Review article

METHODS OF FIELD STUDIES IN BOTANY

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Different methods of collecting, preparation and preservation of plant collections are very important for botanical field studies. Processed and labeled plants are stored in herbaria in which they are conserved and then used for taxonomic, floristic and phytogeographical studies, and then as the evidence and display material. Besides, various analyses of plant communities and their habitats are used in botanical studies of the flora of our surroundings. Field and literature data, as well as, personal communications and oral presentations are very important sources of scientific information used in flora mapping of a certain region.

Key words: botany, field study

INTRODUCTION

Botanical studies of a local region not only are of a didactic value, but they are also the source of information for pupils on diversity of nature and a necessity for protection of the region they live in. Going outdoors, visiting nearby and farther surroundings (on their way from home to school, on playgrounds, sightseeing sites, etc.), pupils, together with their teachers, can observe the principal properties, record and collect plants or analyze and describe plant communities and vegetation of the given region.

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The collection of all plants of a particular region or a period comprises the **flora**, while overall acquaintance with it is encompassed by various floristic studies. On the other hand, the **vegetation** is the collective plant cover over of a certain region. The studies on vegetation are very complicated and encompass investigations of interrelations of numerous plant species that make one plant community or phytocoenosis (phytocenolic studies), as well as, analyses of relations, arrangements and properties of all plant communities related to the given region (vegetational studies).

In order to gain proper knowledge on the flora and the vegetation of a certain region it is necessary to carry different both, field and laboratory, studies. Field studies encompass, first of all, listing/registering and collecting of the plant material, as well as, identification and description of plant communities that are made of that plant material. The collected material is prepared in laboratories for preservation and a further utilization for stereoscopic and microscopic analyses.

The field investigations can also include different microclimatic measurements (solar radiation, solar light, air and soil temperatures, air humidity, evaporation, etc.) that provide data on a habitat conditions under which living creatures live.

I. COLLECTION, PREPARATION AND PRESERVATION OF FLORISTIC COLLECTIONS

The following parameters are determined when floristic studies on a certain region are carried out: **1.** a presence of particular plant species; **2.** an affiliation with certain taxonomic categories; **3.** a distribution over a given region; **4.** a role and a place of individual plant species within a certain plant community; **5.** morpho-anatomical plant traits; **6.** economical significance; **7.** endangerment and z necessity for protection of both, plant species and their habitat.

I.1. Basic principles of working on collecting, preparing and preserving of collections

1. collecting is done in several different habitats; **2.** a sufficient number of plant species that are observe should be collected (except in a case when a species is scarce or endangered in its habitat); **3.** representative, mature, intact and undamaged specimens are collected; **4.** the specimens are determined (if possible) on the spot (of collecting); **5.** collected specimens are fixed (for a wet collection) or their preservation starts with drying (for a herbarium collection); **6.** basic data on the collection spot (habitat) are recorded in the filed labels attached to the collected material.

I.2. Field and laboratory tools and material for collecting, preparing and preserving of herbarial plants

Field tools and material: - **1.** paper for drying and mounting of plants; **2.** cardboard pieces; **3.** herbarium covers; **4.** herbarium plant press-net; **5.** a wooden

handle iron spade; **6.** a spatula, a large and strong knife; **7.** a chisel and a hammer (with one sharp side); **8.** an iron hook or an iron anchor to pull out water plants; **9.** a garden pocket knife; **10.** a garden saw; **11.** garden clippers; **12.** a pocket magnifier; **13.** tweezers; **14.** topographic maps (sections); **15.** a compass and an altimeter; **16.** a notebook - a field log; **17.** labels; **18.** PVC bags; **19.** gallipots and large-diameter test-tubes with plugs.

Laboratory tools and material: - **1.** herbarium paper for storing plants in the herbarium; **2.** herbarium labels; **3.** herbarium (specimen storage) boxes; **4.** envelopes; **5.** glass or PVC tinning jars for a wet collection; **6.** flasks for chemicals use for disinfection and desinsection of the herbarium; **7.** magnifier (hand and a table one); **8.** a stereoscopic microscope; **9.** tools for plant dissection and preparation; **10.** herbarium cabinets (compactor units) and shelves; **11.** literature for the plant identification.

I.3. Planning of expeditions and itineraries

The following should be foreseen by the expedition plan: **1.** the aim and tasks to be implemented; **2.** the duration of the expedition, i.e. the departure and return dates; **3.** the direction of trekking, with places of rest; **4.** the number of participants (per groups); **5.** necessary field equipment; **6.** personal equipment of excursion participants.

Prior to the beginning of detailed studies on the flora, habitats and communities of a particular region it is necessary to gain information of general characteristics of the region - relief, geological substratum, soil, vegetation conditions, adjacency of population settlements and diverse anthropological effects, etc. Furthermore, it is necessary to check available literature data, as well as, geographical maps related to the given region. All stated data, as well as, all field observances are registered into the field log.

I.4. Collection of terrestrial, marsh and water plants

The collection of terrestrial plants. Herbaceous plants are collected as the entire individuals whereby they must not be torn (pulled into pieces), but have to be carefully dug up, if possible with intact underground parts. Branches with leaves, flowers and fruits are cut in trees and shrubs. In order to perform a precise identification it is important to collect several specimens of plants in all developmental stages (prior and during flowering, as well as, during fructifying).

The collection of marsh and water plants. The large water plants with well developed underwater parts, such as water lily is, are collected with 1-2 leaves, flowers, fruits and a portion of rhizome with roots. Smaller and more delicate plants with thin leaves are pulled out of water in a way that plasticized paper (or foil) is placed under the plant and all its parts are arranged on the paper in their natural position. Then, the plant together with the paper is pulled out of water and placed between the herbarium cover or on the plant press-net.

A field label with all necessary data on the habitat has to be attached to each collected plant, because the plant with no label has no scientific value.

I.5. Procedure of plant drying

The following rules are the principle ones in the process of plant drying: **1.** plants have to be dried fast in order to be preserved from deterioration; **2.** it is necessary to use herbarium paper as mach as possible and to replace it with dry paper as often as possible; **3.** dry plants should be kept away from wet ones; **4.** plants on the plant press-net should be often surveyed so that their drying is properly controlled; **5.** labels should not be mixed up when paper sheets are replaced; **6.** plants that have been collected during conditions of dew or rain, should previously be dried by cotton balls or a piece of blotting paper and then placed on the plant press-net.

I.6. Processing, storage and preservation

A **herbarium** is a collection of selected, documented and dried plants. The **herbarium sheet** is a paper sheet of a pale color, **63 x 47 cm** in size, folded by the width; hence the envelope of **47 x 31.5 cm** is formed presenting a **herbarium half-sheet**. There is a firmer paper sheet within the herbarium half-sheet to which a plant is mounted. A **herbarium specimen (exemplar)** comprises several individual plants, collected in a certain location well presenting a given plant species. The herbarium specimen encompasses at least one herbarium sheet; if a plant is of a larger size, the specimen can include several sheets that will encompass certain plant parts (above ground and under ground parts). Herbarium specimens are placed into envelopes and then in the herbarium (specimen storage) boxes and cabinets.

Labeling. The dried and processed herbarium specimen is always accompanied by a herbarium label that differs from a field one by a greater number of pieces of information related to a given plant. The size of a typical herbarium label (usually of a white photocopier paper) approximately amounts to **15 x 10 cm.**

The definite herbarium label contains the following: **1.** Herbarium - the official name, abbreviation or herbarium designation; **2.** Scientific plant name (Latin name and the name given by the author); **3.** Location (broader and narrower determination); **4.** Habitat, i.e. plant community (oak forest, rocks, flooded meadow, etc.); **5.** Altitude; **6.** Medium; **7.** The date of collecting; **8.** The name of the collector (labeled with Leg. - to collect in Latin); **9.** The name of the person who identified the plant (labeled with Det. - to determine in Latin); **10.** Number under which the plant is registered a) collection number - by a collector b) inventory number - under which is registered in the herbarium catalogue.

The herbarium collection is arranged by either the phylogenetic system (by classifying specimens into taxonomic categories from the evolutionary elder towards younger groups) or by the alphabetic order. The herbarium can be arranged according to its specific purposes: herbaria of medicinal, toxic, industrial,

horticultural, indoor, spice plants, etc. At the same time, it is necessary to record all data presented in the herbarium sheet in herbarium catalogues (card index) which always accompany such collections. Data can be transferred from the catalogues to the electronic data bases to provide fast and easy finding, searching and sorting of all data relevant for plant species that are stored in a certain herbarium.

Storage and preservation of herbaria. Herbaria are stored in specimen storage boxes on shelves or in cabinets/compactor units. A common label is stuck on each box with classified material. The ambient conditions in the storage rooms - air temperature of almost always 18^{o}C and relatively low air humidity - should prevent deterioration of the plant material. Preservation of the herbarium means also protection against parasites, first of all, insects that inhabit herbaria and fed on dry plants. Airtight cabinets, isolated premises, as well as, periodical treatments with certain chemical means (TUS bands or naphthalene) are the best and the least damaging ways of protection against pests.

II BASIC PRINCIPLES AND METHODS OF PLANT IDENTIFICATION

The plant identification means recognition of a systematic group to which collected individuals belong based on their traits that are compared with defined diagnoses in "keys" for the plant identification.

The fundamental principle for the plant identification is good knowledge on morphological traits of certain plant organs. The entire plant or its certain organs (especially leaves) can be observed by a magnifier or a stereoscopic microscope in order to make a more adequate identification. Besides, knowledge on anatomical plant traits that can be observed only on microscopic preparations or mucilaginous (macerated) plant tissue is sometimes necessary for the detailed identification of the systematic or ecological affiliation of plants.

II.1. The application of keys and iconography

"Keys" are the books/manuals with introductions for the systematic identifications of plant species. Working by keys is based on the principle that one of two offered properties (a principle of dichotomy) is chosen and opts for and then forwarded to a subsequent level of a dichotomy optioning. The principle of dichotomy is applied until the final goal is achieved, i.e. until the correct Latin name of the plant is determined. Higher plants in Serbia are mostly identified by the Flora of SR Serbia edited by the Serbian Academy of Sciences and Art.

Moreover, **iconographies**, books with drawings (black and white or color) of plants of the certain region, arranged according to their systematic affiliation, can also be used. Higher plants in Serbia are mostly identified by the Iconography of Plants of Southern, Eastern and Central Regions of Europe by Javorka and Csapody edited by the Hungarian Academy of Sciences.

II.2. Making plant microscopic preparations

Preparations of certain plant organs can be temporary or permanent. The preparations of cross-sections of roots, stems/trunks, leaves, fruits or seeds are used for plant identification.

The **temporary preparations** made by a razor blade are the simplest way to obtain prompt, general information on the anatomical properties of certain plant organs. For each new section it is necessary to have a sharp razor blade and additional tools consisting of surgical needle, tweezers, watch glass and dyes - flasks with fluoroglycine, muriatic acid or a reagent after Tucakov (BLAŽENČIĆ, 1979). Manually cut out preparations (if it is dealt with a leaf it is placed in the incised part of elder parenchyma) are shortly immersed into dyes and then transferred to a mount in a drop of glycerin, and then they are protected with a covering hull and observed by the microscope. In such a way, soft, cellulose cells remaining uncolored are easily distinguished from woody, lignified and red colored cell elements (e.g. mechanical and conductive elements). Chloroplasts, i.e. tissues that contain chloroplast, are easily observed on temporary preparations because they are of an intensive green color.

Permanent preparations are made by conventional methods for light microscopy including: **1.** fixing of the material in the field (in 50% ethanol solution with the addition of several drops of formalin); **2.** transferring into adequate fixatives (according to the age of the plant material and the preparation purpose); **3.** preparation of the material for molding (in paraffin or bioplast); **4.** cutting by a hand microtome; **5.** differential dying of the preparation; **6.** observance of finished preparations on microtomes by a video camera with a computer data processor.

III. METHODS OF STUDIES ON PLANT COMMUNITIES

III.1. Methods of test areas (minimum range of distribution)

The identification of a plant community type is achieved by the application of the method of phytocenolic screening on so-called "test" areas. The choice of these areas is done on the basis of the total survey of vegetation of a broader region and selection of the areas in which the observed vegetation is the most excellently and the most typically developed. It means that this area is characterized by relatively consistent ecological conditions; hence the association of plant species characteristic for that vegetation is present there. Simply said, test areas are usually a central part of a certain plant community.

The selected test areas have to be sufficiently large to encompass as a greater number of plants species of the analyzed community as possible. The initial test area of only 0.25m^2 is used to register all present plant species. Then the area is increased to 0.50 m^2, 1 m^2, 2 m^2, 4 m^2, 8 m^2, 16 m^2, 32 m^2, 64 m^2 etc., as long as new plant species occur. In such a way, experience on selection a proper test area for observance of a certain plant community is gradually gained. The test areas in forest communities amount to several hundreds m^2, while they are always less than

100 m^2 in meadow communities, as forests are of a relatively uniform plant composition on great areas, while the plant content in meadow communities is modified with even minimum variations of climatic, geomorphological or pedological conditions.

Plant communities are characterized by a diverse spatial distribution. When recording (in test areas), plants are grouped according to their affiliation to a certain stratum (large trees stratum, small trees stratum, large shrubs stratum, small shrubs stratum, large herbaceous plants stratum, small herbaceous plants stratum).

Phytocenolic screenings, containing the following elements, are made on the basis of data on plants and their habitats: **1.** the number of phytocenolic screenings; **2.** location (narrower and broader surroundings); **3.** very general community properties; **4.** date of screening; **5.** size of screened area (in m^2); **6.** first and family name of a researcher who performed screening.

III.2. Data on habitat

1. Altitude (is measured by the altimeter); **2.** Exposition (determined by a compass); **3.** Slope (expressed in degrees and determined by an inclinometer); **4.** General geomorphological conditions (characteristics of relief); **5.** Geological base (beside rough field estimation, a detailed laboratory analysis of samples is also performed); **6.** Soil (general data on the type, bed depth, hue and texture, etc.); **7.** General conditions of water relationship (presence or absence of water areas, under ground waters, springs, etc.); **8.** Type and traits of adjacent plant communities (that can affect the habitat and the studied plant community in it); **9.** Presence and influences of animals; **10.** Anthropological effects (settlements, different anthropological activities).

III.3. Analytical processing of plant communities - phytocenolic screening

A floristic composition and spatial structure (set of successive strata) of the plant community, as well as, ecological conditions under which the community is developed are determined by the analytical processing. The distribution and importance of each plant species in the formation of a plant community is evaluated on the basis of their abundance, coverage and sociability after the method of Braun-Blanquet. The following parameters are used in such an analysis:

The combined evaluation of abundance and coverage by the 1-6 scale where **5** - the species covers 75-100% of the test area; **4** - the species covers 50-75% of the test area; **3** - the species covers 25-50% of the test area; **2** - the species covers 10-25% of the test area; **1** - the species covers 1-10% of the test area; **+** - the species is scarce with an insignificant coverage.

The evaluation of sociability by the 1-5 scale, where **1** - a plant grows separately, while the estimates **2** to **5** indicate individual plant species that form ever greater associations within a plant community.

III.4. Synthetic processing of plant communities - phytocenolic table

Phytocenolic tables are formed on the basis of several phytocenolic screenings (not less than 10). These tables represent a complex survey of the plant community based on data of all phytocenolic screenings made in different locations of the studied region. The definite status of certain plant species in the formation of the plant community is obtained by the comparison of the presence (the degree of participation) of certain plant species over all phytocenolic screenings. The degree of presence (participation) is determined by the following estimates: **V** - the species can be found in 80-100% of screenings; **IV** - the species can be found in 60-80% of screenings; **III** - the species can be found in 40-60% of screenings; **II** - the species can be found in 20-40% of screenings; **I** - the species can be found in 1-20% of screenings.

In such a way the following can be set aside: **1.** reference species; **2.** differential species and **3.** accompanying species.

Reference species are always related to a certain community is scientifically named after them. These species best reflect ecological conditions of the studied habitat.

Differential species occur in the observed plant community, but only in certain locations and indicate to ecological and floristic specificities of such habitats.

Accompanying species are scarcely or randomly found species in the studied plant communities.

IV ECOLOGICAL MAPPING OF THE FLORA

Ecological mapping of the flora represents recording data on the distribution of plant species of a certain region in the geographical map if possible in the scale of 1:25,000; 1:50,000 or 1:100,000 as they provide the most precise determination of their locations. The sources of data on the plant distribution do not determined the given species only geographically, but also provide information on ecological properties of its habitat.

The following data are sources for the determination of the certain plant species distribution and making maps of ranges of their distributions:

1) Field investigations that encompass systematic **visits** to the studied region in the precisely determined periods (monthly or more often) during the entire growing season including **collection, preparation, identification and labeling of the plant material.**

2) Herbarium collections that can be private ones or can belong to a certain institutions, arranged according to a phylogenetic system or alphabetical order. The herbarium specimens are obvious evidence of the existence of certain plant species in a particular region.

3) Literature data published in Floras of certain regions, floristic and phytocenolic scientific papers, field logs, etc. In some cases, (taxonomically com-

plex genera and species, extinct plants, data of amateur botanists, etc.), these data require a field audit.

4) Personal communications have to include the following information: **1.** Name of plant (scientific/Latin name and the name given by the author); **2.** Locations (broader and narrower determination); **3.** Habitat; **4.** Altitude; **5.** Geological base; **6.** Date of determination; **7.** Person who determined and identified the plant. The personal communications on plant distribution are not always reliable since there is no herbarium material for them.

All data used for mapping (from herbarium labels, published papers or oral presentations and personal communications) are cited in a form of a written comment bellow a map of a range of distribution. For instance:

Example of a herbarium / field label:

Cachrys aplina Bieb. Tara, *Orno-Ostryetum pinetosum nigrae*, 850 m, limestone, (*Tomić, Z.* June 25, 1968, Herb. No. 2564, BEO!)

Citation of a published paper:

Ranunculus acer L., Suva Planina, bottom, *Quercetum frainetto-cerris*, 400 m, limestone, (Diklić, N. 1970: 294)

Citation of a personal communication:

Anemone nemorosa L., Fruška gora, Čerević, *Fagetum montanum*, 800 m, silicate, June 14, 1997, *Stojšić, V.,* **U.S.!**

The following methods can be used to record data in the map of distribution:

1) The dot method where each established findings or locations of the given species is marked by a dot (or any other symbol) in an appropriate geographical map. Hence, a system of dots of different densities will appear on the map in dependence on the map scaling and the number of known locations. The preciseness of data recording is obtained by the use of the geographical maps with a universal transverse mercator grid (**UTM MAPS**) or by the satellite locating of the finding by the **GPS instrument** that records longitudes and latitudes of certain locations expressed in degrees, minutes and seconds.

2) The contour method by which all limits of distributions are linked by one line (all adjacent locations) hence an image of the **shape, ranges and size of distributions** of the given species are obtained.

3) The combined method by which the line and dots are used simultaneously to mark locations and to express the **shape and size of distribution**.

CONCLUSION

All stated methods of field investigations are the basis for obtaining information on the flora of the local environment. If they are used for frequent and permanent monitoring, the obtained data on the status and modifications of the vegetation of the given region will be ever more precise.

These methods are especially important when they are applied in investigations of endangered plant species, because they provide the evaluation of the level of endangerment, as well as, the employment of adequate measures of protection.

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METODE TERENSKIH ISTRAŽIVANJA U BOTANICI

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I z v o d

Za terenska istraživanja u botanici, veoma su važne različite metode sakupljanja, preparovanja i održavanja zbirki biljaka. Obrađene i obeležene biljke deponuju se u herbarijume u kojima se konzerviraju i potom služe za taksonomska, floristička i fitogeografska istraživanja, a zatim i kao dokazni ili izložbeni materijal. Pored toga u proučavanju biljnog sveta u našoj okolini, u botanici se koriste i raznovrsne analize biljnih zajednica i njihovih staništa. Terenski podaci, literatura kao i usmena saopštenja su važni izvori naučnih informacija za kartiranje flore nekog područja.

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