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Plant Exudates and Amber: Their Origin and Uses

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Plants produce and export many different molecules out of their cellular and organismal confines. Some of those chemicals become so abundant that we can see or smell them. The most visible materials oozed by many plants are called “exudates.”

What are plant exudates? Generally, exudates are carbon-rich materials that many plants produce and release externally. When exudates are produced, they are often sticky to human touch. Such plant chemicals can be the visible expression of attack by bacteria, fungi, herbivores, or

some other plant pathology. In other instances, such as in typical underground roots, exudate production appears to be part of the typical metabolism of healthy plants that helps stabilize the soil and foster interactions with other organisms around the roots.

Different plant tissue types and organs can produce exudates. We have collected resins and gums from the above ground portions of plants, or shoots, as well as from the generally below ground portion of plants, or roots. Root exudation has been known for decades and is respon-

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Prolific white, resinous exudation is seen on a tumor-like growth on the trunk of a white pine (*Pinus strobus*) at the Arnold Arboretum.



Resinous exudates on a conifer.



Blobs of white resin on a relatively young shoot of a Japanese black pine (*Pinus thunbergii*, AA accession 11371-O).

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A slab of Great Basin bristlecone pine (*Pinus longaeva*) right out of the microwave oven showing extruded (and very hot!) resinous exudates. Microwave heating experiments were performed at the Laboratory of Tree-Ring Research, University of Arizona, Tucson.

sible for many of the fascinating relationships in the interface of plant roots and soil microorganisms known as the rhizosphere.

Collecting and Analyzing Plant Exudates

After receiving collecting permission (if needed), we spend days walking the grounds of botanical gardens and arboreta, or do field work elsewhere. Exudates are easily collected directly from the trees with no harm to the plant and leaving no doubt about their botanical identity. Occasionally we use more forceful methods, such as carefully microwaving wood slabs to extract the exudates, then letting them resolidify. Once the material is collected, we place it in a small plastic zip-top bag. An additional, external bag is used to hold a paper label containing the collection data. If needed, we let the exudate dry slowly in an oven and, once dried, the materials are ready for subsequent analyses. In other instances, generous collaborators send us materials for chemical analyses.

Carbon-13 solid state Nuclear Magnetic Resonance spectroscopy (ssNMR) is a state-of-the-art research tool that generates spectra

(or chemical signatures) of materials, including plant exudates and amber or greatly fossilized plant resin. The analyses, which use a tiny amount (as little as 50 to 100 milligrams, approximately the volume of a new eraser on a school pencil) of the exudate, are non-destructive. They are performed at Northwestern University (in Evanston, Illinois), one of a few research laboratories in the world with carbon-13 ssNMR capabilities. At times, we observe plants that evidently have produced exudates but the amounts are insufficient for our analyses.

Solid exudates are pulverized manually and undergo two sets of carbon-13 ssNMR analyses: normal decoupling, which gathers signals for all carbon atoms, and interrupted decoupling, which, among others, obtains signals from carbons lacking the attached hydrogens. Just like in spectra used in the health-allied sciences, different regions of the spectra provide valuable information (see Figure 1 on page 4). In the case of NMR, the peaks represent different atoms and reflect their molecular environment. The height of the peaks largely represents rela-

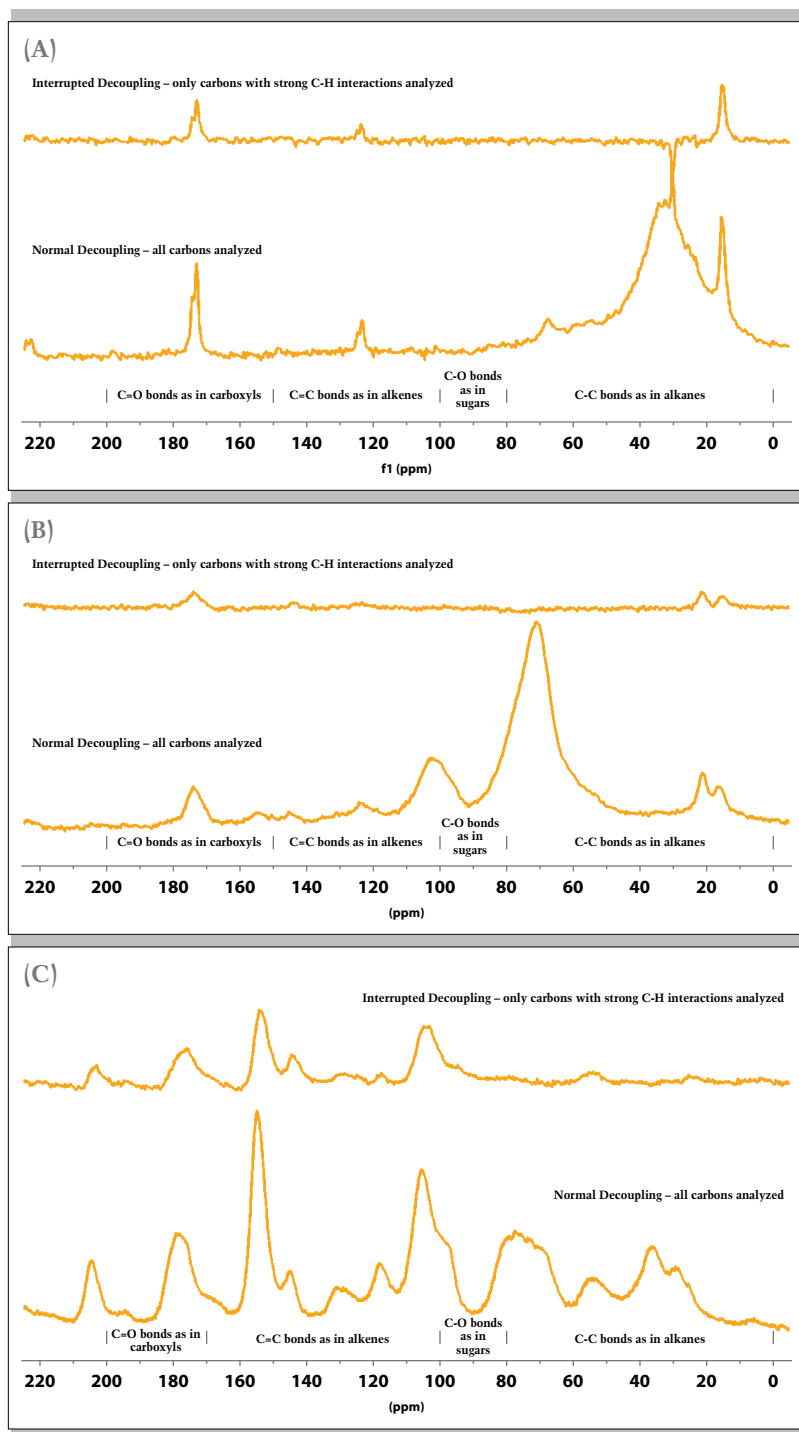


Figure 1. Chemical identity of peaks on a C-13 ssNMR spectra. Panel (A) is a resin, panel (B) is a gum, and panel (C) is a kino (a type of phenolic, often found in *Eucalyptus*). In all panels, the upper result uses interrupted decoupling, which eliminates peaks representing C-H single bonds. The lower result uses normal decoupling in which all carbon-to-atom bonds are represented.

tive abundance of those atoms. The position of the peak along the horizontal axis (parts per million [ppm]) is the resonance frequency characteristic of the atom and its molecular neighborhood. This position is an indication of the chemical identity of the peak as compared to an external molecular reference. In carbon-13 ssNMR, peaks in the 0–80 ppm region are singly bonded carbon atoms (C-C-), or alkanes; signals within the 80–100 ppm region are single bonded carbon atoms with electron-withdrawing neighbors, in particular, oxygen (C-O), as found in carbohydrates, such as sugars. Currently, we have analyzed over 1,800 exudates of all types, including amber, representing most of the major plant groups worldwide. However, a lot more samples still need to be acquired and analyzed.

Types of Plant Exudates

Using NMR, we have determined that there are three major types of plant exudates: resins, gums, and phenolics. Resins are made from terpene molecules. The basic molecular unit of terpenes is a five-carbon molecule, known as isoprene (see Figure 2 on page 6).

When freshly produced, many resins are sticky and smell like Christmas trees or incense. Resins are insoluble in water and thus do not dissolve during rains. As time passes and the resins begin to “mature,” many of their original chemical constituents evaporate. The materials remaining behind in the resin blob form chemical bonds, a process known as polymerization, and the blob begins to harden. With the passage of millennia, the resinous material becomes greatly polymerized and

Not On the Collection List

Not everything that looks like an exudate is an exudate. Some living organisms, particularly fungi, can resemble the kinds of plant exudates we collect. In other instances, the watery—and often foul smelling—material that decomposing portions of plants produce can also resemble exudates. As you may guess, we do not collect those!



Clockwise: Some exudate mimics include a cedar-apple rust (*Gymnosporangium juniperi-virginianae*) fungal fruiting body on *Juniperus virginiana*; an unidentified fungus growing on a *Pinus hwangshanensis* (AA accession 68-76-F)—note its superficial similarity to the yellowish color of some resins; a *Polyporus* fungus on *Quercus palustris* (AA accession 805-87-A); an exudate-resembling, foul smelling material resulting from decomposition by fungi and bacteria on a cut *Cornus kousa* (AA accession 524-49-D) branch.

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Close-up of resinous flow on the trunk of a pine (*Pinus*).

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Latex exudate emanating from a *Euphorbia tirucalli* stem.

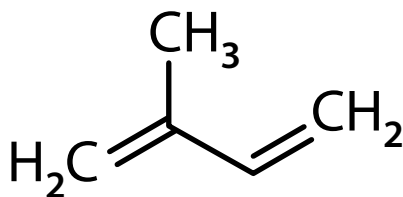


Figure 2. An isoprene molecule, the building block of resins.

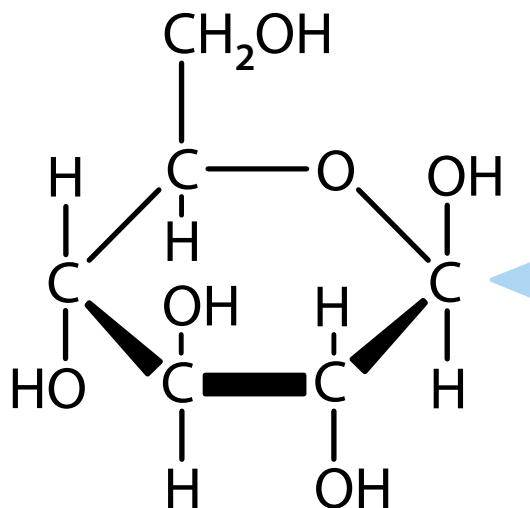


Figure 3. Model of a glucose, an example of a simple sugar molecule. Chemically linked sugar molecules make up carbohydrates. The carbon bound by two oxygen atoms (arrow) is known as anomeric carbon and is characteristic of sugars. Exudated carbohydrates are known as gums.

evolves into the robust gemstone called amber, produced only by specific plant species. Conifers such as pines (*Pinus*), firs (*Abies*), spruces (*Picea*), larches (*Larix*), and some other familiar cone-bearing trees in northern latitudes tend to produce resinous exudates. Many angiosperms (flowering plants) also produce resins.

The term “latex” refers to milky-looking exudates produced by numerous flowering plants, including those in the euphorbia or spurge family (Euphorbiaceae). Latexes can be dangerous to touch, causing dermatitis or other damage, especially to the eyes. Interestingly, all latexes we have examined thus far are resins in suspension.

A second type of exudates is known as gums. Gums are large carbohydrates consisting of myriad sugar molecules linked together chemically (see Figure 3 above). Gums do not



Gum produced by a Yoshino cherry (*Prunus × yedoensis*) growing near the Tidal Basin in Washington, D.C.

tend to smell because of their low volatility stemming from their high molecular weight. When freshly produced, many gums are spongy to touch because of their high water content. Thus, freshly produced gums dissolve easily during rains. If somehow gums manage to survive and dry out, they can then be very hard to dissolve. However, as far as we are aware, gums are not known to survive millions of years as amber does. Gum exudates tend to be produced by flowering plants; fruit trees in the genus *Prunus*, including cherries, plums, peaches, and almonds, commonly produce gums.

The third major type of exudates is known as phenolics. Phenolics are chemically related to terpenes but form unsaturated ring compounds known as aromatics because of their often-pleasant odor. When freshly produced, phenolics tend to be watery and reddish brown, and lack the strong smell of resins. If they survive dissolution, phenolics tend to form brittle solids. As with gums, we are not aware of phenolics that have survived deep time. Phenolics



Reddish phenolic exudates are visible on the trunk of this *Eucalyptus sideroxylon*.

tend to be common in *Eucalyptus* and related plants. Combinations of these major types of exudates, such as gum resins, as well as several other minor kinds of exudates are also known.

Uses of Plant Exudates

In addition to their generally beautiful colors, pleasant aroma, and light weight, resins are water insoluble. These properties make resins, including amber, coveted natural products. Some uses of resins, including amber, include: ceremonial and artistic, as construction materials, ingestive, and, of course, as objects of science because they provide windows into past worlds.

Ceremonial and artistic uses

Amber, that is, greatly polymerized resin, has been used for ceremonial purposes as well as for objects of trade, jewelry, sculptures, and many other items. Although highly valued in the market, amber varies greatly in color and translucency, from white to black and from translucent

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An assortment of typical yellowish amber specimens showing the wide range in color and translucency.

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A group of typical Baltic amber specimens shows varying color.

PATRICK R. CRAIG



Specimens of rare Dominican blue amber from the personal collection of Patrick R. Craig.

to opaque. Because of this variability, color and translucency on their own are generally not good diagnostic traits for identifying amber.

On the other hand, copal (less polymerized resin) and modern resins are still used in some areas of Mexico and Central America for artistic and ceremonial purposes, prized because



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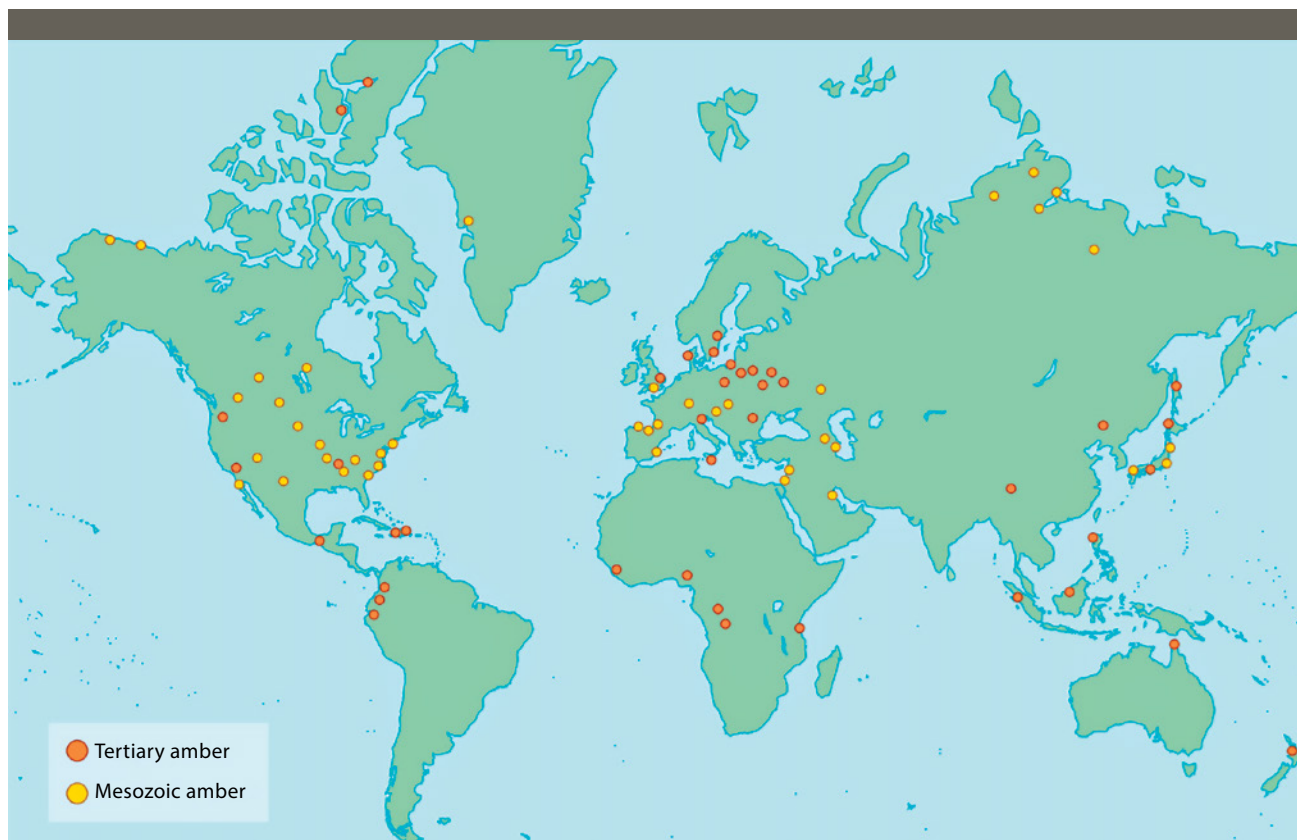
Earrings made from Columbian copal were treated in an autoclave, which applies heat and pressure, resulting in a color change from yellow to green.

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Retsina is a Greek wine traditionally flavored with pine resin.

they smell of incense. Next time you encounter a pine, fir, or spruce tree, look carefully at its bark and you may be able to see some exudate blobs or "teardrops." Pick one of them up and smell it! Pine resin has been used in the preparation of rosin, which is applied to the hairs of bows used to play string instruments such



Is It Amber or Copal?

Amber is greatly fossilized resin. This resinous fossilized material has been found in numerous localities worldwide. The oldest amber has been dated as early as the Carboniferous period, over 300 million years ago. Often, forests whose trees produced resins that eventually became amber tended to be located close to sea level at the time of production.

Partially polymerized resin is known as copal, a Nahuatl or Aztec word that means incense. At times, we have seen the term “semi-amber” used instead of copal. We recommend avoiding the term “semi-amber” because it suggests the material is older than it really is. Although it can be difficult to distinguish copal from resin, a straightforward preliminary way to distinguish between the two is by using a drop of organic chemical such as 95% ethanol or acetone (the solvent used in most nail polish removers). Take a drop of the chemical and place it in a portion of the test sample that has little or no value to the owner. Then touch the wetted portion with the finger. If it feels sticky, the test sample likely is copal; if it does not feel sticky, likely it is amber. We have examined a number of alleged amber samples that turned out to be copal, some of which were in the collections of respectable museums. When finding “amber” specimens of potential scientific value, we recommend testing them by physicochemical means, such as nuclear magnetic resonance spectroscopy (NMR) or others, to gain more confidence on the specimen's true nature.

as the violin (rosin makes the hairs just sticky enough to grip the strings and create sound).

Construction materials

The metallic transatlantic cable that connected the Old and New Worlds telegraphically during the second half of the nineteenth century was insulated by gutta percha, the resinous exudate of *Palaquium gutta*, a tropical Southeast

Asian tree. The modern aviation and aerospace industry uses human-made, lightweight and strong, synthetic resins and phenolics in building airplanes.

Ingestive

An old and interesting use of resins is in the preparation of retsina, a Greek wine that is flavored with a little bit of pine resin (typically from Aleppo pine, *Pinus halepensis*). Gums are also sometimes eaten; in places where the leguminous *Acacia* trees produce copious quantities of gums, these exudates are used as survival foods when other food is scarce. Although it has been alleged that amber has healing and other medicinal properties, we are not aware of scientific studies using a double-blind protocol that demonstrate any medicinal properties of amber.

Science

For reasons that are not known, some forests in the past appear to have produced copious amounts of resins. Although these exudates may have attracted some organisms and repelled others, once small organisms such as insects landed on the sticky material it was difficult to detach from it. When subsequent resin flows covered the specimen it was protected from the action of decomposing organisms and the environment, allowing it to be preserved for a longer time. Subsequent polymerization of the resin preserved a fraction of the resin-entombed organisms, which, when found, now have great value to scientists. Amber encased plant and animal specimens have contributed insights in a number of scientific fields.

Amber specimens that contain larger, rarely found organisms (e.g., scorpions, amphibians, lizards, birds) are of great interest and may command great sums of money. How-

Collecting Competition

Interestingly, sometimes birds, such as the types of woodpeckers commonly called sapsuckers (genus *Sphyrapicus*), compete with us as they also feed on exudates and leave characteristic holes on the surface of some trees. Other birds and some insects are known to use exudates for nest construction.



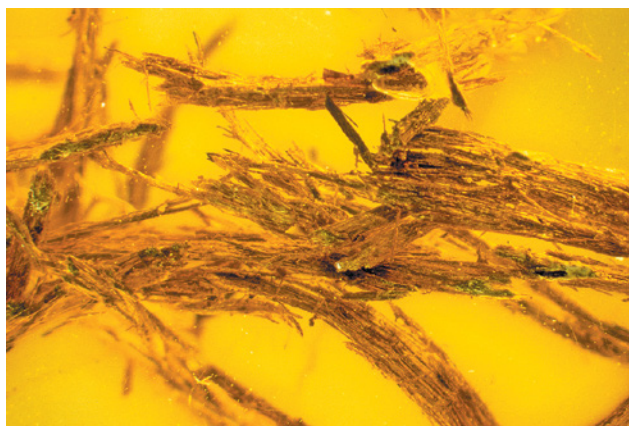
A yellow-bellied sapsucker (*Sphyrapicus varius*) perches on a conifer branch that displays the typical holes created by this and other sapsucker species.

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A drosophilid fly trapped in amber.

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Wood fibers encased in amber.

ever, buyer beware, as there are unscrupulous sellers willing to make money from objects that are not genuine amber.

Ongoing Research Goals

Ultimately, we seek answers to questions because we are curious about nature. Sometimes, our results can help answer a question. For example, along with several other colleagues, including Dr. Lisa Niziolek from the Field Museum of Natural History in Chicago, we answered the question: In what plant family was the tree that produced the blocks of resin found in a thirteenth century shipwreck excavated from the Java Sea? Our studies of many plant exudates have generated a large database of their NMR profiles. When we study a sample of unknown botanical provenance, that database allows us to compare the samples of

unknown botanical origin, like the resin from the Java Sea wreck, with those in our database. With that information, we were able to suggest that the plant whose resins were harvested back in the thirteenth century was from the botanical family Dipterocarpaceae, and perhaps specifically the genus *Shorea*. Having an idea of the botanical provenance of archeological artifacts enriches our knowledge of how our predecessors used plants. In this case, research tells us that aromatic resins were an important commodity at the time and were often imported into China for use in Buddhist rituals as well as medicines, lacquers, and perfumes. We will continue to collect and analyze plant exudates from around the world, including amber and copal, as well as materials associated with anthropological artifacts, adding knowledge for future researchers to use.

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SUZANNE C. SHAFFER



SUZANNE C. SHAFFER

On the lookout even during vacation, author Jorge A. Santiago-Blay (left) noticed resinous exudates on several lodgepole pines (*Pinus contorta*) in Yellowstone National Park, including one partially debarked, possibly by American bison (*Bison bison*) (right). Note the copious exudate production (yellowish color) on the debarked portion of the trunk.

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Dedication

Author Jorge A. Santiago-Blay dedicates this paper to his mother, Ángeles Blay Sálomons, who in the early 1980s suggested to him that he pursue the study of “*las resinitas*” (the little resins) as she used to call exudates. Her memory always lives with him.

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