Mapping the Microbial Universe: The Importance of Living Collections for Fungal Systematics

by E M. Dugan and K. K. Nakasone

The destruction of natural habitats has motivated scientists and environmentalists to collect, measure, and–one hopes–ultimately preserve as much of this planet's diverse biota as possible. Simultaneously, growing human populations have impelled research into how components of this biota can be manipulated to avert a crisis in human food, shelter, and health. Increasingly, collections of germplasm furnish the material basis for applied microbiology (Jong 1997). In addition, living collections also strongly contribute to the basic sciences, including systematics.

Botanical herbaria, fungal herbaria, insect, and animal collections have traditionally stored nonviable, preserved material; this material can be studied at the collection or distributed on loan. While such material has traditionally formed the basis of taxonomic research, these important specimens can and do become depleted, damaged, destroyed or lost.

Culture collections, which can propagate and distribute living material, can distribute without depleting the collections. This is especially true for microbial collections, since many microorganisms readily reproduce in vitro and can be stored for decades. For long-term preservation, living strains are best stored as freeze-dried cultures or in liquid nitrogen vapor (see photos). Culture collections furnish resources for fungal systematics, which in turn, further the progress of applied sciences.

Systematics and the Magnitude of Fungal Diversity

Of the many definitions of systematics we present the following two:

(I) *The classification of living organisms into hierarchical series of groups emphasizing their phylogenetic relationships; often used as equivalent to taxonomy* (Lincoln et al. 1982) and

(2) *The study of the relationships and classification of organisms and the processes by which [organisms have] evolved and by which [organisms are] maintained* (Hawksworth et al. 1995).

Culture collections can be distributed without depleting the collections.

While the first is more narrow and conveys the sense in which the term is most frequently used, the second better conveys the importance of systematics in terms of function: a good classification provides explicit information on phylogeny, and implicit information on evolutionary biology, including physiology and ecology.

What are the dimensions of fungal biodiversity? A commonly cited figure is 1.5 million species, an estimate based on the ratio of fungi to higher plants €or those few areas where species are well known and extrapolated to obtain global estimates for fungi. Even if estimates are confined to more conservative totals for described taxa, the numbers are still daunting: a minimum of approximately 72,000 species, of which about 11,500 were in living collections in 1990.

Mycologists hate long relied on a specialized literature for creating and listing names (Latin binomials and their authors). In addition to individual articles on fungal taxonomy and systematics, there are also more condensed or specialized sources that can be informally categorized (see Box, p. 37).

Conservation of specimens and cultures is accomplished by preservation of dried or fixed material in herbaria or by preserving living inaterial in culture collections. (See Holmgren et a1 (1990)). Herbaria arc extremely important because they are the repositories of type material. Botanical types are dried specimens, or, (recently, for fungi) a limited number of lyophilized specimens.

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Types are the specimens upon which names are founded under the rules of the Botanical Code. But since types can become lost or depleted, and because few physiological tests can be performed with types, many mycologists prefer to work with ex-types, i.e., living cultures derived from types and available from major culture collections. "Authentic" cultures (identified as a given species by the person who first named that species) and "representative" cultures are also available from culture collections.

Housing the World's Culture Collections

The largest and most widely known living collections of fungi and yeasts are: American Type Culture Collection (ATCC), USA; Centraalbureau voor Schimmelcultures (CBS), Netherlands; International Mycological Institute (IMI), UK; arid Mycothèque de L'Université Catholique de Louvain (MUCL), Belgium Each publishes a catalogue, and cultures are available for a fee. Electronic catalogues can be acccssed via ATCC's Internet site: <http://www.atcc.org>. Other collections with catalogued fungal strains include: the Canadian Culture Collection (CCFC), Japan Collection of Microorganisms (JCM), Japan; the Institute for Fermentation, Japan; Deutsche Sammlung von Mikroorganismen (DSM), Germany; National Collection of Yeast Cultures, UK; and the Fungal Genetics Stock Center, USA. These smaller collections also charge a fee for cultures.

The USDA/ARS Culture Collection at the National Center for Agricultural Utilization Research (NRRL) is currently constructing a catalogue. ARS also maintains smaller collections, such as that for entornopathogenic fungi in Ithaca, New York. The Center for Forest Mycology Research maintains a collection of wood-inhabiting fungi at the Forest Products Laboratory, United States Forest Service, Madison, Wisconsin (see DIVERSITY, vol. 12, no.4, 1996, p.4).

Many collections have formed their own organizations. Prominent examples include the United States Federation for Culture Collections, the World Federation for Culture Collections, the Association of Systematics Collections (ASC), and the Microbiological Resource Centers within UNESCO. There are additional organizations for culture collections organized by global region (Europe) or country (e.g., China and UK).

For-profit supply houses sometimes maintain culture collections as well. These vary in size and quality. For-profit businesses differ from non-profit culture collections in that the former need assume no archival responsibilities and need not retain material that does not distribute in high numbers. However, they may constitute reliable sources of material for the clinical, materials testing, and other markets.

Liquid nitrogen tanks at ATCC. Storage in liquid nitrogetn vapor at a maximum of -130°C is state-of-the art technology for the majority of micoorganisms. (Photo by Elmer Davis)

Collections Provide Key Support for Systematics and Biodiversity

Culture collections and herbaria have been active participants in taxonomic inventories, surveys and initiatives in promotion of biodiversity. Conspicuous examples of such efforts include Systematics Agenda 2000, a global initiative undertaken by scientific societies in cooperation with the ASC and supported by the National Science Foundation. Collections personnel were integral to the biodiversity assessment sponsored in part by the United Nations Environmental Programme (UNEP), Global Biodiversity (see DIVERSlTY, vol.10, no.2, p.31-32).

Personnel from IMI, ASC, DSM, the New York Botanical Garden (NYBG), and other collections also participated in a subsequent assessment by UNEP of the scientific and social aspects of biodiversity (Heywood and Watson 1995). IMI is very active in promoting BioNet-International, a project dedicated to the building and sharing of electronic taxonomic databases and to the transfer of taxonomic skills. Personnel from the National Fungus Collection (USA) are also participants. (see DIVERSITY, vo1.13, no.4, p. 35). Similar cooperative efforts are underway to provide necessary tools for conducting biotic surveys; ATCC, CBS, IMI, ARS, and other organizations are collaborating on a methods book for measuring and monitoring fungal biodiversity which will be published by the Smithsonian Institution as part of a series of such manuals.

Collections are also primary centers for the production of taxonomic articles and monographs, including those pertinent to mycology. IMI, which tends to concentrate on fungi of agricultural importance, produces Mycological Papers and other monographs, as well as the IMI Descriptions of Plant Pathogenic Fungi. CBS produces Studies in Mycology and other monographs and concentrates on medical mycology and fungi, and yeasts of importance to food and beverages. NRRL has cooperated in production of the most recent monograph of the yeasts. ATCC has produced taxonomic articles and monographs on several genera of fungi, as have mycologists at the National Fungus Collection and the NYBG. Taxonomists associated with universities, museums, government agencies, or private collections also contribute monographic studies.

Collections materials have been used for basic and applied research in thousands of instances. Review of recent literature indicates that ATCC fungi/yeasts are utilized approximately 100 times annually for publication on taxonomy and identification.

Other large collections have similar impact.

Importance of Collections-Based Systematics for Applied Research

The importance of culture collections as sources of germplasm for applied mycology is clear. However, applied mycology often presupposes adequate identification and classification, and culture collections thereby make further contributions to applied mycology via systematics research.

Screening of well-identified collection cultures is an established strategy for researchers using microorganisms in biotechnology. For example, some white-rot fungi are able to selectively remove lignin from wood. Initial screenings for this ability were conducted with 30 species of white-rot fungi from the CFMR culture collection. These screenings enabled experimental treatment of wood chips with white-rot fungi, producing wood pulp with improved paper strength and doing so at reduced energy requirements relative to mechanical pulping processes.

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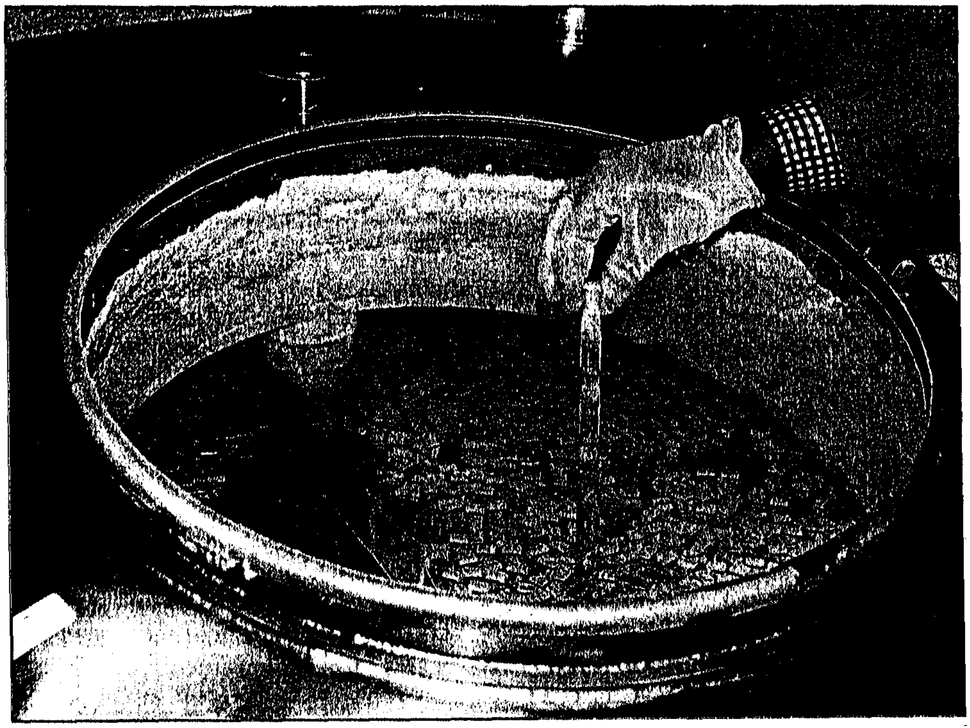
Collections also provide authenticated (properly identified) material for quality control such as clinical and materials testing standards. Among the agencies or organizations for whom the service is provided are the U.S. Pharmacopcia *(Aspergillus niger* ATCC 16404, antimicrobial preservative testing); the International Organization for Standardization (ISO) (eight strains of ATCC fungi); and the Military Standard Environmental Test Methods and Engineering Guidelines (MIL-STD-810E, five strains of filamentous fungi from ATCC). Provision of such well-characterized materials of known species enables accurate comparisons of experimental results and aids further hypothesis formation and testing.

The identification of the organism can be critical for the legal standing of the patent.

Because of the importance of having living material to function as experimental standards, many journal editors now ask or require authors to deposit with culture collections those strains which are experimental subjects and to include the collection numbers in the manuscript. These same strains frequently become the object of subsequent experimentation by other investigators, furnishing a “common denominator” for research so that experimental results can be tied to established names with a high degree of confidence. Even if a strain is re-identified as properly belonging to a different taxon, or if the name of the taxon is modified, results are still of scientific value.



The interior of a liquid nitrogen tank. Cultures are stored on labled canes to facilitate easy location and retrieval.

Photo by Elmer Davis

CATALOGUING NAMES

(1) Catalogs of names Index of Fungi (1920-present), Saccardo's Sylloge Fungorum (1882-1925) Fries' Systema Mycologicum (1821-1832), Persoon's Synopis Methdica Fungorum (1801), and others Hawksworth's 1995 Directory of the Fungi has a section on Catalogues of Names under Literature.

(2) Names for fungi from specific substrates: Host-fungus indices, compendia of soil, seed, or clinical fungi, etc.

(3) Rules pertaining to names: Greuter's International Code of Bontanical Nomenclature (1994), is the most current of a series of codes. Rules are summarized in Dictionary of the Fungi.

(4) Collection catalogs and databases. Several collections have paper or electronic catalogs with entries accompanied by citations. Catalogs of strains from the International mycological Institue (IMI), the American Type Culture Collections (ATCC), and Centraal bureau voor Schimmelcultures (CBS) can be accessed via ATCC's home page (http://www.atcc.org), as can useful databases of the Agricultrual Reseaerch Service (ARS), including the National Fungus Collection. Follow the menu to Mycological Resources on the Internet for dozens of citations pertinent to the location of names, plus other mycolocical literature.

(5) Comprehensive lists of culture collections are found in Sugawara et al. (1993); Hall and Minter (1994); and from mycological organizations (see "For Futher Reading").

Modern classification and nomenclature can be critical for hypothesis formulation, Knowledge of the proper taxonomic position of a fungus is a valid starting point for formulation of hypotheses regarding its physiology, pathogenicity, environmental competitiveness and genetic composition. Screening for metabolites of pharmaceutical interest is often performed on strains known to belong to taxa which have produced valuable metabolites, e.g., *Penicillium, Acremonium, Tolypocladium,* etc.

Systematics research has utilized culture collection resources and produced results of importance for applied science. Collections of other organisms, such as insects, higher plants, bacteria, etc. make similar, valuable contributions of practical importance (see box , p. 38).

Collections as Patent Depositories

Patent law requires that the description of an invention be adequate for the reproduction of the invention by others for purposes of experimentation (not commercial exploitation) during the life of the patent.

Systematics Research and Culture Collection Resources

1. **Industiral Mycology:** The identification of Hypocrea jecorina as the telemorphic (sexual) state of Trichoderma reesei, a hyphomycete known to produce cellulolytic enzymes of industiral importance, although the fungus has only been isolated once from nature. Hypocrea jecornia is more commonly isolated and with the knowledge at the two fungi were intimately related, the enzymatic capabilities of H. jecorina were examined from collections at ATCC, CBS, IMI, and others. Some strains produced cellulase in quantities comparable to T. reesei and their discovery expands to possibilities for microbial cellulase production

2. **Medical Mycology:** For years several plant pathogenic species and clinical (human pathogenic) species were accommodated in the genus Cladosprium. The latter were among the agents of chromomycosis, one of the several names for a complex of difiguring and occasionally fatal disease. For decades the taxonomy of these agents was "one of the most confusing in the field of medical mycology," according to prominent medical mycologist J.W. Rippon. Recent research conducted with culture collection materials has deomonstrated that the plant pathogens and the clinical strains belong to separate and distinct taxonomic groups. The Cladosporium like fungi predominantly responsible for disease in humans and animals are mostly anamorphic (asexual) forms of Capronia or other genera in the Herpotrichiellaceae, (order Chaetothyriales). The true Cladosporium species are largely saprophytes or plant pathogens with teleomorphs in the genus Mycosphaerella a genus in the Dothidaceae (order Dothideales). The Cladosporium-like taxa are now classified in Cladophialiphora, Xylohypha or even Exophiala.

3. **Agriculture:** Strains of fungi which produce no conidia or other nonsexual spores, and which produce the telemorphic state only occasionally, have always presented problems in identification because hyphae alone present few characters useful for identification. Rhizoctonia species are important plant pathogens (and/or occasional symbiots) of higher plants, many of agronomic importance. Traditonally classified as "mycelia sterilia" or "Agonomycetales" strains belonging to these species occasionally produce inconspicuous teleomorphs in Ceratobasdium, Thanatephorus, and a few other genera of basidiomycetes.

Although molecular methods may provide satisfacory practical identification in the future, routine identification is achieved by the presence or absence of anastomosis with known tester strains. Anastomosis occurs when hyphae merge and form ontinuity of cytoplasm. Tester strains belong to designated anastomosis groups (AG-A, AG-B, AGBb, etc.). The American Phytopathological Society published a guide to the identification of Rhizoctonia species. Dozens of Rhizoctonia strains deposited with ATCC and bearing designations for anastomosis group, are described in the publication, and are available as reference strains for use in identification.

The depositor must give a taxonomic description of the organism in the body of the patent. Patents involving the use of living materials which might not be readily available require the deposit of such materials with designated depositories. Both the ATCC and NRRL are such depositories in the U.S. Re-examination of patent materials deposited at NRRL and ATCC has enabled correction of mistakes in the original identification. In one such case, ATCC 34506 was obtained from NRRL and is cited in U.S. Patent 3,230,153 where it was cited in the patent as *Aspergillus restrictus,* but it is really *Aspergillus fumigatus.* Since many strains of the latter are capable of acting as pathogens, the distinction is significant. Another patent strain, ATCC 20611, is the subject of repeated citations in the scientific literature. Deposited as *Aspergillus niger,* 20611 has recently been re-examined by collection staff and identified as *Aspergillus japonicus.*

Novelty is essential for the granting of a patent. Patents involving the use of an organism may be novel by virtue of the use for which the organism is employed, or may be novel because the organism itself has not been previously employed for a given use. Hence the identification of the organism can be critical for the legal standing of the patent (See DIVERSITY vol. 11, no.4, pp.14-15).

Collections Reflect the Spirit of the Earth Summit

Some collections not only accept deposits but also actively sponsor collecting expeditions or employ collectors directly. Subsequent to the Convention on Biological Diversity (CBD), which came into force in 1993, collections have progressively acted in conformity with the spirit of the conference by respecting biological habitats and the rights of indigenous peoples. For example:

- **Royal Botanical Gardens at Kew** (UK) returns 50% of commercial benefits to local collaborators and deposits herbarium vouchers for all collections in local herbaria;
- **New York Botanical Garden** signed an agreement with the Awa Federation, an organization of Native Americans in Ecuador, which carerully defines the conditions under which collecting may be conducted and the custody of collected materials;
- **Association of Systematics Collections** (ASC) has produced a code of conduct for field collecting; and
- **USDA/ARS and ASC** recently sponsored a symposium on germplasm and intellectual property rights.

Collections, Systematics, and Survival

Access to collection materials, living or preserved, presumes that adequate funding sustains the organisms, facilities, and personnel of the collection. Although some microbial collections have more adequate funding than others, most collections in North America have difficulty meeting the demands of their user communities. All but industrial collections are, to some degree, dependent on uncertain government support.

Government funding for collections has suffered declines in absolute terms (e.g., IMI) or is failing to grow in pace with die archival burdens for which collections are responsible (e.g., ATCC). Some collections (CBS, JCM, DSM) are more strongly supported by their governments. However, even for supported collections, such as NRRL or CFMR, funding is very tight relative to mandated responsibilities. Some collections are in search of new sources of support, including new services, products, and partners. IMI is attempting to support itself by increasing its taxonomic and agriculture-related information services and publications. ATCC has established linkage with George Mason University (Fairfax, Virginia) with the objective of becoming a focus of externally funded research in microbial taxonomy and evolutionary biology.

Most collections in North America have difficulty meeting the demands of their user communities.

Collections are also trying to educate the public as to the scientific value of collection materials. The Smithsonian, CAB International, ASC, the American Museum of Natural History, ARS, and other organizations are struggling to inform decision-makers and the public at large about the value of biodiversity and conserving the environment.

Although the larger collections are hard pressed to find adequate funding, smaller collections at universities and other institutions are often even more adversely affected. University collections and other smaller collections are also imperiled when their curators retire or change jobs. In the past, ATCC has absorbed some of the "orphaned" strains into its general collection or taken larger numbers of strains as a special collection. For example, a large number of *Fusarium* strains were collected in the former Soviet Union at the end of World War II. These strains, representative of the fungi causing a large outbreak of mycotoxicosis alimentary toxic aleukia with sickness and death of humans, were widespread throughout affected regions.

The potential impact of losing endangered collections is of great concern to the entire scientific community.

This important coellection was essentially orphaned in 1995 and shipped to ATCC for preservation. The 800+ strains were preserved by addition of 10% glycerol (cryoprotectant) to the original test tubes and placed in a -80C freezer. However, ATCC cannot afford to be the foundling home for all endangered collections and with the current climate may be forced to be increasingly selective in future "rescue" operations.

The potential impact of losing endangered collections is of great concern to the entire scientific community. While study of this threat has begun, further analysis and debate will be required if further erosion of our living collections is to be stemmed (See Colwell 1992).

For further informtation or a complete list of references, contact: Dr. Frank M. Dugan, Collection Scientist for Mycology and Botany, American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209. Tel: +1-703-365-2700. Fax: +1-703-2750. E-mail: <fdugan@ atcc.org>. Internet: <http://www.atcc.org>.

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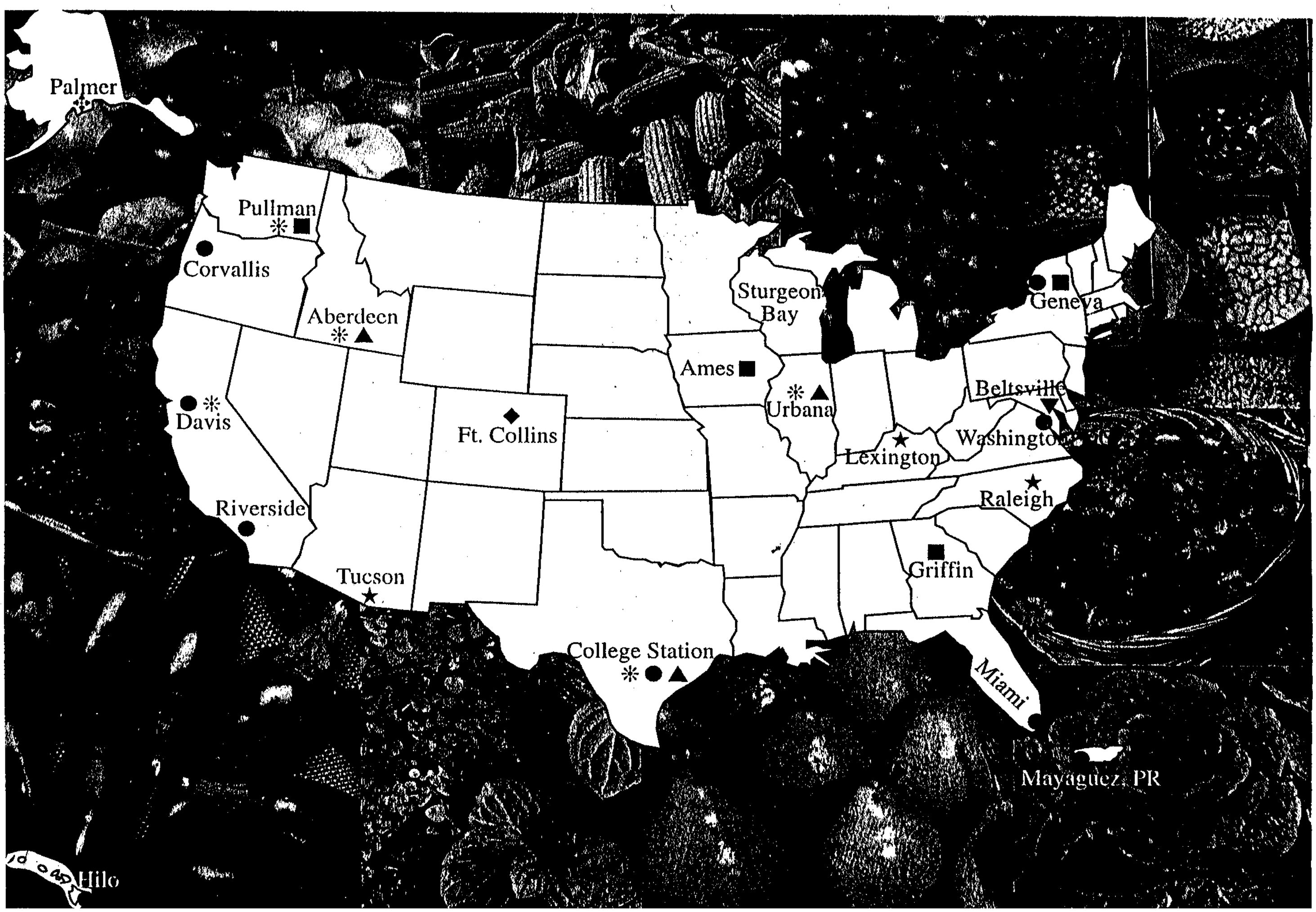

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