Absorption of Gold by Plants

GEOLOGICAL SURVEY BULLETIN 1314-B

Absorption of Gold by Plants

By HANSFORD T. SHACKLETTE, HUBERT W. LAKIN, ARTHUR E. HUBERT, *and* GARY C. CURTIN

CONTRIBUTIONS TO GEOCHEMISTRY

GEOLOGICAL SURVEY BULLETIN 1314-B

A report of experiments on the uptake and movement of Au^{197} *and* Au^{198} *in different sols and solutions by rooted plants and unrooted plant cuttings*

UNITED STATES GOVERNMENT PRINTING OFFICE, WASHINGTON : 1970

UNITED STATES DEPARTMENT OF THE INTERIOR

WALTER J. HICKEL, *Secretary*

GEOLOGICAL SURVEY

William T. Pecora, *Director*

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C. 20402 - Price 30 cents (paper cover)

CONTENTS

ILLUSTRATIONS

TABLES

CONTRIBUTIONS TO GEOCHEMISTRY

ABSORPTION OF GOLD BY PLANTS

By Hansford T. Shacklette, Hubert W. Lakin, Arthur E. Hubert, and Gary C. Curtin

ABSTRACT

Rooted plants and unrooted cuttings of impatiens (*Impatiens holstii*) and garden balsam (*I. balsamina*) did not absorb through roots, or absorb and transport through cut stems, gold sols reduced by glucose or sodium oxalate that contained Au^{198}, as shown by leaf autoradiographs. Radioactive gold from a chloride solution was not found in leaves of rooted impatiens and garden balsam plants but was found in leaves of unrooted cuttings, even though some of the gold was precipitated as a blue coating of colloidal gold on the cut surfaces and in vascular cells. Leaf autoradiographs showed that Au^{198} in solutions of gold cynanide, bromide, iodide, and thiocyanate was absorbed by both rooted plants and unrooted cuttings but that generally more was absorbed by the unrooted cuttings. The concentrations used produced necrosis of the plants. Rooted plants in more dilute $Au^{198}CN$ absorbed radioactive gold and transported it to the leaves while the plants produced new root growth and flowers. Radioactive gold in a thiosulfate solution was not absorbed by rooted plants, but large amounts of it were absorbed by unrooted cuttings. Radioactive gold solubilized by an extract from flax plants that contained hydrogen cyanide derived by enzymatic action on a natural glycoside was absorbed in greater amounts by rooted plants than by unrooted cuttings.

Atomic absorption analyses of stems and leaves of *Impatiens* showed that nonradioactive gold (Au^{197}) chloride, cyanide, bromide, iodide, and thiosulfate were readily absorbed by both rooted plants and unrooted cuttings. Of these compounds, gold cynanide was absorbed in the largest amounts and constituted as much as 320 parts per million of the dry weight of the plant.

Roots of onion (*Allium cepa*) bulbs readily precipitated colloidal gold from solutions of gold chloride and gold bromide. Roots of this plant were able to grow in length in dilute gold cyanide solutions.

The experiments indicated that dilute solutions of gold cyanide were readily absorbed without causing damage to rooted plants. If gold is present in the soil and if cyanogenic plants are rooted in this soil, a mechanism is present for the entrance of gold into the biogeochemical cycling process.

INTRODUCTION

The presence of gold in plants, as determined by chemical analysis, was reported more than a century ago (Malte-Brun, 1824, p. 221). Careful quantitative experiments performed by Harrison (1908, p. 209–210) with tree samples from an auriferous region of British Guiana demonstrated that the concentrations of gold in ash of wood and bark were small, ranging from 0.06 ppm (parts per million), or 1 grain per ton, in bark to 0.6 ppm in wood from the interior of the tree. Later investigators reported the gold content of many species of plants from various localities throughout the world. Although the amounts given in some reports seem too large (as pointed out by Cannon and others, 1968), the fact that plants can absorb gold from the soil solution is well established. The amounts found in plant ash are usually much smaller than 1 ppm. Gold is not known to be essential for plant metabolism, and hence it may be classed as a "ballast" element in plants.

There is no general agreement among investigators as to the forms of gold that are absorbed by plants—that is, whether the absorbed gold is in colloidal-size particles or in water-soluble compounds. The purpose of the experiments described in this report was to determine if plants can absorb some colloidal gold sols and solutions of gold that may be found in the natural environment of plant roots.

The movement of gold in various inorganic geochemical environments was discussed by Chukhrov (1947), Krauskopf (1951), Goldschmidt (1954), Rankama and Sahama (1955), Cloke and Kelly (1964), and other workers and will not be reviewed here. The fact that gold is deposited in plant tissues suggests that it, along with many other metals, enters into the biogeochemical cycling process, which results in an enrichment in metals of the upper soil horizons, as pointed out by Goldschmidt (1937, p. 670–671). Malyuga (1964, p. 67) described the absorption and disposition of metals by plants as follows:

> Depending on the physiological function, the metal enters into the plant tissue or is fixed by organic molecules (proteins, vitamins, enzymes). If the plant does not use all the elements entering together with the basic nutrients, it frees itself of them, discarding them into protective and structural tissues, or, finally, into organs and tissues which are being renewed or are dying (leaves, rootlets). Under all conditions there is a certain accumulation of heavy metals in the organic residue of the soil. With the decomposition of the stems, leaves, and dead roots of the plant, the heavy metals, together with the most stable organic compounds, remain in the humus layer of the soil.

Studies by Curtin, Lakin, Neuerburg, and Hubert (1968) showed that enrichment of gold occurs in mull (humus-rich forest soil) which overlies gold deposits and that analysis of this mull may be a useful method of prospecting for gold.

The physical or chemical state of the gold that moves into plants and through the biosphere has been a subject of much speculation but little experimentation. Lungwitz (1900, p. 501) at first proposed that, in nature, gold is dissolved in cyanide, but he later rejected this theory because not even traces of cyanide had been reported to be produced by the natural decomposition of organic matter. He next considered gold chloride as a compound that might enter vegetation but questioned the effectiveness of this compound because it was not expected to "withstand the reducing action that untold numbers of cell membranes and cell contents must exert." He reported that tree roots had been examined for gold which might have precipitated on them but that no gold had been found, and he emphasized the impossibility of the occurrence of such a precipitation.

The relative concentrations of gold in seeds, seed pods, leaves, upper twigs, stems, and roots, as determined by activation analysis, were studied by Khotamov, Lobanov, and Kist (1966). They found the maximum content to be in leaves and suggested that only the leaves need be analyzed in biogeochemical prospecting for gold.

Chukhrov (1947) concluded that the dissolution of gold as bromide and iodide occurred in the oxidation zone of sulfide-type deposits, but he did not discuss the availability of these compounds to plants.

Listova, Vainshtein, and Ryabinina (1966) reported experiments on the dissolution of gold in solutions formed during oxidation of natural sulfides of lead, zinc, and iron. The presence of carbonates shifted the process of sulfide oxidation toward increased concentrations of intermediate products of sulfide oxidation. The calcium thiosulfate and calcium polythionate that formed during the reaction of carbonates with the products of sulfide oxidation were found to be solvents of gold, and, being easily soluble compounds, they can exist in and migrate with surface waters for a prolonged time. These observations suggest that gold thiosulfate may be absorbed by plants in a natural environment.

In their study of gold in rocks, plants, and waters of the Darasun gold deposit (USSR), Aferov, Zvyagin, Roslyakova, Roslyakov, Shabynin, and Epov (1968) concluded that the ion-complex form is the most probable form of gold migration in contemporary waters and inferred that the gold found in plants on this deposit had been absorbed in this form.

Ong and Swanson (1969) reported that in the interactions of gold with different types of natural organic acids, the gold is not oxidized and complexed by the organic molecules but that organic concentra-

tions in the range of 3–30 ppm can reduce gold chloride solutions to negatively charged colloids of metallic gold. They stated (p. 395):

> For the 30-ppm organic acid solutions, the reduction process is accomplished by the formation of a protective coating of hydrophilic organic molecules around the hydrophobic gold sol making the gold very stable for at least 8 months and not easily coagulated by cations. The gold sols so formed are less than 10 mμ [millimicrons] in size. This protective layer is also formed when colloidal gold is mixed with organic acids. For the 3-ppm organic acid, the organic matter concentration is too low to form the protective coating and the colloidal gold precipitates.

Gold sols of 10 mμ size theoretically could enter into the vascular system of a plant, if the stem were cut so that the conducting tissues were directly exposed to the sols, but most likely could not pass through the membranes of root cells.

Glycosides which yield hydrocyanic acid upon hydrolysis by enzymatic action are found in many species of plants. Conn (1969, p. 519) stated, "Approximately 1,000 plant species representing 90 families and at least 250 genera have been reported to be cyanogenic. Several dozen species have been studied in greater detail, in some instances because of their economic significance, and 11 cyanogenic glycosides have been identified." Hydrocyanic acid and thiocyanates have been isolated from plants which had caused poisoning of livestock (Kingsbury, 1964); both of these compounds are formed from glycosides and both are solvents of gold.

Not only leafy plants but also certain fungi produce cyanide compounds. The mycelium of *Marismius oreades* (Bolt. ex Fr.) Fr., a mushroom of the order Agaricales, was reported by Filer (1965) to produce hydrogen cyanide, and the presence of this compound was linked to the occurrence of patches of dead turf in lawns. Other species of fungi also have been determined to be cyanogenic.

In a study of the resistance of cultivated flax (*Linum usitatissimum* L.) to the fungus *Fusarium lini*, Reynolds (1931) reported that varietal resistance depended on the amount of the glucoside linamarin in the plant tissue. Timonin (1941, p. 406–407) demonstrated that the linamarin in flax is hydrolyzed to form hydrogen cyanide and that in laboratory experiments 25–37 mg (milligrams) of this compound was excreted or diffused from the root system of a single flax plant into the surrounding medium. He stated further (1941, p. 407) that "it can be assumed that the same toxic matter [hydrogen cyanide] is secreted by the resistant variety under natural (field) conditions."

Rovira (1969, p. 44) reported that potassium cyanide may affect permeability of root cells, thereby causing an increase in root exudation of radioactive chloride, a substance generally accepted to move passively (without requiring metabolic action) out of roots. It is not known if hydrogen cyanide causes a similar effect on the permeability

of root cells. According to Rovira (1969, p. 47), plant substances found in soils may have been produced by the leaves, leached from the leaves and deposited in soil, produced from decomposing root residues, and exuded from healthy intact roots.

Because of the widespread distribution of higher plants and other organisms that contain cyanogenic substances, the solubilization of native gold by hydrogen cyanide and the absorption of cyanide compounds of gold by various plant species are likely to occur in natural environments.

MATERIALS AND METHODS

GOLD SOLS AND SOLUTIONS

RADIOACTIVE GOLD EXPERIMENTS

In these experiments, we used sols and solutions of gold that, at lower concentrations, could occur in soils on which plants grow over gold deposits. A list of these gold-bearing preparations and a description of the method used in the preparation of each, follow.

COLLOIDAL GOLD

1. $HAuCl_4$ *reduced with glucose; gelatin used as a protective colloid*
   Heat to incipient boiling 100 ml (milliliters) of a 5-percent solution of gelatin (Eastman 1099, purified calfskin) and 5-percent D-glucose anhyd. Then add slowly 2 ml of 0.6-percent $HAuCl_4$ solution and 4 ml of $HAu^{198}Cl_4$ (0.8 millicurie Au^{198}) and continue heating—but not to boil—until a red sol is produced. Add demineralized water to make final volume 500 ml and adjust pH to 6.2 with 0.2 *N* potassium hydroxide. A gold sol prepared in this manner but without Au^{198} passed through a 0.05-micron filter. The preparation is a modification of the procedure of Henry, Herczeg, and Fisher (1957).

2. $HAuCl_4$ *reduced with sodium oxalate*
   To 500 ml of demineralized water add 2 ml of 0.6-percent $HAuCl_4$ solution, 4 ml of $HAu^{198}Cl_4$, and 6 ml of 1-percent sodium oxalate solution. Heat to boiling and continue gentle boiling until a bluish-purple sol is formed. Cool and adjust pH to 6.2 with 0.2 *N* potassium hydroxide. A gold sol prepared in this manner but without Au^{198} passed through a 0.45-micron filter, but none passed through a 0.05-micron filter.

GOLD SOLUTIONS

1. *Gold chloride solution*
   To 500 ml of demineralized water add 1 ml of 0.6-percent $HAuCl_4$ solution and 4 ml of $HAu^{198}Cl_4$ and stir to mix thoroughly. The pH of the final solution was 6.2.

373-711 O—70——2

2. *Gold cyanide solution*

   Place a sheet of leaf gold (15 mg Au) in 50 ml of 0.2-percent sodium cyanide solution and allow to stand overnight to effect solution of the gold. Evaporate to dryness 4 ml of $HAu^{198}Cl_4$ and heat in a flame to reduce the gold ion to the metal, then dissolve in 10 ml of 0.2-percent sodium cyanide. Combine the two solutions and dilute to 500 ml with water. Adjust the pH to 6.5 with dilute sulfuric acid.

3. *Gold bromide solution*

   Place a sheet of leaf gold (15 mg Au) in a 150-ml beaker, add 4 ml of $HAu^{198}Cl_4$, and evaporate the solution to dryness and heat in a flame to reduce the gold chloride to the metal. Add 10 ml of concentrated hydrobromic acid which contains 0.5-percent bromine and dilute to 500 ml with water. Adjust pH to 6.2 with 0.2 *N* potassium hydroxide.

4. *Gold iodide solution*

   Place a 15-mg sheet of leaf gold in a 150-ml beaker, add 4 ml of $HAu^{198}Cl_4$, and evaporate the solution to dryness. Heat in a flame to reduce the gold chloride to the metal. Add 20 ml concentrated hydroiodic acid and a few crystals of iodine. After solution of the gold, warm on the hotplate to expel the excess iodine. Dilute to 500 ml with water and adjust to pH 6.0 with 0.2 *N* potassium hydroxide.

5. *Gold thiocyanate solution*

   Place a 15-mg sheet of leaf gold in 500 ml of 0.2-percent solution of potassium thiocyanate and allow to stand, but occasionally shake, for 3 days. Filter and add 4 ml $HAu^{198}Cl_4$ solution to the filtrate and adjust the pH to 6.2 with dilute sulfuric acid. The gold content of the solution was found to be 0.08 ppm.

6. *Gold thiosulfate solution*

   Place a 15-mg sheet of leaf gold in 500 ml of 0.2-percent sodium thiosulfate and allow to stand, but occasionally shake, for 3 days. Filter and add 4 ml $HAu^{198}Cl_4$ solution to the filtrate and adjust the pH to 6.2 with dilute sulfuric acid. The gold content of the solution was found to be 2.6 ppm.

7. *Gold cyanide solution via flax*

   Prepare a slurry of wild flax (*Linum lewisii* Pursh), using the above-ground parts at the flowering stage, in a blender and dilute to 800 ml with water. Stir four leaves of gold (60 mg Au) into the slurry and allow to stand for 3 days. Centrifuge and decant slightly turbid supernatant solution free of particulate gold.

Evaporate to dryness 4 ml $HAu^{198}Cl_4$ solution and heat in a flame to reduce the gold to the metal. Dissolve the gold in 20 ml of 0.2-percent sodium cyanide and dilute to 500 ml with the flax extract prepared previously. Adjust the pH to 5.9 with dilute sulfuric acid. The gold content of the final solution was found to be 0.5 ppm.

NONRADIOACTIVE GOLD EXPERIMENTS

In the experiments on the absorption of nonradioactive gold (Au^{197}) by plants, only gold solutions were used, and they were prepared by the methods listed above, except that radioactive gold (Au^{198}) was not added to the solutions. The organization of these experiments, and the solutions that were used, are given in table 2.

NOTE.—*All* pH *values of gold sols and solutions are approximate, obtained by narrow range* pH *(5.0–8.0) paper with visible differences in 0.4* pH *values.*

PLANTS

The experiments on gold absorption by plants were designed to detect gold, if present in the plants, by changes in color of tissues, by autoradiographs of leaves, and by atomic absorption analysis of the stems and leaves. The gross effects of the gold sols and solutions on the plants also were noted.

SPECIES USED

We selected cultivars of impatiens (*Impatiens holstii* Engler & Warb.) and garden balsam (*I. balsamina* L.), shown in figure 1, of the family Balsaminaceae (Bailey, 1937, p. 1641–1644) for tests of the movement of gold through roots and stems and into leaves. These ornamental plants are desirable subjects for these experiments because they (1) readily form roots and root hairs if stem cuttings are placed in tap water, (2) transpire freely and have a rapid rate of water conduction through the stems, (3) have stems that are translucent, and therefore colored solutions moving through vascular tissues of the stem can be easily observed, (4) grow well in the subdued light that commonly is provided by plant growth chambers, and (5) are insensitive to photoperiod, and therefore are vegetative and reproductive at any time of the year.

We chose onion (*Allium cepa* L.) bulbs (family Liliaceae) for studying the effects of roots on gold solutions and sols because these bulbs form abundant roots when partly submerged in water (fig. 2), the roots are large (but with root hairs only near the tips) and white,

FIGURE 1.—Mature flowering plants of impatiens (*Impatiens holstii*), on the left, and garden balsam (*I. balsamina*), on the right.

and marker dyes are readily absorbed by the roots; hence, growth in length can be observed and measured. The roots of both species of *Impatiens*, in contrast, are slender and somewhat fragile and tend to develop pigmentation.

METHODS OF TREATMENT

The *Impatiens* cuttings were rooted in the same test tubes in which they were to remain during the experiments in order to prevent root damage in transferring them to other containers. Many roots formed on the stems during the 2-week period that they remained in a shaded greenhouse, and callus tissue grew over the exposed vascular tissue of the cut ends of the stems. The onion bulbs were rooted by submerging the lower part of the bulb in tap water in disposable beakers; they were kept in a dark growth chamber at a temperature of 14°C. The cool temperature inhibited rotting of the bulbs, and many roots were formed in the 2-week period.

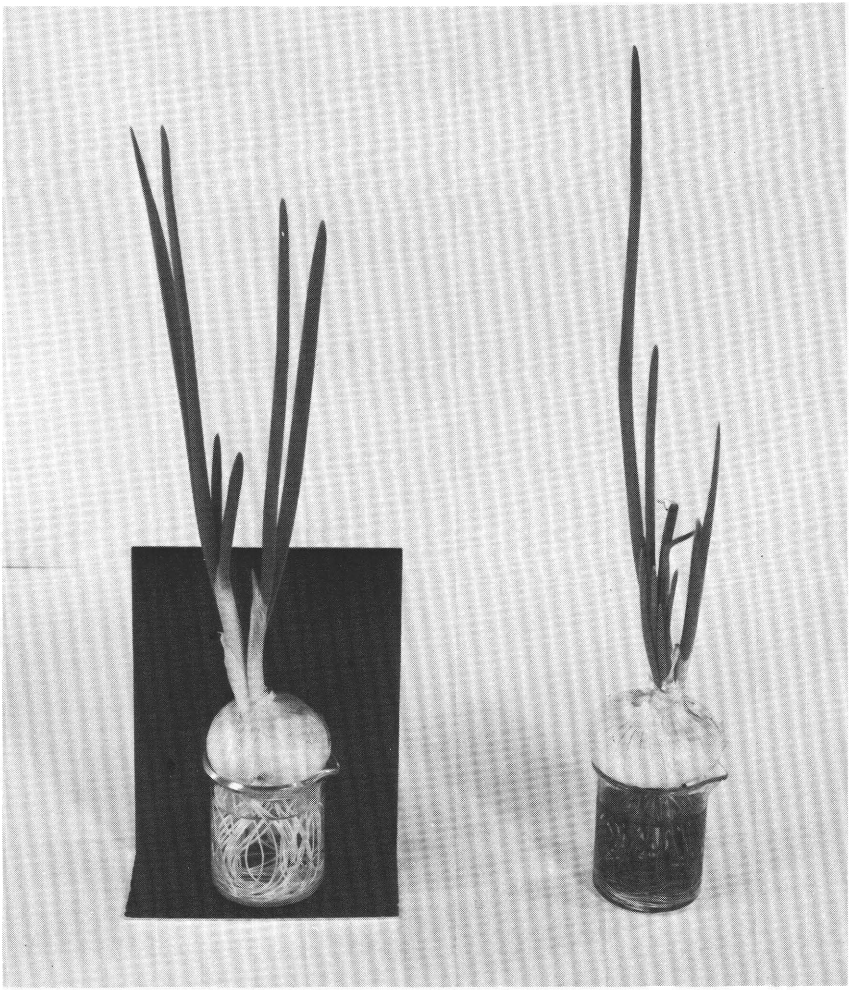


FIGURE 2.—Onion (*Allium cepa*) bulbs with roots that developed in tap water. The roots of the bulb on the left remained in tap water and are a normal white color, but the roots of the bulb on the right were placed in gold chloride solution and are colored dark blue by colloidal gold which was precipitated out of the solution on contact with the roots and deposited on the root epidermis. The gold chloride solution in which onion roots were placed turned blue, whereas the solution remained colorless if it did not contain the roots.

RADIOACTIVE GOLD EXPERIMENTS

At the beginning of the tests for radioactive gold absorption, the tap water in the test tubes of rooted cuttings was poured off, and the gold sols and solutions (listed in table 1) were added. Care was taken to prevent contamination of the leaf surfaces with the solutions.

Unrooted cuttings of *Impatiens* were excised from healthy potted plants (fig. 1) and were immediately placed in the gold solutions. Tap water in the beakers that contained the rooted onion bulbs (fig. 2) was poured off and replaced with the gold solutions. All test plants were then placed in a growth chamber in which 12 hours of fluorescent illumination (approximately 1,000 foot-candles intensity) and a temperature of 24°C alternated with 12 hours of darkness and a temperature of 14°C. A fan in the growth chamber introduced fresh air and promoted rapid transpiration.

After 24 hours, the *Impatiens* plants were removed from the growth chamber, their physical condition was noted (table 1), and one leaf of each plant was excised and placed in a black paper cassette for making the autoradiographs. The plants were then returned to the growth chamber. Industrial G X-ray film was used to record the disintegrations of Au^{198} in the leaves. A sheet of black paper and a sheet of acetate film which acted as a moisture barrier separated the leaves from the sensitive strips of film. The cassettes with their enclosed leaves were placed side by side in a light-tight box and allowed to remain at room temperature for a period of 90 hours. The leaves were removed from the cassettes at the end of this period, and the exposed films were processed in commercial X-ray developer for 5 minutes at a temperature of 20°C, transferred to a fixing solution for 5 minutes, and then washed and dried.

The onion roots were observed after they had been in the gold solutions 24 hours and again, with the *Impatiens*, after 48 hours; their reactions are noted in table 1. At the end of this period, all plants were discarded.

NONRADIOACTIVE GOLD EXPERIMENTS

The same kinds of *Impatiens* plants as those used in the radioactive gold experiments were used for nonradioactive gold experiments; they were also prepared in the same manner and were kept under similar environments. The roots on the cuttings, however, were not as well developed as those on cuttings used in the Au^{198} experiments.

Tap water in the test tubes that contained the rooted plants and unrooted cuttings was poured off and replaced with the solutions of nonradioactive gold (Au^{197}); the plants were then placed in the growth chamber where they remained 24 hours. At the end of this period, the gross effects of the solutions on the plants were noted, and these observations were repeated after 48 hours. The parts of the plants that extended above the tops of the test tubes were then excised (taking care that the gold solutions did not come in contact with these parts), dried 24 hours in an oven with circulating warm air, burned to ash, and the ashes analyzed for gold by atomic absorption methods.

Because of the very small amount of ash produced by burning a sample, the gold content of the plants was computed as parts per million of the dry plant. In order to provide a value that could be used to convert parts per million in the dry plant to parts per million in ash, composite samples consisting of 12 cuttings of each species of *Impatiens* were dried and burned to ash. The percentage of ash in the dry plants is given in table 2.

RESULTS

RADIOACTIVE GOLD EXPERIMENTS

GROSS EFFECTS ON PLANTS

The organization of the experiments and the visible effects of the radioactive gold sols and solutions on the plants are given in table 1.

Wilting of leaves and stems usually was the first noticeable effect of the sols and solutions on *Impatiens*. Leaves may be prevented from obtaining sufficient water to maintain their normal turgor pressure, and therefore wilt, by a failure of the water-absorbing and transporting systems of roots and stems. Leaves may wilt also because of damage to or destruction of the osmotic properties of leaf cell membranes. If leaves wilt but otherwise appear normal, failure of the absorbing and transporting system only is indicated. This failure may be caused by mechanical blocking of the absorbing surfaces or by necrosis of the absorbing and transporting cells. If, however, symptoms of leaf necrosis appear, wilting may be the result of damage to osmotic membranes of the leaf, either with or without failure of the absorbing system.

The sols and solutions used in these experiments may either block absorbing surfaces or exposed vascular elements by depositing colloidal gold or other materials or may cause cell necrosis by means of ionizing radiation or by liberation of phytotoxins. Radioactive gold (Au^{198}) disintegrates into a nonradioactive isotope of mercury (Hg^{198}), and metallic mercury or compounds of mercury generally are toxic to plant cells. Other phytotoxins in the solutions also may have caused necrosis of the *Impatiens;* chlorine, bromine, iodine, and cyanide generally are lethal to plant cells, except when in very low concentrations.

Rooted *Impatiens* in the two gold sols remained turgid or wilted slightly at first but regained turgor pressure in 48 hours. Unrooted cuttings in glucose-gelatin sols were severely wilted, whereas those in sodium oxalate sols remained turgid or only wilted moderately. The reason for this difference in plant reaction to the two sols is not known. The results suggest, however, that some property (perhaps the gelatin) of the glucose-gelatin sol caused a slight to extensive blocking of the exposed vascular cells of the unrooted cuttings which

TABLE 1.—*Organization and results of experiments in radioactive gold absorption by plants*

[Number in parentheses in boxhead is radioactivity, in counts per minute, at beginning of experiments. To determine counts per minute, the activity of 10-ml aliquots of the sols and solutions was measured by a scintillation counter using a well-type 1.5-in. thallium-activated sodium iodide crystal detector]

Experimental plants	Visible effects on plants in radioactive gold (Au^{198}) sols and solutions as observed after (a) 24 hr and (b) 48 hr				
	Glucose-gelatin sol (2,930)	Sodium oxalate sol (3,120)	Gold chloride solution (2,310)	Gold cyanide solution (1,800)	Gold bromide solution (3,500)
Impatiens (*Impatiens holstii*):					
Rooted	a. Leaves slightly wilted	a. Leaves turgid	a. Leaves turgid	a. Leaves moderately wilted.	a. Leaves turgid.
	b. Leaves turgid	b. ----do	b. ----do	b. Leaves slightly wilted	b. Leaves abscissing; stem flaccid.
Not rooted	a. Leaves severely wilted	a. Leaves turgid	a. Leaves turgid	a. Leaves severely wilted	a. Leaves moderately wilted.
	b. ----do	b. ----do	b. Leaves turgid; blue precipitate on cut surface of stem and in stele.	b. Total necrosis	b. Leaves severely wilted.
Garden balsam (*Impatiens balsamina*):					
Rooted	a. Lower leaves slightly wilted.	a. Leaves turgid	a. Leaves turgid	a. Leaves severely wilted, stem flaccid.	a. Leaves severely wilted.
	b. All leaves turgid	b. ----do	b. ----do	b. Total necrosis	b. Total necrosis.
Not rooted	a. Leaves severely wilted	a. Leaves moderately wilted.	a. Leaves turgid	a. Leaves severely wilted	a. Leaves turgid.
	b. ----do	b. ----do	b. Leaves moderately wilted; blue precipitate in stele.	b. Total necrosis	b. Do.
Onion (*Allium cepa*), rooted	a. No effects. Sol remained pink.	a. No effects. Sol remained pale pink.	a. Root epidermis sloughing and covered with dark-blue precipitate.	a. No effects. Solution remained clear.	a. Root epidermis dark blue, no sloughing.;
	b. ----do	b. ----do	b. Clear solution turned light blue.	b. ----do	b. Do.

	Gold iodide solution (2,930)	Gold thiocyanate solution (6,700)	Gold thiosulfate solution (4,000)	Flax plant extract [1] (3,730)
Impatiens (*Impatiens holstii*):				
Rooted	a. Leaves turgid	a. Leaves turgid	a. Leaves turgid	a. Leaf margins necrotic.
	b. Leaves abscissing; stem flaccid.	b. ----do	b. ----do	b. Leaves severely wilted.
Not rooted	a. Leaves severely wilted	a. Leaves moderately wilted	a. Leaves turgid	a. Leaves severely wilted.
	b. Leaves severely wilted and abscissing.	b. All leaves abscissing	b. ----do	b. Do.
Garden balsam (*Impatiens balsamina*):				
Rooted	a. Stem and leaves turgid; leaves abscissing.	a. Leaves turgid	a. Leaves turgid	a. Leaves very slightly wilted.
	b. ----do	b. ----do	b. ----do	b. Do.
Not rooted	a. Leaves very slightly wilted	a. Leaf margins necrotic	a. Leaf margins necrotic	a. Leaves severely wilted.
	b. Leaves severely wilted	b. Entire leaf necrotic	b. ----do	b. Do.
Onion (*Allium cepa*), rooted	a. Root tips brown. Solution remained clear.	a. No effects. Solution remained clear.	a. No effects. Solution remained clear.	a. No effects. Solution remained brownish yellow.
	b. ----do	b. ----do	b. ----do	b. Do.

[1] Gold dissolved in extract prepared from above-ground parts of wild flax (*Linum lewisii*) that was in flower.

impeded the uptake of water but that rooted plants were able to absorb water normally through the root membranes without appreciable interference from the sols. All plants and cuttings remained alive and seemed to sustain no significant cell damage in stems and leaves; this fact suggests that radioactive gold was not translocated through the stems and into the leaves, as was demonstrated by the leaf autoradiographs. The onion roots showed no effects of being submerged in the sols.

Rooted plants in the gold chloride solution remained turgid, as did the unrooted cutting of impatiens. The garden balsam cutting was moderately wilted. A blue precipitate of colloidal gold was deposited on the cut surfaces and in the conducting tissues of the unrooted cuttings. The precipitated gold colloid apparently could not pass through the membranes of the root cells, as is indicated by the autoradiographs, but it did not prevent the movement of water into the roots. The onion roots precipitated the gold; the sloughing epidermis was dark blue, and the solution turned light blue (fig. 2).

All *Impatiens* in the gold cyanide solution were moderately to severely wilted within 24 hours, and all but one were dead within 48 hours; necrosis was evident in roots, stems, and leaves. There was no precipitation of gold on either the *Impatiens* roots or stems or on the onion roots.

The gold bromide solution caused severe wilting of all impatiens within 48 hours, and all plants were moribund or dead soon thereafter. The onion roots precipitated gold from the solution, but the *Impatiens* roots did not.

The gold iodide solution caused all leaves to wilt and to fall from the stems. Neither *Impatiens* nor onion roots caused precipitation of gold.

Rooted *Impatiens* in the gold thiocyanate solution remained turgid, whereas the leaves of unrooted cuttings wilted and either fell from the stems or became necrotic. Neither *Impatiens* roots nor onion roots caused precipitation of the gold.

All *Impatiens* in the gold thiosulfate solution remained turgid; the leaf margins, however, of the unrooted garden balsam became necrotic. None of the plants precipitated gold from the solution.

The solution of gold in flax extract severely damaged all *Impatiens* except the rooted garden balsam. No gold was precipitated from the solution.

Because of our special interest in the transport of gold that is dissolved by cyanide, we performed experiments in addition to those reported in table 1 and figure 3. A dilute solution with respect to Au^{198} was prepared by evaporating a solution that contained 15 mg gold chloride and 1 ml of $HAu^{198}Cl_4$ solution to dryness and dissolving in 20 ml of 0.2-percent potassium cyanide, diluting to 400 ml, and adjust-

ing the pH to 11.0. Rooted plants and unrooted cuttings of impatiens (*Impatiens holstii*) were used for the experiments. The roots of one plant were stained red with food color before the experiment started and then were washed to remove excess color and placed in the gold cyanide solution. Both the rooted plant and the unrooted cutting remained turgid while in the solution for 48 hours, and showed no indication of necrosis. During this period, the rooted plant produced about 5 mm (millimeters) of white new growth on each root that contrasted with the red-stained older part of the root, and a flower opened from a bud on the stem. A leaf from the rooted plant produced an image when exposed to sensitive film for 24 hours, whereas a leaf from the unrooted cutting did not. These plants were kept in the solution several days longer; both remained healthy, and the unrooted cutting began to form roots just as if it were in tap water. Rooted onion bulbs that had their roots stained red were placed in this solution of gold cyanide. They remained healthy and produced 5–7 mm of new root growth in 48 hours.

We performed another experiment on rooted plants and unrooted cuttings of *Impatiens* and on rooted onion bulbs that were placed in the gold cyanide solutions which were prepared as described in the first experiments (solution 2 under the heading "Gold solutions") but which had pH values of 6.2, 8.2, and 11.0 and a greater concentration of Au^{198}. Rooted plants of impatiens and garden balsam in the solutions of pH 6.2 and 8.2 were slightly to severely wilted after 24 hours. The leaves and stems of all plants but the rooted impatiens were severely wilted and necrotic after 48 hours. The leaves of rooted plants in the solution that had a pH of 11.0 remained turgid for 24 hours but were wilted and necrotic after 48 hours, whereas leaves of unrooted cuttings were severely wilted and necrotic a few hours after being placed in the solution. Autoradiographs showed that a large amount of Au^{198} had been deposited in the leaves of all the plants. These solutions caused no appreciable change in the onion roots, and no gold was precipitated from the solutions.

AUTORADIOGRAPHS

The relative amounts of radioactive gold in leaves of the experimental plants are indicated by the darkness of the image—up to solid black—that is produced on the film. It should be mentioned that the sols and solutions contained many times more nonradioactive gold than Au^{198}, and that if both kinds of gold were absorbed in proportion to their abundance in the solutions, the autoradiographs indicate only a very small part of the total amount of gold in the leaves. Autoradiographs of leaves from rooted plants and unrooted cuttings of impatiens and garden balsam are given in figure 3. A series of controls, consisting

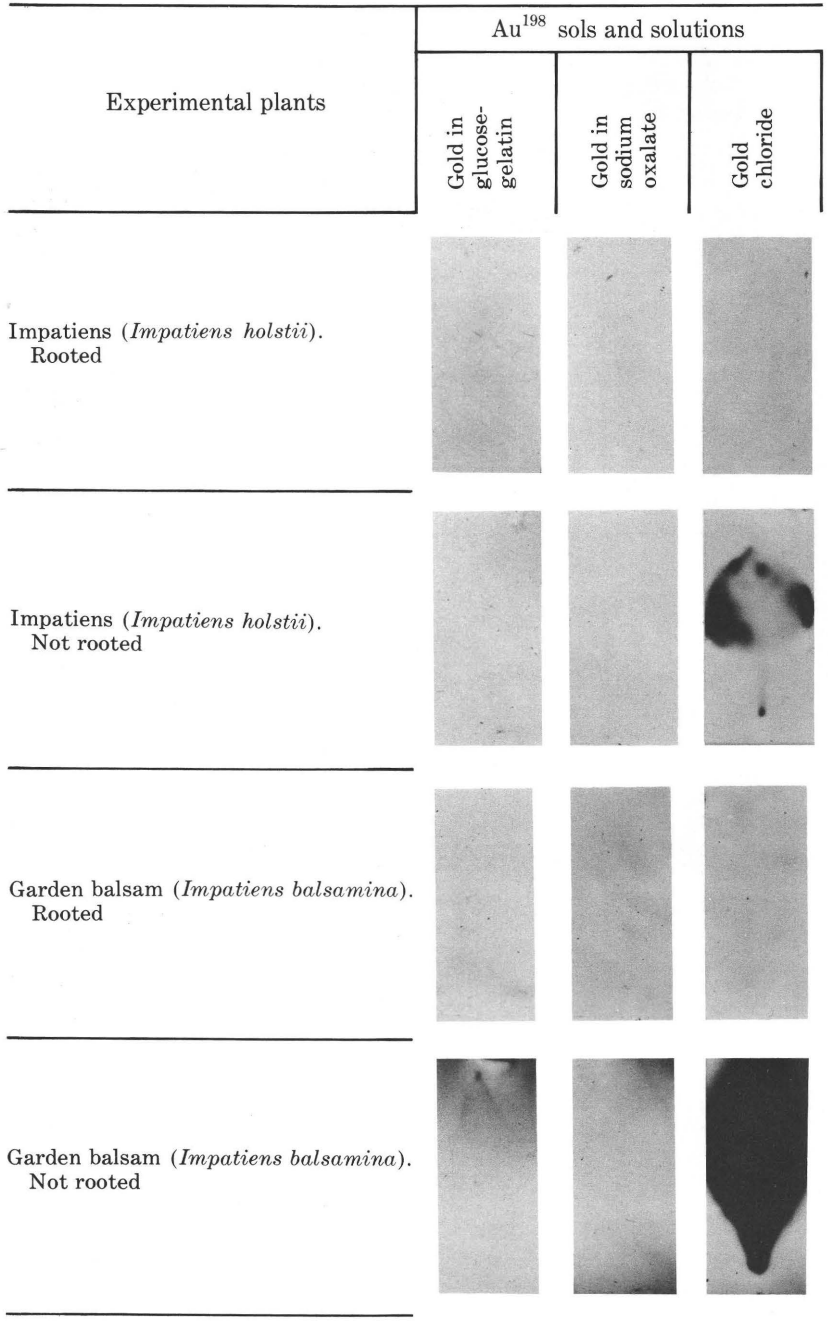
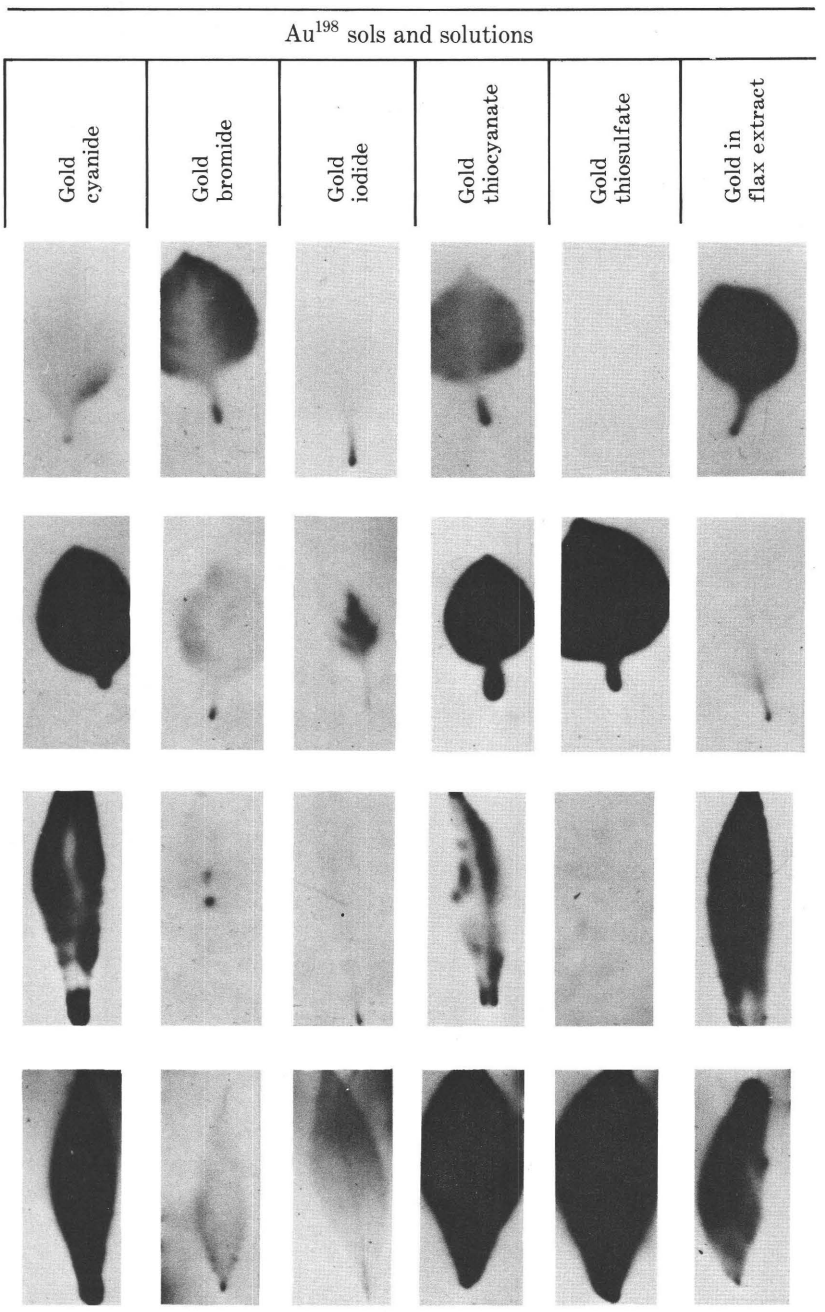


FIGURE 3.—Autoradiographs of *Impatiens* leaves from rooted plants



and unrooted cuttings that were in radioactive gold sols and solutions.

of rooted plants and unrooted cuttings in tap water, was kept under the same environment as that of the experimental plants. Autoradiographs of leaves of the controls were negative and are not shown in figure 3.

Radioactive gold of colloidal size in the two sols was not transported to the leaves of either rooted plants or unrooted cuttings. A very dim leaf image was formed by a leaf from an unrooted garden balsam that was in the glucose-gelatin sol; probably the sol contained traces of Au^{198} in solution which produced the image.

The rooted plants in gold chloride solution transported little or no Au^{198} to the leaves, whereas the unrooted cuttings transported large amounts, in spite of their having precipitated colloidal gold on the cut surfaces and within the vascular system of the stems. These results suggest that gold chloride is reduced to colloidal gold on contact with the plant cells and that the colloidal gold cannot pass through the root membranes but can enter the cut vascular tissue and be transported to the leaves. If this supposition is correct, the reason is not apparent why colloidal gold in the two sols cannot likewise enter the conducting tissues of the cut stems.

Radioactive gold in gold cyanide, bromide, iodide, and thiocyanate solutions was absorbed by both rooted plants and unrooted cuttings, but in all experiments (except the autoradiograph of the plant in a gold bromide solution) more was absorbed by the unrooted cuttings. This fact indicates that the compounds damaged the absorptive mechanisms of the roots; normally, a rooted plant will absorb more water and solutes than will an unrooted cutting of comparable size because of the greater surface for absorption that the roots afford.

Very little or no radioactive gold in the thiosulfate solution was absorbed by the rooted plants, but large amounts were taken up by the unrooted cuttings. Apparently, membranes of the root cells will not permit this compound to pass through them.

The radioactive gold that was dissolved in flax extract was absorbed by both rooted plants and unrooted cuttings, but more was absorbed by the rooted plants. This difference in absorption is the normal condition for plants. The compound in the flax plant extract that dissolved the gold is hydrogen cyanide that was formed by the action of plant enzymes on a glycoside that is present in flax.

NONRADIOACTIVE GOLD EXPERIMENTS

The experiments with impatiens and garden balsam, as reported in table 1, were repeated substituting nonradioactive gold (Au^{197}) for the radioactive gold (Au^{198}) in the solutions (gold in flax extract being omitted) in order to determine if ionizing radiation or the toxic

nature of the compounds caused the damage to plants that is reported in table 1. In addition, we wished to substantiate the uptake of gold by plants as shown by autoradiographs by atomic absorption analysis of the plant stems and leaves for gold; nonradioactive gold solutions were used for analysis experiments to avoid radioactive contamination of the analytical apparatus.

GROSS EFFECTS ON PLANTS

The organization of the experiments and the visible effects of Au^{197} solutions on the *Impatiens* plants are given in table 2.

The rooted plants and unrooted cuttings were observed 24 hours and 48 hours after they were placed in the solutions. Of the plants and solutions that can be compared in tables 1 and 2, visible effects on only unrooted impatiens in the gold chloride solution, on rooted garden balsam in the gold bromide solution, and on unrooted garden balsam in the gold iodide and gold thiocyanate solutions differed significantly in the two experiments. The reasons for these differences are not apparent. Similar visible effects on the plants used in the two experiments indicate that the toxic nature of the solutions alone can cause the wilting and necrosis that occurred—ionizing radiation is not necessary to produce these effects.

ATOMIC ABSORPTION ANALYSES

The concentration of gold in the dried plants is given in table 2. These values can be converted to approximate gold content in ash of the plants by considering dry impatiens to yield 8 percent ash and dry garden balsam to yield 10 percent ash when burned.

Although the amounts of radioactive gold, as indicated by autoradiographs, and of nonradioactive gold, as determined by atomic absorption analysis, do not always correspond for all plants and all solutions used in the experiments, gold cyanide is the compound that usually was absorbed in the largest amounts in both experiments. The pH of the gold cyanide solution appears to affect the amount of gold that is absorbed by the plants—small amounts generally are absorbed at pH 7.7, and much greater amounts are absorbed at pH 11 and 6.5.

Nonradioactive gold chloride was absorbed in small amounts by all plants, whereas radioactive gold was absorbed only in unrooted cuttings.

Gold bromide and gold iodide were absorbed in small to moderate amounts in all plants used in both experiments.

Only very small amounts of nonradioactive gold thiocyanate were absorbed by the plants, whereas much larger amounts of the radioactive compound were indicated by the autoradiographs.

TABLE 2.—*Organization and results of experiments in nonradioactive gold absorption by plants*

[Dry impatiens yielded 8 percent ash, dry garden balsam 10 percent ash, when burned. ----, no data available. Concentrations of gold in solutions, in milligrams per liter. Gold determined by the method of Thompson, Nakagawa, and VanSickle (1968)]

Experimental plants	Visible effects on plants in nonradioactive gold (Au^{197}) solutions as observed after (a) 24 hr and (b) 48 hr, and the parts per million (ppm) gold in dry plants after they had been in solutions 48 hrs.			
	Gold cyanide (29 mg/l)			Gold chloride (pH 6.2; 5.7 mg/l)
	pH 11	pH 7.7	pH 6.5	
Impatiens (*Impatiens holstii*):				
Rooted	a. Leaves slightly wilted	a. Leaves severely wilted, necrotic.	a. Leaves very slightly wilted	a. Leaves turgid.
	b. Total necrosis	b. ----do	b. Leaves turgid, stem necrotic	b. Do.
	160 ppm Au	1.7 ppm Au	180 ppm Au	1.0 ppm Au.
Not rooted	a. Leaves moderately wilted	a. Leaves severely wilted	a. Leaves severely wilted	a. Leaves severely wilted.
	b. Leaves severely wilted, necrotic.	b. ----do	b. Leaves severely wilted, necrotic.	b. Leaves severely wilted, necrotic.
	320 ppm Au	4.2 ppm Au	130 ppm Au	7 ppm Au.
Garden balsam (*Impatiens balsamina*):				
Rooted			a. Total necrosis	a. Leaves turgid.
			b. ----do	b. Do.
			78 ppm Au	7.5 ppm Au.
Not rooted	a. Severe necrosis	a. Leaves moderately wilted	a. Leaves severely wilted; stem flaccid.	a. Leaves moderately wilted.
	b. Total necrosis; plant brown	b. Plant necrotic, but green	b. Total necrosis	b. Leaves slightly wilted.
	32 ppm Au	32 ppm Au	260 ppm Au	7.5 ppm Au.

Experimental plants	Gold bromide (pH 6.2; 29 mg/l)	Gold iodide (pH 6.0; 29 mg/l)	Gold thiocyanate (pH 6.2; 0.1 mg/l)	Gold thiosulfate (pH 6.2; 2.2 mg/l)
Impatiens (*Impatiens holstii*):				
Rooted	a. Leaves turgid	a. Leaves slightly wilted	a. Leaves very turgid	a. Leaves turgid.
	b. Leaves very slightly wilted; blue deposit on stems and roots.	b. Leaves severely wilted, leaf abscission.	b. ----do	b. Leaves very turgid.
	28 ppm Au	64 ppm Au	<1 ppm Au	1.4 ppm Au.
Not rooted	a. Leaves severely wilted	a. Leaves severely wilted and necrotic.	a. Leaves severely wilted	a. Leaves severely wilted; leaf abscission.
	b. ----do	b. ----do	b. ----do	b. Do.
	55 ppm Au	33 ppm Au	<0.8 ppm Au	6.6 ppm Au.
Garden balsam (*Impatiens balsamina*):				
Rooted	a. Leaves turgid	a. Leaves turgid	a. Leaves turgid	a. Leaves turgid.
	b. Leaves turgid; roots necrotic	b. ----do	b. ----do	b. Do.
	39 ppm Au	2.7 ppm Au	0.9 ppm Au	3.3 ppm Au.
Not rooted	a. Leaves moderately wilted; slight necrosis.	a. Leaves turgid	a. Leaves turgid	a. Leaves turgid.
	b. ----do	b. ----do	b. ----do	b. Do.
	160 ppm Au	45 ppm Au	<0.4 ppm Au	28 ppm Au.

Nonradioactive gold thiosulfate was present in small amounts in rooted plants, but the radioactive compound was not shown in the autoradiographs. Large amounts were indicated in unrooted cuttings used in both experiments.

SUMMARY

1. If plants can absorb a particular sol or solution of gold, a sufficient amount enters the leaves to produce a positive autoradiograph and to be measured by atomic absorption analysis even though severe wilting and necrosis of stems and leaves occur within 24 hours after the plants have been placed in the solutions.

2. Colloidal Au^{198} reduced by glucose and sodium oxalate could neither pass through root membranes nor enter the cut vascular systems of the experimental plants. The tests indicated, however, that colloidal gold precipitated from a gold chloride solution probably enters the vascular systems of cut stems and is transported into the leaves.

3. Contact of gold chloride with cell membranes of roots, cut stems, and cells of the vascular system causes a reduction of the compound and precipitation of the gold on the cell surfaces as a blue deposit of colloidal gold. This reaction limits or prevents the absorption of gold chloride by rooted plants.

4. Despite the extreme toxicity of concentrated solutions of radioactive gold cyanide to all *Impatiens*, leaves of all plants in these solutions contained radioactive gold. Solutions of low Au^{198} content, in contrast, were not toxic; plants in these solutions remained turgid and produced new root growth even though autoradiographs showed that Au^{198} was present in the leaves. Of all the solutions tested, greater amounts of gold were absorbed from gold cyanide solutions than from any other solutions.

5. Radioactive gold bromide solutions were very toxic to *Impatiens*, and gold was precipitated from the solutions by onion roots. The nonradioactive solutions were toxic, and gold was precipitated from the solutions by impatiens roots; nevertheless, moderate to large (160 ppm) amounts of gold were absorbed.

6. Radioactive gold iodide solutions were severely toxic to all experimental plants. Radioactive gold was present, however, in the leaves of all *Impatiens*. The nonradioactive solutions were less toxic, and leaves of impatiens contained as much as 64 ppm gold.

7. Radioactive gold thiocyanate was toxic to unrooted cuttings of *Impatiens* but was absorbed by both rooted plants and unrooted cuttings. Nonradioactive solutions were less toxic, but very small amounts of gold were absorbed from the solutions.

8. Gold thiosulfate solutions were only slightly toxic to all *Impatiens* but were not, or were only slightly, absorbed by rooted plants. Unrooted cuttings generally absorbed large amounts.

9. The radioactive gold in the flax extract was absorbed by all *Impatiens*, but more was absorbed by the rooted than by the unrooted plants. This pattern of absorption resembles that which occurs in natural soil solutions. The concentration of the solution used was, however, very toxic to all *Impatiens*.

CONCLUSIONS

Colloidal gold was not absorbed by the roots of plants used in these experiments. Of the solutions of gold that are most likely to occur in the root zone of the soil under natural conditions, gold cyanide is the most readily absorbed by roots and transported in largest amounts to the leaves. If gold is present in the soil and if cyanogenic plants are rooted in this soil, a mechanism is present for the entrance of gold into the biogeochemical cycling process.

REFERENCES CITED

Aferov, Yu. A., Zvyagin, V. G., Roslyakova, N. V., Roslyakov, N. A., Shabynin, L. L., and Epov, I. N., 1968, Gold in rocks, plants, and waters of the Darasun deposit: Vop. Geol. Pribaikal. Zabaikal, no. 3, p. 146–149 (in Russian).

Bailey, L. H., 1937, Impatiens, *in* Bailey, L. H., ed., The standard cyclopedia of horticulture: New York, Macmillan Co., v. 2, p. 1641–1644.

Cannon, H. L., Shacklette, H. T., and Bastron, Harry, 1968, Metal absorption by *Equisetum* (horsetail) : U.S. Geol. Survey Bull. 1278–A, 21 p.

Chukhrov, F. V., 1947, Gold migration in the oxidation zone: Akad. Nauk SSSR, Izv. Ser. Geol. no. 4, p. 117–126 (in Russian).

Cloke, P. L., and Kelly, W. C., 1964, Solubility of gold under inorganic supergene conditions: Econ. Geology, v. 59, p. 259–270.

Conn, E. E., 1969, Cyanogenic glycosides: Agr. and Food Chemistry, v. 17, no. 3, p. 519–526.

Curtin, G .C., Lakin, H. W., Neuerburg, G. J., and Hubert, A. E., 1968, Utilization of humus-rich forest soil (mull) in geochemical exploration for gold: U.S. Geol. Survey Circular 562, 11 p.

Filer, T. H., Jr., 1965, Damage to turfgrasses caused by cyanogenic compounds produced by *Marasmius oreades*, a fairy ring fungus: Plant Disease Reporter, v. 49, no. 7, p. 571–574.

Goldschmidt, V. M., 1937, The principles of distribution of chemical elements in minerals and rocks: Chem. Soc. Proc. Jour., Jan.–June, 1937, p. 655–673.

——— 1954, Geochemistry: Oxford, Clarendon Press, 730 p.

Harrison, J. B., 1908, The geology of the goldfields of British Guiana, *with* historical, geographical, and other chapters, by Frank Fowler and C. W. Anderson: London, Dulau & Co., 320 p.

Henry, R., Herczeg, C., and Fisher, C., 1957, Une nouvelle méthode de préparation d'Or colloïdal radioactif: Internat. Jour. Appl. Radiation and Isotopes, v. 2, p. 136–139.

Khotamov, Sh., Lobanov, E. M., and Kist, A. A., 1966, The problem of the concentration of gold in organs of plants within ore fields: Akad. Nauk Tadzhik SSSR Doklady, v. 9, no. 11, p. 27–30 (in Russian).

Kingsbury, J. M., 1964, Poisonous plants of the United States and Canada: Englewood Cliffs, New Jersey, Prentice-Hall, Inc., 626 p.

Krauskopf, K. B., 1951, The solubility of gold: Econ. Geology, v. 46, no. 8, p. 858–870.

Listova, L. P., Vainshtein, A. Z., and Ryabinina, A. A., 1966, Dissolution of gold in media forming during oxidation of some sulfides: Metallogeniya Osad. Osad.-Metamorf. Porod, Akad. Nauk SSSR, Lab. Osad. Polez. Iskop., p. 189–199 (in Russian).

Lungwitz, E. E., 1900, The lixiviation of gold deposits by vegetation: Eng. and Mining Jour., v. 69, p. 500–502.

Malte-Brun, M., 1824, Universal geography: Boston, Wells and Lilly, v. 1, 635 p.

Malyuga, D. P., 1964, Biogeochemical methods of prospecting: New York, Consultants Bur., 205 p.

Ong, H. L., and Swanson, V. E., 1969, Natural organic acids in the transportation, deposition, and concentration of gold: Colorado School Mines Quart., v. 64, no. 1, p. 395–425.

Rankama, Kalervo, and Sahama, Th. G., 1955, Geochemistry: Chicago, Univ. Chicago Press, 912 p.

Reynolds, E. S., 1931, Studies on the physiology of plant disease: Missouri Bot. Garden Annals, v. 17, p. 57–95.

Rovira, A. D., 1969, Plant root exudates: Bot. Rev., v. 35, no. 1, p. 35–57.

Thompson, C. E., Nakagawa, H. M., and VanSickle, G. H., 1968, Rapid analysis for gold in geologic materials, *in* Geological Survey research 1968: U.S. Geol. Survey Prof. Paper 600–B, p. B130–B132.

Timonin, M. I., 1941, The interaction of higher plants and soil microorganisms, 3, Effect of by-products of plant growth on activity of fungi and actinomycetes: Soil Sci., v. 52, p. 395–413.