratory, interest attaches to both agricultural and range land species typical of the area. The following acreage, based on Washington State agricultural reporting statistics, are representative of irrigated land in the Pacific Northwest, including Oregon and parts of Idaho. Fruit tree acreage is, however, small; as are several specialty crops.

Crop	Acres
*Barley	4,114,000
Wheat	2,258,000
*Dry peas	143,000
*Alfalfa hay	523,000
Clover timothy	230,000
Grain hay	91,000
Miscellaneous hay	83,000
Corn (grain silage)	87,000
*Sugar beets	63,000
Fruit trees (estimated)	14,000
TOTAL:	7.61 million acres

Based on considerations of growth rate, leaf structure, surface area, and pubescence, the starred items should be systematically compared.

Not all land in the Pacific Northwest is under irrigation. Substantial areas around eastern Washington and Rocky Flats remain in desert condition. Native grasses and the shrubs are used as livestock forage, including bluebunch wheatgrass, Agropyron spicatum and cheatgrass, Bromus tectorum. At the Nevada Test Site, Bromus rubens is an important grass at low elevations; and Poa nevadensis is an important grass at high elevations. Probably the most important livestock forage shrub at the Nevada Test Site and the Hanford Reservation is winterfat, Eurotia lanata.

Referances

1. Burton E. Vaughan and John A. Strand. 1970. Bioogical implications of a marine release of ^{90}Sr. Health Physics 18:25-41.

2. J. T. Cummins, J. A. Strand, and B. E. Vaughan. 1969. The movement of H^+ and other ions at the onset of photosynthesis in Ulva. Biochim. Biophys. Acta 173:198-205.

3. Keith R. Price. 1973. A review of transuranic elements in soils, plants and animals. J. Environ. Quality 2:62-66.

4. Keith R. Price. 1971. Critical Review of Biological Accumulation, Discrimination and Uptake of Radionuclides Important to Waste Managmment Practices, 1943-1971. BNWL-B-148. Battelle-Northwest, Richland, Washington. pp 13, 23.

5. R. E. Wildung, H. Drucker, T. R. Garland, and G. S. Schneiderman. 1973. Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants. Quarterly progress report to the National Institute of Environmental Health Sciences, June-Septmeber 1973 (Contract No. 211B00844).

6. R. Scott Russell. 1966. Radioactivity and Human Diet. Pergamon Press Ltd.; Bell and Bain, Ltd., Glasgow. pp191-211

7. Joseph K. Soldat. 1971. The Radiological Dose Model. In: J. F. Fletcher and W. L. Dotson (eds.), Hermes - A Digital Computer Code for Estimating Regional Radiological Effects from the Nuclear Power Industry. HEDL-TME-71-168. Westinghouse Hanford Co. , Richland, Washington.

19. G. S. Dehnel, B. E. Vaughan and W. J. Vaughan. 20 March 1968. Foliar Uptake and Translocation of ^{22}Na-labelled Monodisperse Glycerol Aerosol Particles (0.6 Micron). USNRDL-TR-68-40. Springfield, Virginia.

20. Betty Klepper and D. K. Craig. 1974. Deposition characteristics of aerosol particles onto foliage and other surfaces, pp51-54. In: Pacific Northwest Laboratory, Annual Report for 1973. BNWL-1850, Vol. 1 -Life Sciences, Part 2-Ecological Sciences. Battelle-Northwest, Richland, Washington.

33. Romney, E. M., H. M. Mork and K. H. Larson. 1970. "Persistence of plutonium in soil, plants and small mammals." Health Physics 19:487-491.

34. Romney, E. M., R. G. Lindsey, H. A. Hawthorne, B. G. Bystrom and K. H. Larson. 1963. "Contamination of plant foliage with radioactive fallout." Ecology 44:343-349.

35. Volchok, H. 1971. Presentation at Symposium on Plutonium on Plutonium in the Environment. Los Alamos. August, 1971.

21 ~~1.~~ Ahlgren, G. E. and T. W. Sudia. 1967. The mechanism of the foliar absorption of phosphate. In Isotop. Plant Nut. Physiol., Proc. Symp., Vienna 1966, pp 347-369.

~~2. Altman, P. L. and D. S. Dittmer (eds) 1966. Biology Data Book, Federation of American Societies for Experimental Biology, Bethesda, Maryland; Table 69.~~

~~3. Altman, P. L. and D. S. Dittmer (eds) 1966. Environmental Biology, Federation of American Societies for Experimental Biology, Bethesda, Maryland; Table 72.~~

32 ~~4.~~ Ambler, J. E. and R. G. Menzel. 1966. Retention of foliar applications of Sr^{85} by several plant species as affected by temperature and relative humidity of the air. Radiat. Botany 6: 219-223.

13 ~~5.~~ Ariz, W. H. 1963. Influx and efflux by leaves of Vallisneria spiralis. Protoplasma 57: 5-26.

28 ~~6.~~ Biddulph, O. and J. Markle. 1944. Translocation of radiophosphorus in the phloem of the cotton plant. Am. J. Botany 31: 65-70.

17 ~~7.~~ Bukovac, M. J. and S. H. Wittwer. 1957. Absorption and mobility of foliar applied nutrients. Plant Physiol. 32: 428-435.

29 ~~8.~~ Canny, M. J. and M. J. Askham. 1967. Physiological inferences from the evidence of translocated tracer: a caution. Ann. Botany 31: 409-416.

30 ~~9.~~ Eglinton, G. and R. J. Hamilton. 1967. Leaf epicuticular waxes. Sci. 156: 1322-1335.

~~10. Epstein, E., W. E. Schmid, and D. W. Rains. 1963. Significance and technique of short-term experiments on solute absorption by plant tissue. Plant and Cell Physiol. 4: 79-84.~~

~~11. Evans, E. C. III and B. E. Vaughan. 1966. Wounding response in relation to polar transport of radiocalcium in isolated root segments of *Zea mays*. Plant Physiol. 41: 1145-1151.~~

12. Handley, R., R. K. Schulz, H. Marschner, R. Overstreet, and W. M. Longhurst. 1967. Translocation of carrier-free ^{85}Sr applied to the foliage of woody plants. Radiat. Botany 7: 91-95.

25 ~~13~~. Hanson, J. B. and O. Biddulph. 1953. The diurnal variations in the translocation of minerals across bean roots. Plant Physiol. 28: 356-370.

27 ~~14~~. Hutchin, M. E. and B. E. Vaughan. 1967. Relation between calcium and strontium transport rates as determined simultaneously in isolated segments of the primary root of *Zea mays*. Plant Physiol. 42: 644-650.

12 ~~15~~. Jyung, W. H., S. H. Wittwer, and M. J. Bukovac. 1965a. Ion uptake by cells enzymically isolated from green tobacco leaves. Plant Physiol. 40: 410-414.

~~16. Jyung, W. H., S. H. Wittwer, and M. J. Bukovac. 1965b. The role of stomata in the foliar absorption of Rb by leaves of tobacco, bean and tomato. Proc. HortScience 86: 361-367.~~

16 ~~17~~. Jyung, W. H. and S. H. Wittwer. 1964. Foliar absorption—an active uptake process. Am. J. Botany 51: 437-444.

22 ~~18~~. Kylin, A. 1960. The accumulation of sulfate in isolated leaves as affected by light and darkness. Botan. Notiser 113: 49-81.

26 ~~19~~. Lepeschkin, W. 1930. Light and the permeability of protoplasm. Am. J. Botany 17: 953-970.

23 ~~20~~. van Lookern Campagne, R. N. 1957. Light-dependent chloride absorption in *Vallisneria* leaves. Acta Bot. Neerlandica 6: 543-582.

21. Middleton, L. J. 1958. Absorption and translocation of strontium and caesium by plants from foliar sprays. Nature 181: 1300-1303.

31 22. Middleton, L. J. and J. Sanderson. 1965. The uptake of inorganic ions by plant leaves. J. Exp. Botany 16: 197-215.

24 23. Rains, D. W. 1967. Light-enhanced potassium absorption by corn leaf tissue. Science 156: 1382-1383.

24. Salam, M. S. A. and M. A. Sowelim. 1967. Dust deposits in the city of Cairo. Atmos. Environ. 1: 211-220.

36 25. Sinclair, D. and V. K. La Mer. 1949. Light scattering as a measure of particle size in aerosols: The production of monodisperse aerosols. Chem. Rev. 44: 245-267.

14 26. Smith, R. C. and E. Epstein. 1964a. Ion absorption by shoot tissue: Kinetics of potassium and rubidium absorption by corn leaf tissue. Plant Physiol. 39: 992-996.

15 27. Smith, R. C. and E. Epstein. 1964b. Ion absorption by shoot tissue: Technique and first findings with excised leaf tissue of corn. Plant Physiol. 39: 338-341.

28. Spector, W. S. (ed). 1956. *Handbook of Biological Data.* W. B. Saunders Co.; Table 120.

8 29. Vaughan, W. J. and B. E. Vaughan. 1968. Lung deposition of labelled, monodisperse submicronic particles. Naval Radiological Defense Laboratory Technical Report TR-68-108 of 4 October 1968.

9 30. Yamada, Y., M. J. Bukovac, and S. H. Wittwer. 1964. Ion binding by surfaces of isolated cuticular membrane. Plant Physiol. 39: 978-982.

10 31. Yamada, Y., W. H. Jyung, and S. H. Wittwer. 1965. The effects of urea on ion penetration through isolated cuticular membrane and ion uptake by leaf cells. Proc. HortScience 87: 429-432.

11 32. Yamada, Y., S. H. Wittwer, and M. J. Bukovac. 1964. Penetration of ions through isolated cuticles. Plant Physiol. 39: 28-32.

~~33.~~ Yamada, Y., S. H. Wittwer, and M. J. Bukovac. 1965. Penetration of organic compounds through isolated cuticlar membranes with special reference to C^{14} urea. Plant Physiol. 40: 170-175.