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Preparation and Use of Voucher Specimens for Documenting Research in Weed Science

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Voucher specimens and herbarium collections provide the foundation for many aspects of research in the plant sciences. Available for study and verification by contemporary and future workers, voucher specimens promote reproducibility in scientific method because permanent records document identification, distribution, and interspecific and intraspecific variation of species. The utility and importance of voucher specimens and herbarium collections in supporting research in weed science are discussed, and the collection, preparation, documentation, storage, and shipment of voucher specimens are detailed.

Key words: Herbarium; herbarium specimen; documentation of research; use of herbarium specimens; preparation of herbarium specimens; handling of herbarium specimens; storage of herbarium specimens.

Properly prepared voucher specimens are fundamentally essential in documenting occurrences and distributions of plant species. The specimen itself is tangible, permanent, and verifiable evidence, and its label includes geographical and ecological data. Vouchers also provide evidence of hybridization, seed set, flowering, and fruiting dates and may even be sources of seed for germination studies. Voucher specimens should be deposited in an officially recognized public herbarium (Holmgren and Holmgren 1998; Holmgren et al. 1990), where they will receive proper care and will become permanent records available to other researchers. An herbarium is a collection of dried plant specimens, a permanent repository of specimens and data, and a component of most state universities, natural history museums, botanical gardens, and federal plant-research facilities. Excellent background and introduction to the herbarium are provided by von Reis Altschul (1977) and Simpson (2006). Herbarium specimens will last indefinitely if properly prepared, cared for, and protected from water, humidity, and a variety of pests, such as insects and fungi. Each specimen is a voucher, providing a permanent record of the occurrence of a species at a particular geographical location and time; thus, specimens without associated data are of limited use. Although often neglected by weed scientists (cf. Muenscher 1955; Zimdhal 1999), voucher specimens and the herbarium fulfill a vital role by enabling the accurate identification of weeds and documentation of research. The essential role of vouchers and herbarium collections in scientific research and the importance of citing voucher specimens in publications are emphasized by Funk and Morin (2000) and Funk et al. (2005).

Herbarium specimen data have been used to map historical distributions and to elucidate pathways and means of dispersal in North America of introduced weeds, such as dog mustard [*Erucastrum gallicum* (Willd.) O. E. Schulz] (Luken et al. 1993) and European brooklime (*Veronica beccabunga* L.) (Les and Stuckey 1985). Voucher specimens routinely provide documentation about the introduction and dispersal of newly introduced weeds (e.g., Carter and Mears 2000; Carter et al. 1996), and morphometric data taken from herbarium specimens were analyzed to establish the origin of the introduced weed bloodscale sedge (*Cyperus sanguinolentus* Vahl) and thereby resolve its nomenclature (Carter and Bryson 2000). Herbarium specimen data were used by Petřík (2003) to analyze dispersal dynamics since 1854 of an introduced weed, lovegrass sedge (*Cyperus eragrostis* Lam.), in Europe and by Rosen et al. (2006) to revise concepts relating to the historical distribution and introduction of deeproot sedge (*Cyperus entrerianus* Boeck.) in the United States, to map its distribution, and to evaluate its status. Barney (2006) used herbarium specimen data to recreate the historic phytogeographical distributions of two invasive plant species, mugwort (*Artemisia vulgaris* L.) and Japanese knotweed (*Polygonum cuspidatum* Sieb. & Zucc.), in North America. Observations of characters present on herbarium specimens were used to explain increased vigor (heterosis) through hybridization or introgression in a highly competitive invasive weed (Carter 1990). Phenology data documented by herbarium specimens have recently been used to investigate climate change (e.g., Lavoie and Lachance 2006; Miller-Rushing et al. 2006), and DNA studies of herbarium specimens are possible under certain conditions (e.g., Drábková et al. 2002; Ribeiro and Lovato 2007; Šmarda and Stančik 2006).

Weed science research is greatly enhanced when substantiated by voucher specimens available for study by contemporaries and scientists in the decades and centuries to come. Therefore, preparation of voucher specimens should be a routine part of research in weed science. The purpose of this article is to provide information to the weed science community on basic procedures for preparing and handling plant specimens.

Preparing Voucher Specimens

The following steps are normally involved in preparing a voucher specimen from start to finish: (1) locate the plant,

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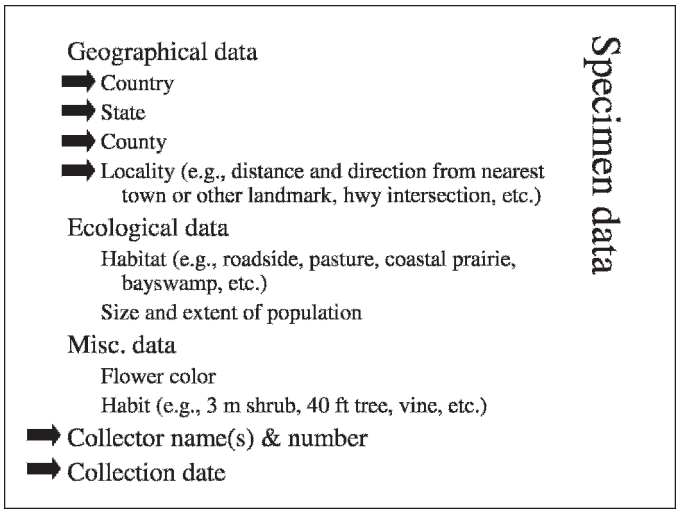

Figure 1. Data elements normally recorded in the field notebook when preparing voucher specimens; arrows indicate essential elements.

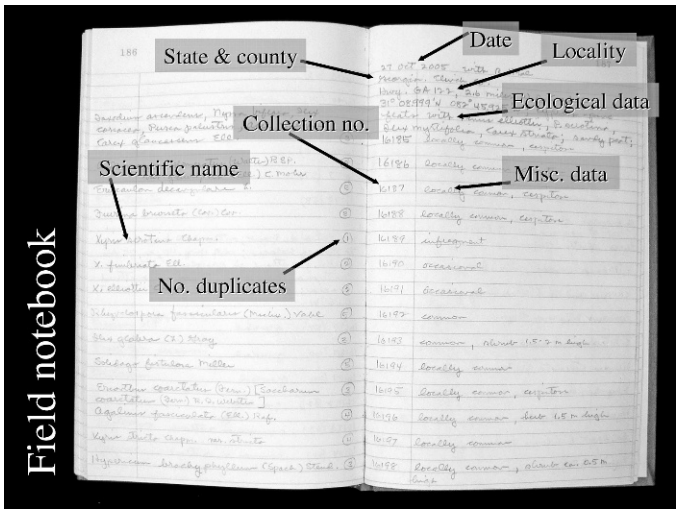

Figure 2. Field notebook showing organization of the basic data elements: date, state, county, locality, ecological and miscellaneous data, collection number, number of duplicate specimens, and determination (scientific name) of voucher.

(2) prepare the plant for pressing, (3) record geographical and other data in a field notebook, (4) press the specimen, (5) dry the specimen, (6) identify the specimen, (7) prepare the label, (8) mount the specimen, (9) apply a serial accession number to the herbarium sheet, (10) sort the specimens, and (11) file the specimen systematically into the herbarium. Steps 9 through 11 are normally done by herbarium staff. Most herbarium curators will accept well-prepared, unmounted voucher specimens so long as they include adequate data, and many will accept unidentified vouchers and identify them in exchange for the specimen as a contribution to the herbarium collection. Therefore, it is generally acceptable to complete only steps 1 through 5 or 6 and still send the voucher to an herbarium where it will be further processed and properly stored. Now available online, *Index Herbariorum* (Holmgren and Holmgren 1998; Holmgren et al. 1990) is a catalog of the world's officially recognized herbarium collections and a useful source with addresses and contact information for herbaria.

Recording Specimen Data. Ideally, the following kinds of data are recorded: geographical data, including country, state, county, and specific locality with latitude and longitude coordinates; ecological data, particularly type of habitat, size, and extent of population, soil type, names of associated plant species, and the type and identity of plants on which parasites and epiphytes are growing; miscellaneous data denoting features of the plant that might not be evident in the finished specimen, such as flower color or plant height; collector's name; collection number; and date of collection. If the voucher provides documentation for a particular project, then the name of the project or a brief statement about it might be included in the specimen data. Voss (1999) provides useful guidelines for recording data and preparing specimen labels. The arrows in Figure 1 indicate essential data elements.

Although field botanists normally record collection data in a field notebook, data could be included in any research record book. The field notebook should be small enough to be conveniently slipped into a daypack and should have a permanent binding and high-quality paper. Field notebooks are available from most forestry, engineering, or scientific supply houses. Figure 2 shows how the basic data elements might be organized in the field notebook. Also, after the voucher specimen is identified, its determination is normally recorded in the notebook correlated with a unique collection number. Although a variety of systems are used for designating collection numbers, we recommend beginning with "0001" and increasing serially with each new voucher collection. Normally, duplicate collections made from the same population at one site on the same day are given the same serial collection number. Most field botanists use serial collection numbers because they are a convenient means of keeping track of large numbers of specimens and data, and we would hope researchers who routinely collect vouchers for preservation in the herbarium would see their value and use some system for numbering specimens sequentially. However, the absence of collection numbers should never deter a scientist from submitting voucher specimens to an herbarium. Figure 3 shows a data sheet for voucher specimens that might be adapted to accompany unlabeled specimens sent to an herbarium. Herbaria may or may not have the resources to produce labels for extrinsic specimens, but processing of specimens at any herbarium is expedited when properly prepared labels are included. Nevertheless, specific details about responsibilities and requirements with regard to contributions of voucher specimens should be arranged in advance with the individual herbarium curator.

Duplicate Specimens. In most cases it is desirable to collect more than one voucher specimen per population. Disseminated to multiple herbaria through specimen exchange programs by herbarium curators, duplicate vouchers are readily available to a wider field of researchers. Duplicates are especially important in the documentation of newly introduced weeds, as they greatly facilitate identification at new geographical stations by other scientists. Also, with problematical specimens, it is sometimes desirable to send a duplicate to a taxonomic specialist for determination or

VOUCHER SPECIMEN DATA
Required elements shown in bold at top of form

Date of collection:

Collector name(s): Collection number:

Country:

State:

County (Parish):

Locality (e.g., 3.5 miles west of intersection of Interstate 75 and Hwy. US 84 in Valdosta):

GPS coordinates:

Habitat type (e.g., bayswamp, marsh, pasture, roadside):

Size and extent of population:

Misc. data (e.g., flower color, plant height):

Figure 3. Sample data sheet for voucher specimens sent without labels to an herbarium.

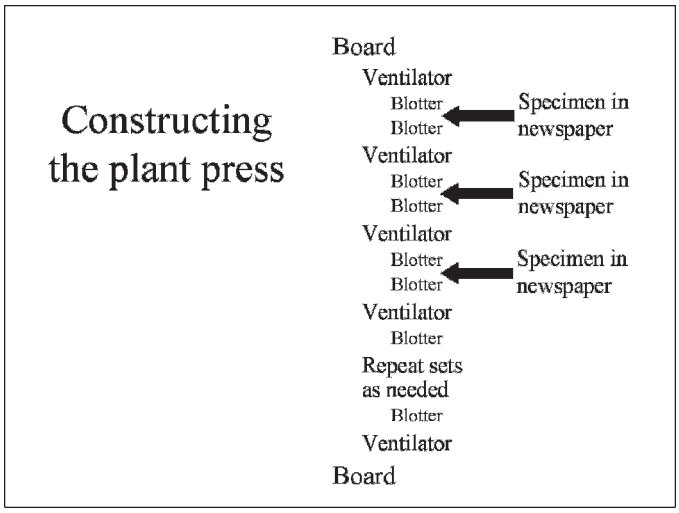

Figure 4. Schematic showing how the press is constructed with each specimen enfolded in a newspaper page inserted between press sets.

confirmation. Under standard practice, the specialist would keep the duplicate for addition to his or her institutional herbarium in exchange for the identification service.

Pressing Specimens. The two main objectives in pressing plant specimens are (1) to flatten the plant in a size and conformation that display important characteristics and are convenient for storage in standard herbarium cabinets and (2) to dry the plant material as quickly as possible maximizing preservation of structures and chemical compounds as well as preventing degradation by organisms that grow or feed on organic substrates (i.e., fungi, bacteria, etc.). Certain types of plants require special treatment (e.g., aquatics and succulents), but for most plants, preparing specimens is a simple process.

Specimens are normally pressed enfolded in single newspaper pages. Whereas most any absorbent paper may be used, newspaper is inexpensive and readily available, and a folded single sheet of newspaper is slightly smaller than the standard herbarium sheet and, thus, is a practical guide for preparing properly sized voucher specimens. The entire newspaper section (e.g., the sports section) is easily reduced to individual pages by tearing lengthwise in half along the vertical center crease. The collection number is then written along the margin of the folded newspaper page, and the newspaper page with enfolded specimen is then placed between two ventilator-blotter sets in the plant press.

Plant press components may be purchased from scientific or herbarium supply companies or constructed and assembled from basic materials. The standard press consists of two straps or ropes, two plywood or lattice header boards [1.3 by 30.5 by 45.7 cm (0.5 by 12 by 18 inches)], paper blotters [30.5 by 45.7 cm (12 by 18 inches)] to absorb moisture from the specimen, and ventilators [30.5 by 45.7 cm (12 by 18 inches)] with channels oriented parallel to their 30.5-cm (12-inch) edges. The ventilators allow warm air to flow through the press as the specimens are dried. Ventilators are commonly constructed of corrugated cardboard. Although relatively inexpensive and lightweight, corrugated cardboard ventilators become crushed with repeated use and must be routinely inspected and replaced. This is especially a problem under wet, humid field conditions; therefore, more expensive corrugated aluminum sheets are sometimes employed as ventilators in the tropics. Heavy-duty press straps with parachute buckles are recommended because they tend to be more durable than rope and are not prone to slipping. Open-cell foam sheets [0.5 by 30.5 by 45.7 cm (0.2 by 12 by 18 inches)] may be used in place of paper blotters for specimens with both thick, hard parts and thin, delicate structures. Figures 4–7 show how to construct the plant press and place the voucher specimen for efficient drying.

Additional Considerations in Preparing Specimens. The plant base should always be rinsed free of soil before the specimen is placed in the newspaper fold. Ideally, to the extent possible, the appearance of the finished specimen should conform to the living plant, and it is important to include parts and life stages that are useful for identification. If specimens are too large to fit the newspaper page, their stems and leaves should be carefully broken and folded or cut to fit. Cutting or breaking and folding are preferable to bending the stems, because with cutting or breaking there is usually no doubt about how the specimen was altered during preparation. In contrast, artificial bending of the stem is to be avoided because with bending one cannot so easily discern whether the condition is natural or artificial.

Small Herbs. With small herbs ($<$ 1 m tall), the entire plant is generally preserved. If they are small enough, several plants should be pressed within the newspaper fold, although crowding plants within the newspaper will prolong the drying period. Include as much of the plant base as practical or at least a representative portion of the root system, rhizome, or other subterranean organ. Plants should be dug from the ground because important underground structures are often broken off by pulling.

Large Herbs. With larger herbs, the stem may be broken and folded one or more times to fit the newspaper page or may be cut into two or more sections, each pressed in a separate

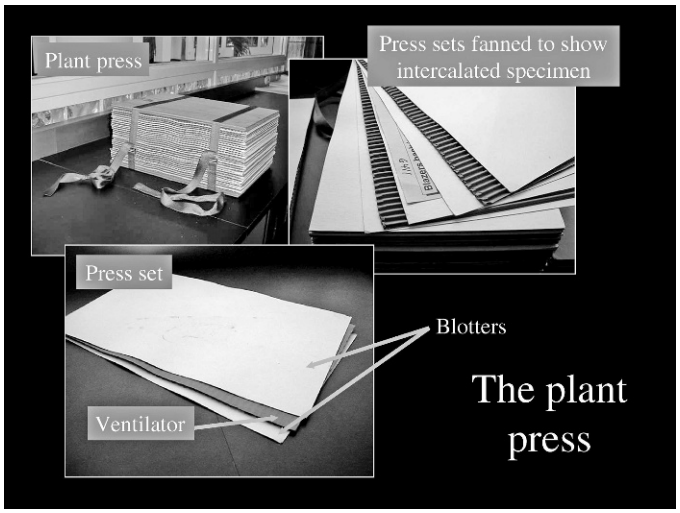

Figure 5. Each press set consists of a ventilator placed between two blotters, and each voucher specimen, enfolded in a newspaper page, is intercalated between two press sets.

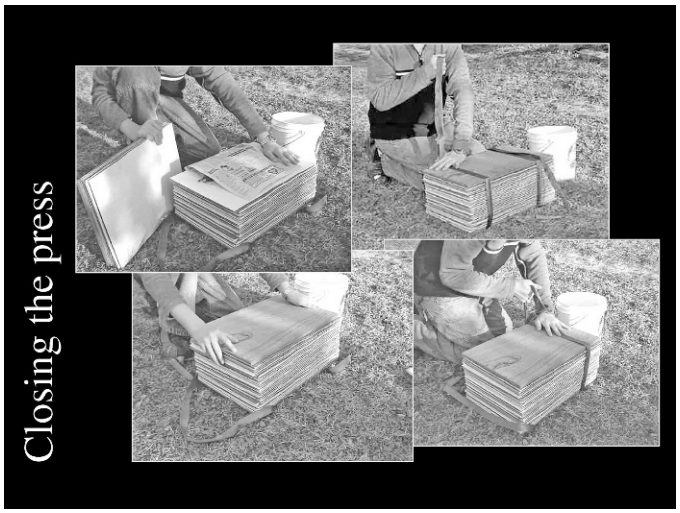

Figure 7. Steps in closing a press for drying.

newspaper fold. If it is impractical to preserve the entire plant, then cut it in pieces and include representative portions: the plant base, a portion of the midstem with attached leaves, and the upper stem with leaves and flowers or fruits. If the entire plant is not preserved, then its height should be estimated and recorded in the field notebook.

Trees, Shrubs, and Vines. Only representative portions of trees, shrubs, and vines are preserved. Be sure to include enough of the stem to show the pattern of leaf arrangement. Also, position the leaves to show both upper and lower surfaces, and include flowers or fruits. Break and fold the stems, and estimate the plant height and record it in the field notebook.

Large fleshy fruits, stems, or subterranean organs (e.g., taproots, corms, tubers, bulbs) can be especially difficult to dry, and they are normally sliced into two or more sections before placement into the newspaper fold and pressed for drying. Normally, large dry fruits and seed cones are not pressed. Instead, they are tagged separately with the same collection number as the pressed voucher specimen.

Figure 6. Steps in preparing and pressing specimens.

Well-preserved voucher specimens with intact flowers or fruits are essential for positive identification, especially of poorly known species, newly introduced nonindigenous species, or other species not represented in the herbarium. Voucher specimens should include the anatomical structures necessary to identify each particular group. Mature fruits are essential for positive identification of grasses, sedges, rushes, and similar kinds of plants, and characteristics of the plant base are also critically important in identifying such plants. Therefore, care should be taken to include representative portions of rhizomes or other subterranean structures when the specimen is removed from the ground.

Jones and Luchsinger (1986) discuss general plant collection techniques, and Hicks and Hicks (1978) provide a thorough review on practices of herbarium curation and plant collection. The wet, humid environment of lowland tropical areas presents special challenges in drying voucher specimens and preserving them against pests, especially in remote locations. Under such conditions, numbered specimens enfolded in newspaper are doused with alcohol, bundled together, and placed in large, heavy-duty plastic bags to prevent decomposition before access can be found to a dryer. Blotters are changed daily to remove moisture from specimens during drying. Additionally, specialized collection methods are employed for certain kinds of plants, e.g., aquatics (Haynes 1984), succulents (Baker et al. 1985), aroids (Croat 1985), and palms (Dransfield 1986).

Special Considerations for Invasive Weeds. During and after collection, every precaution should be taken to prevent dispersal of seeds or other reproductive parts of plants, especially of noxious weeds. This would normally include cleaning of footwear, trowels, mattocks, buckets, or other collecting gear in the field; proper disposal of plastic bags used to hold specimens; and proper housekeeping indoors in areas where specimens are dried and handled. A stiff brush is useful

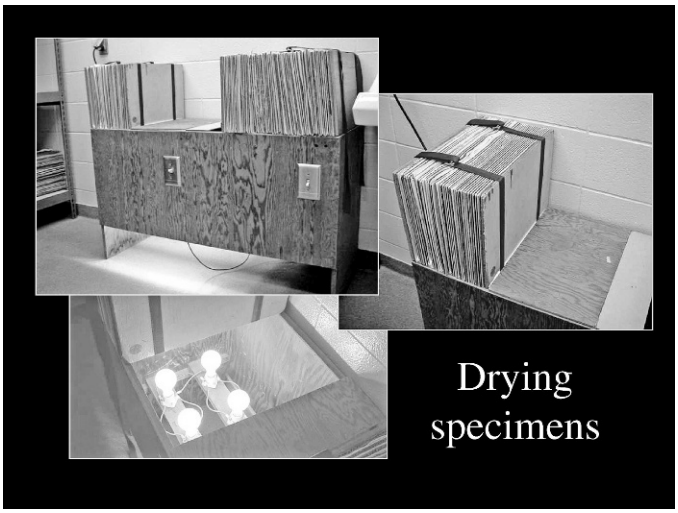

Figure 8. Drying voucher specimens with a simple plant dryer.

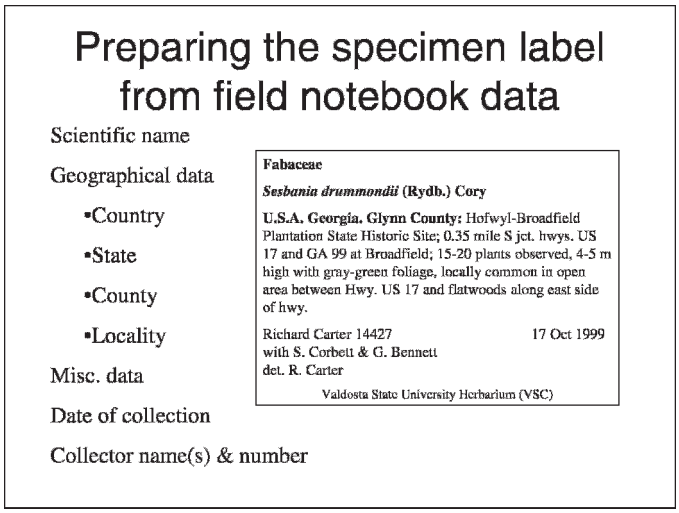

Figure 9. A sample label showing organization of the various data fields.

in cleaning footwear, clothing, and collecting equipment in the field, and equipment can be heat treated with an autoclave to prevent dispersal of seeds or other propagules. Plastic sleeves are useful to prevent dispersal of seeds and to protect the collector from stinging hairs or toxic compounds.

In the United States, federal laws provide for control and management of importation, transportation, and commerce of noxious weeds (Tasker 2007). Additionally, individual states have laws preventing transportation, possession, or sale of harmful weeds. All federal, state, and local regulations should be followed in preparing, handling, transporting, and shipping of jurisdictional weeds and their propagules.

Refrigerating Specimens. Refrigeration (4 C) is a convenient means of keeping fresh specimens for short periods (i.e., several days) when immediate processing is not possible. The fresh specimen should be kept in a closed plastic bag during refrigeration, and precautions should be taken to prevent freezing. Also, it may be beneficial to place a dry paper towel in the bag during refrigeration to absorb excess moisture.

Drying Specimens. Once the press is assembled with a header board and ventilator on each end (Figure 4), the straps are positioned and tightened. The plant press is then placed on a dryer so that warm air rises up through the ventilators taking moisture away from the specimens as it passes between them. The simple dryer shown in Figure 8 is essentially a plywood box open at the top and bottom. Heat, generated by 150-watt incandescent bulbs or some other source, rises by convection and passes through the presses above. For increased efficiency and safety, the dryer should be used in a well-ventilated room and precautions should be taken (e.g., installation of a hardware-cloth or screen barrier beneath the press) to prevent paper components of the press or parts of the specimen from coming into contact with heating elements. Frequently, at colleges and universities, arrangements can be made to use drying facilities at the local institutional herbarium. When a dryer is unavailable, a fan may be positioned to circulate air through the press and speed moisture removal. This can be an effective alternative under conditions of low relative humidity, but is largely ineffective in humid environments.

Identifying Specimens. A stereo-dissecting microscope is useful when identifying plant specimens, and regional floristic manuals are usually employed for routine determinations. However, newly introduced, nonindigenous plants present much greater difficulty, and their reliable identification usually requires access to a wide variety of primary literature (e.g., scientific journals and monographs), exotic floras, or the assistance of a taxonomic specialist. Because most taxonomic identification keys are based largely upon characteristics of flowers and mature fruits, it is essential that specimens of poorly known or newly introduced species possess these structures. Reference specimens already deposited in the herbarium are indispensable and greatly facilitate the determination of problematic specimens.

Preparing Labels. Data taken from the field notebook are used to prepare labels for the voucher specimens, as shown in Figure 9. Labels are permanently printed on archival-quality paper. Word processors and databases are widely available, easy to use, and can greatly expedite label preparation. With laser printing the ink is bonded to the paper and is less susceptible to fading; therefore, laser printers should be used in label production instead of inkjet printers. As indicated previously, many herbarium curators accept well-prepared specimens without labels, so long as they are accompanied by adequate data. A form for recording collection data to submit with a voucher specimen in the absence of a finished label is shown in Figure 3.

Mounting Specimens. This discussion is not intended to provide complete instructions on mounting herbarium specimens. Instead, its aim is only to provide some general background about how the dried voucher specimen is processed into a finished herbarium specimen. Mounting of the plant on a sheet of stiff herbarium paper (Figure 10) enables convenient storage while providing a certain degree of protection to the

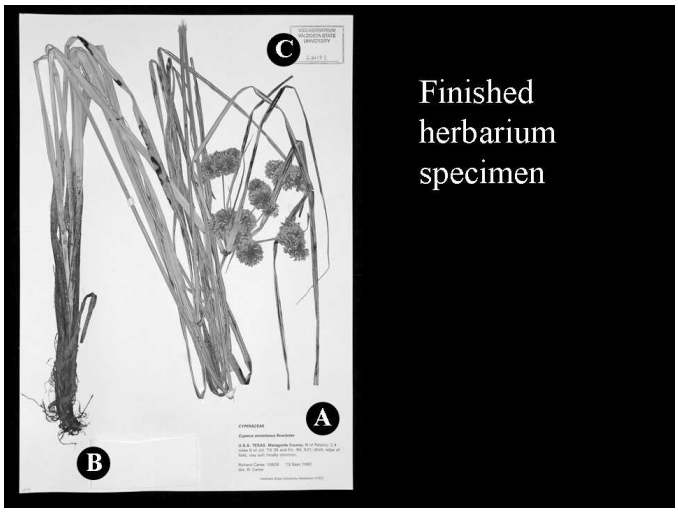

Figure 10. A finished herbarium specimen showing (A) label, (B) fragment packet, and (C) herbarium stamp with accession number.

specimen during handling. To ensure longevity, only archival-quality materials (i.e., buffered, neutral pH) are used in preparing herbarium specimens. The dried voucher specimen is mounted on a sheet [29.2 by 41.9 cm (11.5 by 16.5 inches)] of archival-quality herbarium paper with a specimen label printed on archival-quality paper. Specimen fragments and loose seeds are normally preserved in archival-quality paper packets, and glue or linen tape is used to affix the specimen, label, and fragment packet to the herbarium sheet. Archival-quality herbarium materials are available from most herbarium, museum, and library supply companies.

A variety of mounting methods are used to affix the dried plant specimen to the herbarium sheet. In the ''spot welding'' technique, the specimen is inverted and drops of glue are placed on its lower side, then it is carefully turned back over and placed onto the herbarium sheet and weighted down with metal weights (e.g., large washers, rebar segments) until dry. In the glass-plate method, a sheet of glass or a plastic tray is coated with a thin layer of glue using a paintbrush; the specimen is placed on the layer of glue and carefully lifted out with forceps and placed on the herbarium sheet. To prevent unwanted sticking after application of the glue, a sheet of wax paper is temporarily placed on top of the specimen between it and a piece of cardboard, then pressure is applied through the cardboard until the glue dries. Specimens may also be strapped to the herbarium sheet using strips of archival-quality adhesive-linen tape or strands of glue extruded from a plastic applicator bottle. Archer's adhesive, and modifications (Croat 1978), dry to form clear plastic straps. These polystyrene polymers have been used extensively in herbaria in the past, but because their use requires exposure to volatile organic solvents, they have fallen into disfavor. Water-soluble glues are best because they are usually nontoxic and can be loosened or removed if needed. Whatever the mounting technique, care must be taken to avoid obscuring plant structures, encasing small fruits or flowers in the adhesive, or rehydrating tissues thorough excessive application of water-based adhesives. Plastic tape and staples should never be used to attach specimens to the paper.

Sorting and Filing Specimens. Once mounted, the finished voucher specimens are given serial accession numbers, sorted by taxonomic group, and filed sequentially in herbarium cases using archival-quality genus folders.

Storing, Handling, and Shipping Voucher Specimens

Storing and Handling Specimens. Dried voucher specimens, both before and after mounting, are properly stored in a dry, pest-free environment. Generally, they should be kept in tight herbarium cases at a temperature below 21 C with relative humidity below 50% (Lull and Moore 1999). Unprocessed or partially processed specimens should be isolated from the herbarium collection, and all incoming specimens should be frozen to eliminate pests before transfer into the herbarium collection. As an additional precaution, repellents or insecticides, such as naphthalene or dichlorvos (DDVP), are kept in the herbarium cases (Hall 1988), although health concerns about exposure to any such compound should always be taken into consideration. If facilities are not available for proper storage, voucher specimens should be sent to an herbarium as soon as possible. Metsger and Byers (1999) provide additional recommendations for proper storage of herbarium specimens.

Freezing Specimens to Control Pests. Freezing is a safe and effective means of controlling insect pests in herbarium specimens. Rapid freezing is essential to prevent acclimation of pests (Hall 1988). To ensure rapid freezing, specimens are frozen in packets no more than 15 cm (about 6 inches) thick, and to reduce condensation problems, the specimen packet is placed in a plastic bag before freezing. In a conventional domestic freezer, the specimens should be held at a temperature of −18 C or lower for at least 48 h. For control of resistant dermestids, refreezing is recommended after rapidly bringing the packet to 15–20 C. If available, an ultracold (−40 to −80 C) freezer is most effective. Herbarium pest-control methods are reviewed by Hall (1988) and Strang (1999).

Shipping Voucher Specimens. Unmounted, dried voucher specimens are easily mailed. Even international shipment generally does not require a permit, although some types of plants may be restricted under certain conditions. The dried specimens in newspaper folds are sandwiched between reinforcing pasteboards and secured with tape before posting. Additional pasteboards should be used as necessary for reinforcement. Depending on the number of specimens being sent, suitably sized cardboard cartons are useful for shipping.

If drying facilities are not available, it is possible to ship a ''fresh'' specimen. The specimen is first placed within a folded newspaper section (e.g., section A, sports section, arts section) and then flattened by placing books or other heavy objects on the newspaper section for several days. The specimen still in the newspaper section is then sandwiched between reinforcing pasteboards, secured with tape, and mailed. This method should be used only as a last resort, when a plant dryer is not available, and the recipient should always be given prior notice before shipment. Following are

some additional precautions that should be observed in preparing voucher specimens.

- Do not tape or staple specimens to paper.
- Do not mail fresh specimens in zip-lock or other plastic bags.
- Do not leave specimens in zip-lock or other plastic bags at room temperature for prolonged periods.

Long-Term Storage of Specimens. Properly prepared voucher specimens can be kept indefinitely in the herbarium if stored under ideal conditions. The oldest European herbaria date to the 16th and 17th centuries (von Reis Altshul 1977, Holmgren et al. 1990). Although valid use of herbarium specimens by researchers is certainly encouraged, special care must be taken in the handling of historically significant and unique specimens. Type specimens, which are especially valuable in taxonomic research (McNeill et al. 2006), or very old or rare specimens are often photographed to minimize unnecessary handling, thereby reducing the risk of damage.

Conclusions

One of the most basic attributes of science is that it be repeatable. The absence of a voucher specimen indicating exactly which species is the subject of a research project presents a dilemma of the most fundamental sort should the research ever be questioned or new information suggest the need for reappraisal. The voucher specimen, permanently preserved in an herbarium, can be critically examined and reexamined, and its identity can be verified, refuted, or disputed by other researchers. Even if shown to be misidentified, the voucher provides tangible supporting evidence for the research and allows for correction by future workers. Thus, voucher specimens and herbarium collections are essential components of any well-designed research project. Additionally, such specimens provide a broader sampling of biological data that may be important in completely unrelated studies.

Much of what we know about the distributions of plant species is based upon label data on voucher specimens in herbarium collections, and there is enormous potential for using herbarium specimens and associated data to elucidate much about distributions, patterns of dispersal, and origins and relationships of weeds. We encourage weed scientists to work more closely with herbarium botanists, to support herbarium collections, and to document their research by depositing properly prepared voucher specimens in publicly accessible herbaria.

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