Chapter 27: Collecting herbarium vouchers

A. P. Davis
Royal Botanic Gardens, Kew, TW9 3AB, UK
E-mail: A.Davis@kew.org

Abstract

The constantly developing uses of herbarium resources and advances in technology necessitate an update and partial revision of Chapter 27: Collecting Herbarium Vouchers of the Technical Guidelines. Examples of modern uses of herbarium specimens and associated data, and the application and methodology of new technologies are detailed and discussed. Several updates of the 1995 version of chapter 27 are provided, including some basic health and safety information. Anticipated future requirements and prospects for collectors of herbarium vouchers are briefly discussed. The gathering of high-quality herbarium-voucher specimens (particularly when they are accompanied with DNA samples and accurate georeference data) and related data are regarded as a critical and integral part of germplasm collection.

Introduction

The role of herbarium-voucher collectors has expanded considerably since 1995, due to improvements in technology, the emergence of new scientific methodologies and the extension of scientific and practical uses for herbarium vouchers and related data. Electronic equipment, such as global positioning systems (GPS), digital cameras and computers, have all become smaller and lighter, and battery life has improved. This has enabled herbarium collectors to take many new devices into the field as part of their everyday equipment, extending the amount and quality of the data that can be gathered. The use of DNA sequencing, and other molecular methods, utilized for a wide spectrum of scientific enquiries (population genetics, systematics, biogeography, rapid diversity assessments), has developed immensely since 1995, so that for many collectors, DNA sampling is now considered part of their regular protocol. The very nature of herbarium specimens, as permanent and verifiable records of organisms in space and time, makes them a fundamentally useful resource for several fields of enquiry, extending their use and value very much beyond their original and primary remit of taxonomy and systematics. Voucher specimens, in conjunction with accurate locality (georeference) data, are now also being used as the primary resources for conservation and diversity assessment (e.g., Golding 2004; Lister et al. 2010; Rivers et al. 2010; Roberts et al. 2005; Rondinini et al. 2006; Willis et al. 2003;), niche modelling, predictive mapping (e.g., Graham and Hijmans 2006; Phillips et al. 2006) and climate change (e.g., Pearson and Dawson 2003; Primack et al. 2004; Raxworthy et al. 2008). In order to maximize the potential of new technologies and novel scientific methodologies, for genebanking and germplasm science and the development of crop genebank management, it has been necessary to update and extend conventional protocols for voucher collecting. This information is outlined below.

This chapter is a synthesis of new knowledge, procedures, best practices and references for collecting plant diversity since the publication of the 1995 volume *Collecting Plant Diversity; Technical Guidelines*, edited by Luigi Guarino, V. Ramanatha Rao and Robert Reid, and published by CAB International on behalf of the International Plant Genetic Resources Institute (IPGRI) (now Bioversity International), the Food and Agriculture Organization of the United Nations (FAO), the World Conservation Union (IUCN) and the United Nations Environment Programme (UNEP). The original text for Chapter 27: Collecting Herbarium Vouchers, authored by A. G. Miller and J. A. Nyberg, has been made available online courtesy of CABI. The 2011 update of the Technical Guidelines, edited by L. Guarino, V. Ramanatha Rao and E. Goldberg, has been made available courtesy of Bioversity International.

Current Status

New technology and equipment

Global positioning systems (GPS)

The latest generation of GPS units is lightweight, extremely accurate, has a long battery life and is capable of storing and downloading large amounts of georeference data. Earlier systems (mainly pre-2006) did not work well under the tree canopy, requiring the user to place the unit above or close to the canopy or to seek higher ground. In many cases, this considerably reduced the accuracy of readings, and users were often likely to take fewer georeferences. In May 2000, the degraded accuracy (about 100m) of civilian GPS was removed, increasing accuracy to about 15m. By 2006/7 most GPS units were fitted with highly sensitive receivers, more advanced microchips, and ground and space augmentation systems ("EGNOS" in Europe, "WAAS" in North America and "MSAS" in Japan). These changes have increased the accuracy to around 3m or better, with 95% confidence, given good satellite coverage. A good-quality modern GPS unit will now take an accurate reading under the tree canopy and in other previously problematic locations, such as in ravines and valleys. The long battery life means that multiple (e.g., 100s) of data points can made on a daily basis, and the unit can be left switched on for hours instead of minutes. Many collectors will now make an extended series of data points, capturing not only the exact position at which their herbarium vouchers were collected but also many other features, including records of absence, roads and paths, limits of primary vegetation (forest, native grassland), new villages and other settlements, and changes in land use. Most GPS units feature a track-log function, which will record the whole collecting journey in space (location) and time, which is an excellent means of geo-tagging specimens and photographs. These data are easily downloaded and stored electronically for later use in conjunction with global information system (GIS) software.

The most readily accessible and easiest tool for mapping your data is GoogleEarth™, which enables free and easy access to recent high-resolution satellite imagery, in conjunction with numerous tools and applications. The georeference data, or data points (also known as "waypoints"), are easily plotted manually, one by one or in large numbers, showing, for example, the route and extent of a collecting mission. In the first instance, this tool enables collectors to visualize their data points, check for accuracy and see whether they have applied the correct methodology for collecting the required spatial data.

All GPS units come with detailed instructions; the user needs to be familiar with the unit before collecting. Most important, the unit should be set up/configured for use in the correct manner. In most cases, the units should be set to metric, so that the data produced is given in metres (altitude) and kilometres (distance) and the time is set to local, although the latter is often set automatically. For the georeferences, the most common format is to set the GPS unit at degrees, minutes and seconds (e.g., N 49° 21′ 41.4″ E 30° 6′ 22.0″) or degrees and decimal minutes (e.g., N 49 21.690 E 30 06.367). The former is preferred for herbarium voucher labels and, as good practice, should be copied into the field collecting notebook at the point of voucher collection.

The GPS unit will usually store the data in various ways, but the most useful for the user will be digital latitude and longitude (e.g., 49.36151 and 30.10612), which is used by most GIS software. Setting the GPS unit to the correct datum is essential. The World Geodetic System 1984 (WGS 84) is probably the most common datum for GIS data sets and is the one used for visualization with GoogleEarth™. Because the Earth is misshapen, when creating a datum to suit a specific country or region, geodesists have a "local" or "regional" datum. For some mapping projects, the use of the correct regional datum is critical, so consultation with project partners or the project manager on this point is very important. It is essential to note the datum used, even if it is WGS 84. It should also be noted that even when using local datum, the GPS unit might also record and store data in WGS 84.

Any investigation involving spatial analysis of distribution data necessitates accurate georeferencing of specimens, which in turn means that the collector of voucher specimens should be adequately trained and provided with the right equipment.

Digital Cameras

The advent of reasonably priced and easy-to-use digital cameras has accelerated the amount of imagery now available for understanding and visualizing germplasm and its natural environment. In conjunction with the voucher specimen, images can improve the chances of accurate identification by providing details of shape, colour and form, which are mostly lost when herbarium specimens are made. Other images can also be captured, such as habitat, associated species and possible threats to the population or individuals. Both types of images provide useful resources for publicity and media use. As with all other voucher information, it is important for the images to be linked to the voucher specimen by a unique identifier (e.g., the collector's name and number, or a barcode), which in turn will provide a link to associated data, the most important of which is the accurate locality reference. Some cameras have a GPS processor as part of the unit; some GPS units incorporate a camera. It is also possible to geo-tag images using the GPS track log facility, linked to a digital camera, and there are numerous packages and software programmes available to do this.

Computers

Reasonably priced laptops, electronic notebooks and tablet computers are now widely available, and some have the additional advantage of long battery life. They all provide a means of recording and storing data directly in the field, with the possibility of linking to various software packages and the internet. In particular, many collectors like to download their photographic images on a daily basis, to free up camera memory cards, provide a back-up system and as a means of editing images. Hand-held data recording devices have been available for several years, performing similar functions as traditional notebooks but with added electronic functionality. Such devices have not yet replaced the field notebook, however, and field-testing during extended field studies suggests that they are currently of rather limited application. The main problem, and one that is common to electronic devices, is battery life, and the need for constant recharging, which is a particular problem where there is limited access to a reliable power source. However, with the advent of smart phones and similar technology, these problems will no doubt soon be overcome.

Other

Water- and solution-tight resalable bags, such as Whirl-Pak®, are now widely used for collecting spirit material in the field. These bags have the advantage that numerous spirit collections can be carried in a much smaller space, compared to rigid plastic bottles. Moreover, the close contact of plastic with sample means that very little spirit has to be used. Once the plant tissues have totally absorbed the spirit, the samples can be maintained for a few weeks with very little liquid, which is particularly important for transportation by plane. The spirit should be drained from the bag and the bag resealed; then several samples can be placed in a larger bag, the latter being totally sealed with non alcohol-soluble tape. Once the samples arrive at the herbarium or place of permanent storage, they can be decanted into a suitable archival liquid (Copenhagen Mixture or Kew Mixture) in appropriate storage jars (see Foreman and Bridson 1992).

DNA sampling

Before 1995, DNA samples would usually only be collected as part of a specific request or for target groups. With the increased demand for DNA samples, many collectors are now routinely collecting high-quality DNA samples for each herbarium voucher. If not required for an ongoing study, the DNA samples are banked in long-term storage facilities, either as silica-dried leaf material or as purified extracted DNA, for later use. Some herbaria now list their DNA sample collections via the internet so that researchers can access material that would otherwise not be available to them. The methodology for DNA sampling has not changed essentially since the silica-dried methodology was formalized (Chase and Hills 1991), although there has been a shift from collecting relatively small (less than 1g of fresh leaf material) to larger amounts of plant material for DNA extraction. This is due to the realization that samples might have to be used for multiple analyses under different protocols, and the advent of next-generation sequencing, all of which require more DNA. For this reason, it is often advisable to collect at least 2g–6g of fresh leaf material, depending on the plant and the methods being employed. A simple method for the collection of leaf material (adapted from Chase and Hills 1991) is given below.

Fresh leaf material is torn into small pieces (not exceeding $2cm^2$), to a total 4g–10g, and placed in small (12cm x 8cm) sealable plastic bags containing 50g–100g of fine-particle silica gel, including some indicator silica gel (which will change colour when wet, indicating whether the samples are rehydrating by absorbing moisture from the atmosphere). The ratio of fresh leaf material to silica gel should be about 1:10. Shake the bags to distribute the silica gel between the layers of leaves. If leaves are not completely dry within 12 hours, remove the silica gel and add an appropriate amount of new silica gel. Good-quality silica gel can dry 6 to 8 samples before being saturated.

To assess whether a leaf sample is thoroughly dry, bend one of the leaf pieces: if it is dry, it should snap and break cleanly. The leaf material may now be removed from the silica and placed in a new sealable plastic bag, or similar holder, with a small amount of silica gel, including some indicator type. The remaining silica gel can now be reused but must be checked very carefully to make sure that all fragments from the previous sample are removed. As the capacity of the silica gel is used up (i.e., as it absorbs water), the pieces of indicating gel turn from a dark violet-blue to pale blue and finally to a pale pinkish purple, or from amber to pale yellow, depending on the type of indicator gel used. At this point, the silica gel can be regenerated in an oven at 175°C for one hour. It may also be dried using other heat sources, although longer periods of heat exposure are required (4 to 5 hours, overnight, or 1 to 2 days, depending on the heat source). The indicator silica gel should return to its original colour when regenerated.

Silica gel is an irritant, especially to the respiratory tract, and can cause irritation of the digestive tract. Fine-particle silica gel causes irritation to the skin and eyes. There are also long-term health issues associated with its use. For this reason, avoid contact with the skin, and only use it in a very well-ventilated environment. In addition, the blue indicator gel contains cobalt, which has also been associated with long-term health issues.

Other updates and comments on the previous (1995) version

Basic equipment for herbarium collecting

In addition to the basic equipment for herbarium collecting given in box 27.1 of the 1995 version of this chapter, it is good practice to include the items of equipment shown in the box below, which are divided into two categories: consumables and non-consumables. This does not include camping equipment, other field apparatus and safety equipment.

Preparing material in the field

In addition to the notes on preparing material in the field in the 1995 version of this chapter, it is strongly recommended that for larger samples, such as trees and shrubs, where a shoot or branch is selected as the specimen, at least one leaf be turned over so that the under-surface of the leaf is exposed. This is done by twisting the leaf at the petiole and then applying pressure after the pressing paper (e.g., newspaper) has been folded over the plant. Done in this way, the specimen will not have to be harshly manipulated prior to herbarium mounting. The under-surface of the leaf is often critical for correct species identification. For smaller plants, where more than one individual is collected per specimen, a whole plant can be turned over at the time of mounting.

Generally, any material over 3cm thick, such as wood and large fruits, should not be placed in the press, as it will cause the other specimens to become misshapen upon drying. Instead, such material should be wrapped in newspaper, or similar material, and placed separately on the plant drier. As with all voucher parts, this material should be numbered with a unique identifier (e.g., collector's name and number), using a jeweller's tag and/or written on with a pencil or permanent non-alcohol-soluble marker.

Basic Equipment for Herbarium Collecting

Consumables

- Large (90cm x 60cm) heavy-gauge (500+) clear plastic bags (used to temporarily hold the plants until they are ready to prepare and press)
- New, large (90cm x 60cm) heavy-gauge (500+) clear plastic bags*
- Durable (waterproof) pocket-sized field notebook(s) for use with a pencil
- Several pencils (HB to 2B) and a sharpener
- Large and small, waterproof and non-alcohol-soluble marker pens (e.g., Sharpie® brand)
- Newspapers and corrugated cardboard sheets (43cm x 28cm) (required for preparing specimens for the Schweinfurth method and conventional air drying)
- Alcohol (preferably ethanol), 60%–70% (or higher that can be diluted to 70% in the field)*
- Good-quality plastic parcel tape or other non-alcohol-soluble tape*
- Parcel string

Recommended or essential, depending on the project

- Whirl-Pak® plastic bags or similar for collecting spirit material
- Wide-mouthed plastic bottles (assorted sizes), such as Nalgene®, for collecting spirit material
- 70% ethanol or other temporary preserving fluid, for collecting spirit material
- Small bags (Ziploc® type) for silica gel DNA samples
- Silica gel
- Small (10cm x 7.5cm), medium (20cm x 19cm) to large (35cm x 20cm) bags (Ziploc® type), for various applications
- Rice sacks and rice sack needle (useful if bundling lots of alcohol-processed specimens, see below)

Note: A good supply of all of the above items is required, the amounts of which should be carefully calculated according to the number of specimens anticipated.

* Only required if Schweinfurth (alcohol) method is being used.

Non-Consumables

- Professional plant press(es) with heavy-duty straps
- A sharp pocket knife
- Scissors
- Hand lens (magnification x10)
- Tape measure or ruler
- Global positioning system (GPS), preferably including a barometric altimeter
- Mechanical altimeter

Recommended or essential, depending on the project

- Digital Camera for recording appearance of the live plant, habit and habitat
- Batteries (for digital camera) or alternative power source(s), such as solar charger
- Small field binoculars (for looking into the tree canopy, etc.)
- Pole clippers/pruners (essential if collecting trees or where tree climbers are unavailable)
- Machete/parang (required if bark and “slash” information is needed)
- Heavy-duty thorn-proof gloves (if dealing with spiny plants such as rattans)
- Sturdy boxes for specimen transportation (required if plants are dried in the field)

Drying specimens

In addition to the notes on drying plant specimens in the 1995 edition of this chapter, the following information is given for completeness. Many collectors now use naked flames (including gas, charcoal and spirit burners) to dry plant specimens, particularly in remote areas. These methods come with inherent risks, as drying papers and plant material are readily combustible, particularly when previously treated with alcohol. Thus, the following information also includes notes on safety. A useful guide on collecting herbarium specimens is provided by Carter et al. (2007), with illustrations that demonstrate many of the main points given below.

1. Plant presses should be made up in the following sequence: corrugate – blotter (drying sheet) – specimen (between a sheet of folded newspaper) – blotter – corrugate – blotter – specimen – blotter – corrugate,…etc. Always end with a corrugate, which goes directly against one side of the press. The folded side of the newspaper should be lower-most in each press, so that no plant material can fall out of the press (and onto the heat source). Each press may be made quite large, but it should not be unstable or overly bulky; there should be nothing (such as specimen parts) sticking out of the press. When metal corrugates are used, the press size can be extended to the limits of the straps. If metal corrugates are not available, corrugated cardboard can be used, although then the press size will have to be much smaller, because the airflow with cardboard is considerably less and the plants will not dry properly.

2. The straps should be fastened as tightly as possible: tighten the first strap moderately firmly, fully tighten the second strap, then return to the first strap and fully tighten. If the straps are fastened in opposite directions, the pressure will be more even, although this not always the case. The long slats of the plant press should be outermost when the press is assembled and tightened, so that even pressure is applied to the specimens. (In most cases this will also avoid damage to the press.)

3. Expose the presses to a gentle (35°C to 45°C) heat source for as long as is necessary to dry the specimens properly, but they should not be over-dried or brittle. A variety of heat sources can be used in the field (e.g., gas, oil and charcoal heaters). Where there is a permanent facility, purpose-built and thermostatically controlled, fan-assisted drying ovens are the best, although alternative set-ups using electric fan heaters, electric elements and gas stoves can be adapted. A steady flow of warm air around and through the presses (via the corrugates) is just as important as temperature. Excessive initial heat can cause specimens to become "stewed" (recognized by discoloration and a "cooked cabbage" smell), especially if the drying papers are not frequently changed or if the plant is succulent. Some collectors warn against rapid drying, but generally it is considered desirable. The presses (and any loose material) must be well supported so that they cannot fall onto the heat source and start a fire. Make sure that the straps are securely tucked away so that they do not hang down and catch fire.

4. Each press should be examined regularly for tightness and turned so that the heating is even, although the edges of the newspaper should still remain lowermost. If allowed to become loose, undue distortion or shrivelling will occur, which might result in items being lost or falling onto the heat source. If necessary, a second folder of newspaper may be added to each specimen (the opposite way around) at the time of pressing. Examine the press at least twice a day and change the blotters (drying papers) as necessary, but not the flimsies. The dry specimens should be removed as soon as possible and the blotters (drying papers) made ready for re-use. Allow 18 hours to four days (or more) for complete drying. The specimens should be rigid when dry, unless very delicate. If possible, allow about 12 hours before handling the specimens after drying, especially for specimens prepared using the Schweinfurth (alcohol) method, as they are often very brittle and require some time to reabsorb moisture from the atmosphere.

Equipment for drying specimens in the field

Two comments on box 27.2 in the 1995 version of this chapter are required:

- Newspaper is not the best type of drying paper (blotter). Thick blotting paper, or similar paper, 1mm–3mm thick, is preferable.
- If plants are dried in the field, as opposed to the Schweinfurth (alcohol) method, they will be brittle and very fragile after drying. In this case, sturdy boxes are required for carrying the dried plant specimens out of the field and for later transportation.

Chemical treatment: the Schweinfurth (alcohol) method

Additional comments for this section are also necessary. It should be pointed out that once the collector has access to plant-drying equipment, the alcohol-treated specimens can be carefully removed from the plastic bags and then dried in the conventional manner (see the 1995 version of this chapter 27 and the text above on drying specimens). Polythene tubes, as recommended in 1995, are generally not easy to obtain and, therefore, large (90cm x 60cm) heavy-duty (500+) clear plastic bags can be used – and in many cases are

preferable. Inferior quality plastic bags can come apart at the seams if they are exposed to alcohol, so it is always a good idea to seal the seams with strong, non-alcohol-soluble parcel tape.

The most convenient way to tie up the bundles of plants, prior to placement in a plastic bag and wetting with alcohol, is to tie them with parcel string, using the "herbarium knot" (Forman and Bridson 1992). Roughly pillow-sized bundles are about right. It is important to wet all the specimens with alcohol, but the bundles should not be heavily saturated: any alcohol not absorbed within 10 to 20 minutes should be poured off. Likewise, always check the inside of the bundle to make sure that the flimsies (e.g., newspaper folders) are not dry. If they are, add more alcohol. It is good practice to add alcohol to the centre of the bundles first and let it absorb outwards through the bundle. Three or four bundles can be placed in a 90cm x 60cm plastic bag, which should be securely sealed with tape after removing as much air as possible. In difficult terrain or on long journeys, it always a good idea to double-bag the main plastic bag. Two or more completed packages can then be placed in a rice sack for extra durability and ease of transport. It is also advisable to keep the packages out of direct sunlight. Properly prepared and carefully stored Schweinfurth-prepared specimens will last for many months prior to drying.

Recording data

In addition to recording the features of the plant (see 1995 chapter), it is essential (for the production of herbarium labels and for subsequent scientific analysis) for all the basic information to be recorded at the time of collection. The following guidelines are provided:

Information Required for Each Specimen Entry, Where Applicable

A specimen is only useful if it is accompanied by adequate field notes and has a unique identifying number to accompany it. The best system of unique identification is a consecutive number series for each collector. This should start at 1, and each number should refer to only one collection, and never be repeated. Therefore, the collector's name(s) + their collection number comprise the unique identifier. For common surnames, or where joint collectors have the same surname, initials are used to provide the specimen with a unique identifier (e.g., Jones [W.P.] 14962; Dransfield [J.] & Dransfield [S.] 17346). All duplicates (parts of the same plant) should bear the same unique identifier.

- Collectors name(s) and number (see above)
- Precise location: country, state/province/division (or any meaningful/useful division or divisions within the country); kilometres and direction from an obvious landmark (e.g., town, large village, mountain summit, river mouth); georeference (latitude and longitude) recorded with a GPS (set GPS to degrees, minutes, seconds; and metric units)
- Altitude: record with mechanical altimeter, equilibrated against a known altitude (i.e., sea level) (Some modern GPS units have accurate barometric altimeters.)
- Habitat:
    1. Vegetation type (humid, evergreen forest, mangrove, savannah) (If possible add dominant species to more precisely indicate vegetation type/association.)
    2. Geology: rock or substrate type (This is not always possible if the soil is thick or if geology is complex.)
    3. Soil type (iron-rich, silty, sandy)
    4. Other: topography, slope, exposure
- Plant description: record features that will not be present or evident from the dried pressed specimen (tree, shrub, or herb; height; shape, structure, architecture); notes on root system; features that may be lost on drying (e.g., colour of flower parts and other parts that are coloured); odour (flowers, leaves, wood and bark); exudates, oils and waxes; if it is a tree or large shrub, record the bark characteristics (texture, slash, exudates) and diameter at breast height (dbh)
- Date of collection: use a system that is unambiguous (e.g., 21 Aug. 2008)
- Associated material: if the main specimen is accompanied by additional items that will be linked together at a later date, record these in the notebook; e.g., photographs (image number can be recorded in notebook), fruit (carpological specimen), DNA samples, spirit material, wood sample, ethnobotanical artefact, medicinal product
- Number of duplicates: record the number of duplicates that have been collected for each accession number
- Additional collectors: note the full names or surname plus initials of the collecting team; for ethnobotanical collections or when vernacular names are being collected, be sure to record the language used, village/tribal affinities (if relevant) and the name of the informant

Storing and dispatching vouchers

In the 1995 version of this chapter, the use of para-dichlorobenzene (i.e., moth balls) prior to storage and dispatch is recommended as an effective deterrent against pests. Although there is no direct evidence, para-dichlorobenzene may be reasonably anticipated to be a carcinogen, and it is banned by some institutes. Likewise, naphthalene is classified as possibly carcinogenic to humans and animals, and exposure to large amounts may damage or destroy red blood cells.

Future challenges/needs/gaps

Developments in the use and application of herbarium data have extended the role and value of this recourse for germplasm science and genebanking. Today's collectors need to be aware of these changes so that they can develop their equipment and protocols accordingly. This is particularly true for the collection of DNA samples and the use of new electronic equipment, especially geo-location hardware (GPS) and GIS software. At present, the use of some electronic equipment, such as portable computers, is limited by battery life and problems with recharging in the field, although these issues will no doubt be overcome. GPS devices will become more accurate and increasingly sophisticated. Hand-held electronic notebooks and other data-recording devices have improved and may become mainstream in the future. Protocols for long-term storage of silica-dried and extracted DNA material requires further investigation and investment, including looking into aspects related to data-basing and future access. As with all extra-locally gathered resources, further work on prior informed consent, access and benefit-sharing agreements is also required. One of the biggest challenges for the future, however, is the maintenance and development of herbaria and other voucher storage facilities, which include the training and retention of skilled curators and specimen-based scientists.

Conclusion

The collection of herbarium-voucher specimens and associated data is an integral component of germplasm collection and genebanking, and where possible, best-practice procedures should be followed and maintained. Developments in the use and application of herbarium data have extended the role of these resources, and the modern herbarium-voucher collectors will need to be aware of these changes so that they can develop their equipment, skills and protocols accordingly. The value of high-quality, accurately georeferenced herbarium specimens and associated material, particularly DNA, should not be underestimated.

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