

was highest in sheaths and lowest in developing heads. Ash content of all plant parts except stems was higher in spring than in fall. Stems were only slightly lower in the spring. Percentage of silica in the ash was highest in the roots in spring, averaging 43.3%, and was lowest in developing heads (average of 9.80%).

Spodograms representative of the silica deposition in various parts of the barley plants are shown in Figure 1. Considerable difference existed between the depositional pattern occurring in leaves and in sheaths. Rows of crenate edged silica particles, like those observed in barley sheaths, also, occurred in wheat sheaths (6, 8). The results showed no direct relationship between silica content and resistance to greenbugs, cold, or diseases. Both resistant and nonresistant varieties had high silica

contents. There may be a relationship between early depositions and type of deposition to such resistance, as has been indicated by other workers.

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SELENIUM

Release of Volatile Selenium Compounds by Plants. Collection Procedures and Preliminary Observations

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Release of volatile selenium compounds by two plant species, *Astragalus racemosus* (Pursh.), a selenium-accumulating plant, and alfalfa, *Medicago sativa* (L.), a nonaccumulator, was studied using trace amounts of Se⁷⁵ in solution culture. Methods for collection of the volatile selenium compounds are described. Amounts of volatile selenium released by both the accumulator and nonaccumulator species were qualitatively related to the amounts of selenium within the plant. With intact plants the release of the volatile compounds was mainly by the foliage and the rate of release varied throughout the day.

PLANTS growing on soils or in solution cultures containing substantial amounts of selenium often have a characteristic garlic-like odor. Indeed, many of the so-called primary accumulators have especially offensive odors, and it has been suggested by some workers that the intensity of the odor might be used as a qualitative indication of selenium in the plants (14).

It has been generally assumed that the characteristic odor of these plants was due to the release of one or more volatile selenium compounds, although the identity of these compounds has not yet been established. However, certain fungi produce dimethyl selenide when cultured in media containing selenate and a methyl donor such as methionine (2, 4). Also, dimethyl sel-

enide is a respiratory excretion product of rats dosed with selenate or with dimethyl selenide (10, 11). Rosenfeld and Beath (14) have suggested that dimethyl selenide may also be released by higher plants, but experimental evidence on this point is lacking.

Evidence from numerous studies suggests that selenium may be lost from plant material during storage, oven drying, or chemical analysis. Thus Moxon and Rhian (12) reported losses of 4 to 73% when the grains of barley, corn, and wheat were stored for 3 to 5 years. In studies with *Astragalus bisulcatus*, Beath, Eppson, and Gilbert (7) found not only that analytical values for selenium decreased during drying at 20° C., but that the selenium compounds released could be trapped by bubbling air from the drying plant material through concentrated sulfuric acid. However, Hurd-Karrer (8) reported that when wheat seedlings were dried in the oven at 60° C. for 24 hours the

odor characteristic of drying seleniferous plant material was not detected.

For the most part, volatilization of selenium was studied with plant tissues containing relatively large amounts of the element—so-called selenized plants containing from 5 to several thousand p.p.m. of selenium. These studies were of considerable interest because of the known toxic effects of selenized vegetation on animals.

However, with the more recent recognition of animal requirements for trace amounts of selenium, it became desirable to investigate the problem of volatilization of selenium by plants containing low level or trace amounts of the element. [The role of trace amounts of selenium in animal nutrition has been discussed in detail by a number of workers and is not considered here (7, 13-16).]

Analytical values for selenium in plant materials suspected of contributing to selenium-responsive disorders of ani-

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mals have been quite variable. In part, these variations may be attributed to great technical difficulties in determining submicrogram amounts of the element in biological materials, but it is also possible that unrecognized and uncontrolled volatilization losses occurring during harvest, storage, and preparation of the materials for analysis have contributed to the conflicting results. Thus, an examination of volatile losses from plants and plant materials of low selenium status is warranted.

This paper reports results from experiments on the release of volatile selenium from *Astragalus racemosus*, a selenium-accumulating species, and from alfalfa, *Medicago sativa*, a nonaccumulating species, when both were grown in media containing only trace amounts of selenium.

Methods

Plant Culture. *Astragalus racemosus* (Pursh.) and alfalfa (*Medicago sativa* L.) plants were cultured in nutrient solution by the procedure of Johnson *et al.* (9). The salts used in the preparation of the nutrient solutions were analytically low in total selenium, providing less than 0.01 μg . of Se per liter of culture solution. An additional 1.0 μg . of Se was added as high specific activity Se^{75} (26 μc .) to the alfalfa cultures when the seedlings were 2 weeks old. Radioactive selenium was supplied as selenite from selenious acid obtained from Oak Ridge National Laboratory, Oak Ridge, Tenn., and from Tracerlab, Waltham, Mass. *Astragalus* plants were grown for 6 weeks without added Se, after which Se^{75} (3.4 μg . of Se, as 100 μc . of Se^{75}) was added. Collection of volatile selenium was commenced immediately after the addition of Se^{75} .

To minimize possible health hazards from volatile radioactive selenium, the plants were cultured in a well ventilated greenhouse. Illumination varied with weather conditions, reaching a maximum of 9000 foot-candles on clear days.

Collection of Volatile Selenium.

During the collection period the intact plants were placed in a large bell jar or in a plastic glove box. Air was drawn from around the enclosed foliage through a variety of trapping solutions contained in gas-washing bottles or tubes using a vacuum pump to provide an air flow of about 3 liters per minute.

A variety of liquid trapping media was investigated. Some systems were chosen to determine whether the volatile compound was soluble in water or simple aqueous solutions, such as 0.1M ammonium ethylene(dinitrilo)tetraacetic acid (EDTA) at pH 5; other solutions—for example, 1M KOH and 1M NH_4OH —were selected for the purpose of retaining any acidic compounds released. Lead acetate (10% aqueous solution) was used to determine whether the volatile compound might form an insoluble lead salt, as suggested by Gorsuch (5) in his study of losses of Se on wet ashing.

Two per cent KMnO_4 in 1M KOH was tried, in the hope that reduced selenium compounds might be oxidized to selenite or selenate. Sodium ethylate (2% in absolute ethanol) and benzene were used to measure effectiveness of organic solvents. Diaminobenzidine, which has been used as an analytical reagent in determination of selenium [Handley and Johnson (6) and Cheng (3)], was also tried as a 0.5% solution of the hydrochloride in HCl at pH 1.8.

The known effectiveness of activated carbon granules in removing offensive odors and smog constituents from the atmosphere suggested that charcoal might effectively trap the selenium compounds. A borosilicate glass tube, 15-mm. i.d., 5 cm. long, containing 0.8 gram of activated charcoal granules (Cliffchar activated carbon, grade 4 \times 10 mesh, technical, Cliffs Dow Chemical Co., Marquette, Mich.), was inserted in the air stream following a cold trap immersed in dry ice-acetone to remove most of the transpiration water.

Collections were made over a 24-hour period. Aliquots of solutions were taken at 4 hourly intervals for counting of Se^{75} . Charcoal was replaced at 4-hour intervals for similar counting. In all cases, a deep-well gamma scintillation system, with NaI(Tl) crystal and Tracerlab spectrometer and scaler components, was used. Count rates were corrected for background and decay.

Results and Discussion

None of the liquid media retained significant amounts of Se^{75} activity. However, the charcoal granules proved effective in retaining radioactivity, which was shown by half-life measurements and energy spectrum measurements to be authentic Se^{75} .

To evaluate the efficiency of the charcoal in absorbing the Se^{75} -labeled compounds, a tube was filled with weighed portions of charcoal separated by Dacron batting, so that successive portions of the charcoal could be removed for counting. Over a 6-hour collection period and with an air flow rate of 3 liters per minute, 83% of the total Se^{75} recovered was found in the first 0.8-gram portion, and 12, 4, 1, and 0% in successive 0.4-gram portions of charcoal.

Very little radioactivity was retained in the transpiration water frozen out in

the dry ice-acetone trap, which preceded the charcoal tube. Immersion of the charcoal tube in liquid nitrogen did not increase efficiency of absorption of Se^{75} compounds on the charcoal.

Desorption or extraction of Se^{75} from the charcoal was studied by shaking 0.8-gram samples of the charcoal with 4 ml. of solvent for 1 hour, filtering the solution to remove suspended charcoal, and counting the filtrate. Individual portions of charcoal were taken for each solvent. Results obtained with six solvents are given in Table I.

The results indicate that the Se^{75} -labeled compounds contain polar groups and are thus at variance with the suggestion that the volatile selenium compound from plants is dimethyl selenide (14). However, published values for solubility characteristics of dimethyl selenide may not be applicable to the submicrogram amounts of selenium compounds collected in this study. The amounts of material so far obtained are too small to permit any further characterization.

With both species of plants used in this study, evolution of volatile selenium compounds commenced soon after the application of Se^{75} to the nutrient solution. With *Astragalus*, detectable Se^{75} activity was trapped on the charcoal within 24 hours after supply to the roots in the solution culture (Figure 1). With alfalfa, where the Se^{75} was applied at an earlier stage of growth and at a lower concentration, release of volatile Se became measurable about 1 week after the Se^{75} was introduced into the nutrient solution (Figure 2).

Data of Figure 2 indicate that both the selenium content of the fresh plant material and the evolution of volatile selenium varied with the age of the plant, and that the amount of volatile selenium released was a function of the selenium content of the plant.

Within a single set of alfalfa plants the rate of evolution of Se^{75} fluctuated during the day (Figure 3). Here volatile Se^{75} was collected at approximately hourly intervals. During the first day,

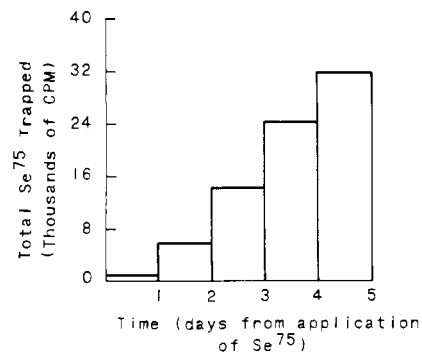


Figure 1. Collection of volatile Se^{75} from *Astragalus* plants of low selenium status

Cumulative counts

Table I. Extraction of Se^{75} from Charcoal

(Volatile Se released by alfalfa plants)

| Solvent | Se^{75} Activity Extracted, % |
|----------------------|--|
| Decahydronaphthalene | 0 |
| Toluene | 0 |
| Benzene | 9 |
| Ethanol (100%) | 60 |
| Ethanol (50%) | 97 |
| Distilled water | 94 |

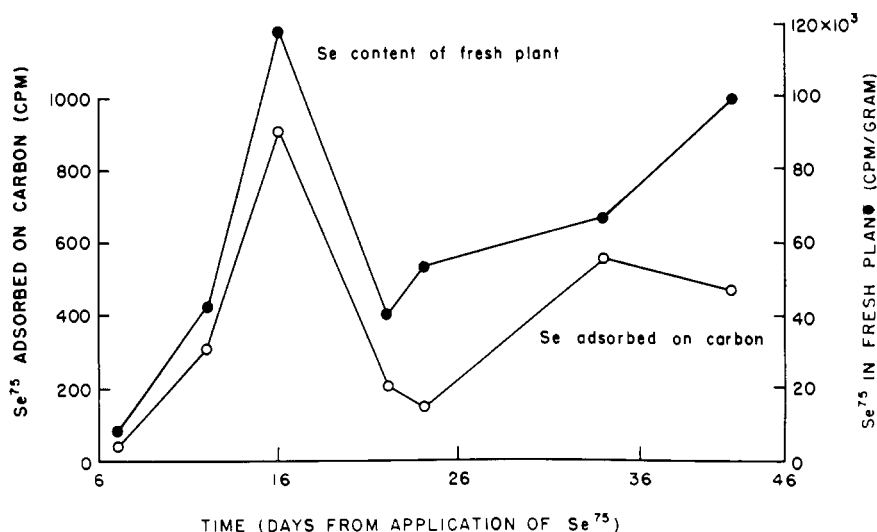


Figure 2. Selenium content of alfalfa plants and amounts of volatile Se^{75} collected with time after Se^{75} application

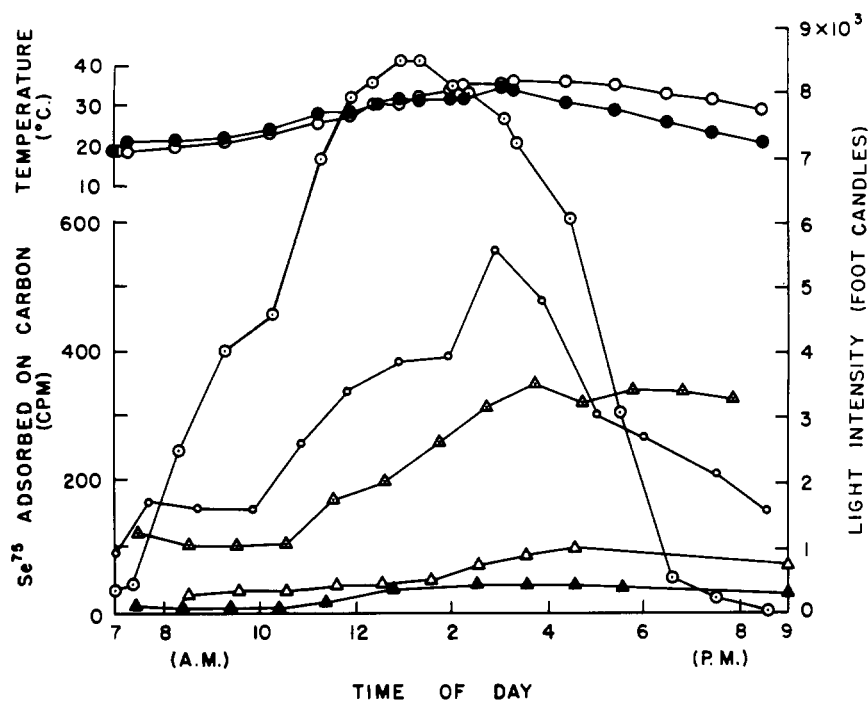


Figure 3. Effect of light intensity and temperature on evolution of Se^{75} by alfalfa

- Light intensity, foot-candles
- Se^{75} from photosynthesizing plant system
- △ Se^{75} from dark plant system
- △ Se^{75} from roots and nutrient solution
- ▲ Se^{75} from nutrient solution after removal of roots
- Temperature of nutrient solution
- Temperature of ambient atmosphere

the plants received full greenhouse illumination, while on the second day the bell jar which enclosed the plants was covered with heavy aluminum foil to exclude light. On the third day the tops of the plants were severed and volatile Se^{75} from the culture solution containing the decapitated roots was collected as before. On the fourth day the roots were removed, and volatiles were collected from the nutrient solution only. Light intensities and temperatures of the ambient air and nutrient solution were recorded. These values were similar on each of the collection days. Con-

sequently, temperature and light data for the first day only are plotted.

Figure 3 shows that the foliage of the plant released most of the Se^{75} which was collected on the charcoal, since removal of the tops reduced the collection of volatile selenium to very low levels. Both shaded and unshaded plants reached maximum rates of volatile production in the early afternoon, but the unshaded plants produced more volatile selenium. The maxima lagged about 2 hours behind the light intensity maximum, roughly coinciding with the temperature maximum; however, the data

do not permit separation of possible light and temperature effects. Only very small amounts of volatile selenium were released by decapitated roots and by the nutrient solution.

These results clearly show that the release of volatile selenium compounds by intact plants is not restricted to selenium accumulators such as *Astragalus racemosus*, but can also occur in a non-accumulator such as alfalfa, and that the production of these volatile compounds is not dependent on high internal concentrations of selenium in the plant, since the release of volatile selenium was readily demonstrated when only trace amounts of the element were supplied to the experimental plants. Additional studies are needed to identify the volatile compounds and the mechanisms by which they are produced in the plant, and to determine the cause of the variations in output of volatile selenium with time of day which were observed in the alfalfa experiments.

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