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PLANT MICROTECHNIQUES EMPLOYED IN HERBIVORE FOOD HABIT STUDIES

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ABSTRACT. *Microscopic analysis of stomach contents and fecal material have been used to determine the food habits of a variety of herbivores including the nutria,* Myocastor coypus, *in southeastern Louisiana. Identification of ingested plant matter was based on diagnostic cell patterns of the epidermis of leaves, stems and roots. A reference collection was made of epidermal sections of potential food plants. Several different techniques used to prepare reference material are reviewed.*

INTRODUCTION

Plant microtechniques have been employed in herbivore food habit studies for quite sometime. Essentially, an investigation of this sort involves identifying ingested plant matter recovered from stomach or fecal samples. One of the first such studies was done in 1939 by Baumgartner and Martin (1) determining the food habits of squirrels. Since then, other studies have determined the food habits of eastern cottontails, *Sylvilagus floridanus* (2, 3), whitetail jackrabbits, *Lepus townsendii* (4), muskrats, *Ondatra zebethicus* (5), white-tailed deer, *Odocoileus virginianus* (6), caribou, *Rangifer tarandus* (7), and even kangaroos and wallabies (8). In 1979, Willner et al. (9) reported on the food habits of nutria, *Myocastor coypus,* in the marshes of Maryland. The techniques employed by these researchers were similar to those used by this author to determine the food habits of nutria in the fresh marshes of Southeastern Louisiana (10).

MATERIALS AND METHODS

Identification of plant fragments collected from either stomach or fecal samples is facilitated by certain diagnostic characteristics of the various plant tissues. Of course only those tissues which retain diagnostic characteristics after mastication and persist through partial or complete digestion are of use to the researcher. The epidermis and some vascular tissues are usually the only plant fragments which can be recovered in stomach or fecal samples. Xylem fibers and phloem vessel elements can sometimes be identified as such but usually do not possess unique characteristics necessary for specific identification. The epidermis, on the other hand, does have many unique diagnostic characteristics.

The epidermis is a layer of cells that covers all parts of the primary plant body including leaves, stems and roots. It acts to restrict transpiration and control gaseous exchange. This function results from

the presence of cutin in the outer cell walls and a layer of cutin on the outer surface of the epidermis known as the cuticle. Epicuticular waxes are also present (11). These features which inhibit transpiration also make the epidermis resistent to digestive enzymes. Therefore, any diagnostic characteristics present in the epidermis are usually preserved during the course of digestion.

Results and Discussion

Epidermal characteristics are usually distinct enough to identify plants to genus and in some cases to species. Characteristics which facilitate identification include size, shape and arrangement of epidermal cells as well as the presence or absence of trichomes and idioblasts. Such characteristics though may vary from one plant part to another. Monocotyledon plants typically have a parallel arrangement of rectangular-shaped cells on both leaves and stems. Leaf epidermis can often be distinguished from that of the stem on the basis of number of stomata, shape of cells and the presence of trichomes. The epidermis of dicotyledonous plants exhibits more diversity in size, shape and arrangement of cells. Dicotyledons are usually easier to separate into genera and species, and their leaf epidermis is easily distinguished from that of the stem.

A prerequisite of stomach content or fecal material analysis is the assemblage of a reference collection of possible food plants. Any plants which an animal may utilize should be collected and microscopically examined to identify distinguishing characteristics. It is important to include in the reference material any plant parts which the animal may eat including leaves, stems, roots and even flowers or seeds.

There are a variety of plant microtechniques which can be used to make slides of reference materials. Dusi (2) and others (12, 8) have described how pieces of plants can be macerated in acid solutions to separate the epidermis from underlying parenchyma tissue. The epidermis can then be stained and mounted on a slide.

This author in his nutria study used a razor to section off the epidermal tissue of various plant parts. In some cases, the epidermis of succulent plants was simply peeled away. The tissue samples were then dehydrated in a series of alcohols, stained in aqueous safranin, dehydrated again and cleared in xylene. Specimens were then permanently mounted with Permount mounting medium (10). Willner et al. (9) used this same technique in their study. Some studies have found the staining process unnecessary and simply mounted freshly sectioned material in a water miscible mounting medium (13, 14).

Willner et al. (9) used one other technique which bears mentioning. They used "Duco" cement to obtain an impression of the epidermal cell pattern. This procedure is much quicker and easier than some of the other techniques mentioned above. Shirley (10) experienced some problems with glue impressions due to stretching or cracking of the