TECHNIQUES FOR IDENTIFYING RUMEN MATERIAL FROM DEER

Thesis for the Degree of M. S.

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DEAN PAUL LONGRIE

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ABSTRACT

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MATERIAL FROM DEER

By

Dean Paul Longrie

Eleven species of winter browse were given two or three treatments, *in vivo* digestion, *in vitro* digestion, and dry-grinding to produce relatively small plant particles whose size ranged from approximately 2.5 mm to .25 mm. These particles were then given several histological treatments prior to microscopic analysis. Of the limited number of histological treatments used, those found to be most practical for observation of specific structural characteristics were treatments with either a macerating solution, 10 per cent acetic and 10 per cent chromic acid, and mounting in glycerin-jelly or mounting in glycerin-jelly without prior histological treatment. Characteristics which differentiated one species from the other ten species were determined. The structural characteristics used for identifying each species were identical, regardless of the technique used to produce the small plant particles examined. It was therefore concluded that specific

structural characteristics of the species examined are evident in rumen material and that these characteristics may be determined by microscopic analysis of dry-ground material.

TECHNIQUES FOR IDENTIFYING RUMEN MATERIAL FROM DEER

By

Dean Paul Longrie

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INTRODUCTION

Qualitative and quantitative identification of ruminoreticulum contents is a potentially valuable research technique for establishing which foods deer are consuming in the wild. Such techniques will probably become more important as research in wildlife nutrition is increased (Dietz 1965). Currently, inadequate botanical techniques are available for positive identification of rumen contents (Stewart 1967). One limitation is that different foods may be digested at differing rates due to variable factors such as the amount of food consumed, the degree of mastication, and the effectiveness of rumen microorganisms (Norris 1943, Smith 1952, Anderson et al. 1965, Dietz 1965, Stewart 1967, Church 1969). This could result in invalid qualitative and quantitative results, especially, if a high proportion of material that was broken down rapidly was classified as unidentifiable. This unidentified finely digested material could compose 10 to 12 per cent of the total analyzed material (Buss 1967, Stewart, 1967, Segelquist 1969).

Because of this botanical problem and because winter is considered a critical period, when browse is most limited (Dahlberg and Guettinger 1956), one objective of this research was to determine distinguishing characteristics of several species of winter browse found as finely digested material in the rumen, with special emphasis on the techniques used to determine generic and/or specific characters. The other was to test if actual mastication and partial digestion by deer would result in particles having the same appearance as those obtained by a simulation of mastication and digestion.

The species of browse chosen were selected from those recommended by professional wildlife biologists familiar with the winter food preferences of Michigan deer. Digestion of the browse was achieved by dry-grinding and by *in vitro* and/or *in vivo* fermentation. The material subjected to digestion was washed through a series of fine mesh sieves and treated singly or with a combination of the following: stain, acid, and/or a clearing agent. It was subsequently mounted in one of three media and examined microscopically to isolate identifying features, which were then photographed.

MATERIALS AND METHODS

Production of Particulate Material

Three methods were used to produce very small plant particles. Two of the methods produced particles similar, at least in size, to particles found in the ruminoreticulum of a deer. The particles were produced from the following browse species; white cedar (*Thuja occidentalis*), trembling aspen (*Populus tremuloides*), white pine (*Pinus strobus*), flowering dogwood (*Cornus florida*), smooth sumac (*Rhus glabra*), red osier dogwood (*Cornus stolonifera*), gray dogwood (*Cornus racemosa*), red maple (*Acer rubrum*), red oak (*Quercus rubra*), basswood (*Tilia americana*) and staghorn sumac (*Rhus typhina*).

In Vivo

The first method used to obtain finely digested plant material involved the feeding of browse to captive white-tailed deer (*Odocoileus virginianus*). Because a great deal of time was required to collect enough browse to feed a deer for a short period of time, only five species of browse were fed. A 3.5 year-old buck with a rumen fistula was fed white cedar, aspen, white pine, and flowering dogwood. A 1.5 year-old doe was fed smooth sumac. Another doe, 2 years old, was fed white pine. The procedure was as follows. Fresh browse was collected and frozen to preserve its digestibility (McDonald 1968). After a sufficient quantity was collected, it was thawed and fed *ad libitum* for 24 to 30 hr. Sugar water was sprayed on the browse to increase its palatability. A 270 ml rumen sample was taken between 24 and 30 hr after the food was presented to the deer. The sample was frozen for later preparation and analysis.

In Vitro

The second method used for the production of small plant particles, *in vitro* fermentation (Johnson 1966), included chemical (Annison and Lewis 1959) as well as mechanical means of breakdown. Twigs from all browse species collected were fermented *in vitro*. The materials (Figure 1A) used were:

1. A tank of carbon dioxide, with a regulator providing the anerobic atmosphere required by the cellulose digesting microorganisms.
2. A manifold and flexible tubing permitting the distribution of the carbon dioxide.
3. Several #7 rubber stoppers with long input tube and short exhaust tubes permitting the bubbling of carbon dioxide into the fermenting mixture.

4. One rubber stopper with a long input tube and a V-slot cut in the edge permitting carbon dioxide input and exhaust while subsamples and pH were taken.
5. A 160 ml wide mouth graduated bottle permitting volume maintenance during fermentation.
6. A water bath used to maintain the fermentation bottle contents at the normal rumen temperature of 39° C.
7. A single probe pH meter used to determine pH.
8. A large mouth pipette permitting samples of large particles to be taken.
9. "Ohio" media, providing required nutrients and buffers normally provided by the animal.
10. Sodium carbonate, used to buffer the fermenting solution.
11. A solution of 5 per cent sulfuric acid used to terminate fermentation.
12. A 40 cm flexible plastic tube 3 cm in diameter used to remove rumen material from a fistulated deer.
13. Cheesecloth used to strain the rumen material.

The procedure followed was:

1. Freshly collected dormant twigs were cut into pieces less than 3 cm.
2. Some species were broken up with a mortar and pestle to further simulate mastication.

3. The plant material was weighed and 1 to 3 g was placed in each of two bottles containing 75 ml of "Ohio" media.
4. The bottles were refrigerated overnight at approximately 4° C.
5. The bottles were put into the waterbath and carbon dioxide was bubbled through the contents for 45 min before inoculation with rumen liquor.
6. Material was taken from the rumen of a 1.5 year-old male white-tailed deer which had been fistulated (Figure 1B) by the author and an associate.
7. The rumen material which had been removed without apparent discomfort, was strained through cheesecloth.
8. Fermentation was initiated with the addition of 25 ml of the strained rumen liquor to each bottle.
9. The pH was checked and if necessary the fermenting rumen material was buffered to a pH of 6.7.
10. A 10 ml sub-sample which was put into a test tube containing 0.5 ml of 5 per cent sulfuric acid, was usually taken from the bottles at 12, 24, and 48 hr after the start of fermentation.

11. At the end of 48 hr of fermentation 2 ml of 5 per cent sulfuric acid was added to each bottle.
12. The bottles were refrigerated for further treatment and analysis.

Prior to the start of the *in vitro* fermentation of the browse, a check of the procedure and materials used was made by determining the digestibility of Solka Floc,[1] a standard source of cellulose. Digestibility was expressed as the weight of cellulose minus the remaining weight of cellulose, divided by the initial weight of cellulose, the quotient multiplied by one hundred (Church 1969). The digestibility of Solka Floc was determined both with sheep rumen liquor and with deer rumen liquor (Figure 2A).

Dry-Grinding

The third method, dry-grinding, was strictly mechanical (Figure 2B). Twigs from each of the species listed above were treated as follows. Approximately 10 g of dormant twigs were air dried. The dry twigs were ground in a Wiley mill, grinding 5 g through the 40 mesh screen and 5 g through the 20 mesh screen. The ground material was stored in air-tight vials for further treatment and analysis.

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[1]Brown Company, Chicago, Illinois

Analysis of Digested Material

Preparation for Analysis

The materials used in preparation of the plant fragments for analysis were as follows: (1) a series of U.S. Standard Sieves--No.'s 8 (2.380 mm opening), 20 (.841 mm opening), 35 (.500 mm opening), and 60 (.250 mm opening)--was used to separate different size plant particles; (2) a 1 per cent Safranin solution was used to stain the lignified cell walls (Johansen 1940); (3) a solution made of 10 per cent nitric and 10 per cent chromic acid was used to dissolve some of the lignin that was cementing the cell walls together; (4) Hertwig's solution, a clearing agent, was used to remove artifacts from the cells (Dusi 1949); (5) several mounting media--Hoyer's (Becke 1955) or glycerin-jelly (Guyer 1936) for permanent mounts and glycerin for temporary mounts--were used.

The procedure followed in treating each species was as follows. The digested material was washed through a series of sieves. A small sample from each or all sieve sizes was placed in a test tube, containing 2 ml of the acid solution, for 10 min. A sample from each or all sieve sizes, except No. 8, was placed on a slide and mounted in one of the mounting media. Half of the species digested were treated with 1 per cent Safranin stain and/ or Hertwig's solution. The plant fragments treated with acid were mounted in one of the three media or treated with Safranin and/or Hertwig's solution and then mounted.

Microscopic Analysis

The procedure used in microscopic analysis was to draw distinctive features as observed at 44 X, 125 X, or 500 X magnification and note the location of characteristic structures for later comparison and photography.

References used in identifying morphological structures were Esau (1965), Gleason and Cronquist (1963), Panshin and De Zeeuw (1964), and Winton (1916).

Microphotography

Microphotographs were made of at least one distinguishing characteristic of each species observed. All microphotographs were made with a Lietz 35 mm camera, microscope, and adapter. The microphotographs were taken on Kodak Plus-X film.

RESULTS

Digestion Methods

All of the methods used--dry-grinding, *in vitro* and *in vivo* fermentation--resulted in the production of finely divided plant particles. Plant particles taken from the rumen and washed through the series of sieves provided almost equal quantities of material in each sieve. Grinding dry material through the 40-mesh screen produced very finely fragmented material that was almost exclusively retained by the No. 60 sieve. Therefore, only dry material ground through the 20-mesh screen, which resulted in almost equal proportions of ground material in each sieve, should be used in preparing air dried browse for analysis.

The determination of Solka Floc digestion described by Crampton and Maynard (1938) demonstrated that the *in vitro* equipment and procedure used were functional. The similarity in digestibility of Solka Floc by sheep and deer (Figure 2A) rumen inoculum indicated that the micro-organisms from the deer used were able to readily digest cellulose. The fermentation of the cut-up freshly collected browse was enhanced by grinding the plant material with mortar and pestle. The plant particles collected after 48 hr of fermentation had the desired gradient of sizes which indicated that it was unnecessary to collect subsamples at 12 and 24 hr after the start of fermentation.

The rumen contents obtained from the fistulated and non-fistulated deer provided a sufficient quantity of finely digested browse at every sieve size class, if the sample were thoroughly mixed before washing a portion of it through the sieves or, if the entire sample were washed through the sieve series.

Preparatory Treatment

Washing the particles through the sieve series provided a common base, particle size, from which plant material given various treatments could be examined for specific characteristics. The first slides prepared held material taken from a single sieve. The largest particles examined, retained by the No. 20 sieve, were mostly opaque but they did permit the observer to see how different cells and tissues were associated. The next size sieve, No. 35, primarily retained multicellular fragments that were clear, transmitted light, but did not have a great deal of cellular detail. The smallest sieve size, No. 60, held small multi-cellular or unicellular fragments which frequently exhibited cellular detail. It was found by experience that it was more practical, timewise, to take materials from each sieve size and mount them all on one slide.

Seventeen slides of white cedar particles, which were treated with all possible combinations of stain, clearing agent, and acid, were prepared for analysis. It was concluded, after analyzing each slide, that particles treated with either acid and then mounted or mounted without further preparation have the most discernible structural characters.

Adequate slides were prepared using all three of the mounting media, Hoyer's, glycerin, and glycerin-jelly. Hoyer's medium is harmful to human skin and dried relatively slowly. The low viscosity of glycerin made it difficult to work with. The fast-drying glycerin-jelly was the most manageable media used. Regardless of what media is used care must be taken not to use too much. After putting the coverslip on the slide a hard rounded object, such as a spatula handle, should be gently pressed on the surface of the coverslip to insure a uniform distribution of the mounting media.

Microscopic Analysis

The species characteristics noted (Table 1) were found in material given each treatment--dry-grinding, *in vitro* and/or *in vivo* fermentation.

A minimum of six and an average of 10 slides were prepared for each species analyzed. Before a cellular feature was designated as characteristic it was observed several times on slides prepared from each particle producing treatment.

The species characters found for white cedar were papillate epidermal cells that appeared as almost transparent wedge-shaped structures on the margin of the leaf fragments observed (Figure 3A), and stomates which had guard cells with obvious cell wall thickenings at their conjuncture. The cell wall thickenings frequently appeared as bone-like structures with a distinct epiphisis (Figure 3B).

Obvious specific indicators were difficult to find for trembling aspen. The following characters, however, when used together, separated trembling aspen from the other ten species analyzed. Numerous alternatively arranged bordered pits were frequently observed on the vessels (Figure 4A). When the focus was changed the pits appeared to form bands perpendicular to the long axis of the vessels. Rows of rectangular cortical parenchyma associated with fragments of vascular material were also frequently observed (Figure 4B) as was the square appearing epidermis (Figure 4C).

Several species characteristics were observed for white pine. The almost transparent prong cell which appeared barb-like on the margin of the needle particle (Figure 5A) was obvious. Other obvious characteristics were rows of stomates, whose aperatures appeared as columns of dark squares, and epidermal cells which appeared as long columns with thick walls (Figure 5B). At 500 X, the thick epidermal cell walls appeared wavy.

One diagnostic character of flowering dogwood was the epidermal cell configuration which was demonstrated most clearly when only the single layer of epidermal cells was observed (Figure 5C). The epidermis was usually observed when it was associated with several other layers of cells (Figure 6A). Other frequently observed structures were linearly arranged bordered pits on the vessels which appeared in apparent bands across the plant fragment (Figure 6B). Refocusing the microscope was required to observe the banding effect.

The abundance and size of the cortical parenchyma cells of staghorn and smooth sumac were identical and apparently representative (Figure 7A). Vessels with sclariform bordered pits were frequently occurring structures on slides of smooth sumac particles. The sclariform bordered pits appeared as dark oblong figures (Figure 7B) in one focus and as smaller, clear slits at another focus. This differed from the relatively abundant bordered pits found in the vessels of staghorn sumac. The bordered pits appeared as circular dark areas at one focus and smaller clear dots at another. The characteristic most clearly distinguishing staghorn sumac from the other species examined was the abundance of pubesent (hair covered) epidermis (Figure 7C).

Red osier dogwood had characteristically dense bud scale hairs which were relatively thick (Figure 8A). The epidermal cells had a characteristic appearance when they occurred as a single layer of cells (Figure 8B).

The rigid appearing and relatively short bud scale hairs of gray dogwood were observed on all slides of gray dogwood particles (Figure 9A). Another frequently occurring gray dogwood structure was the cortical parenchyma which had numerous simple pits (Figure 9B). The simple pits appeared as dark ovals at one focus and light ovals, of the same size, as the focus was changed. The configuration of the epidermal cells (Figure 9C), which occurred frequently, could also be used, in conjunction with the other characters mentioned, to identify gray dogwood particles.

The almost oval bud scale, with hairs located primarily at the scale apex (Figure 10A), indicated the presence of red maple. Another specific indicator, the epidermal cells, appeared to have partial walls (Figure 10B) when only one layer of cells was observed.

Red oak had characteristically long, dense, and flimsy appearing bud scale hairs (Figure 10C). Another frequently occurring structure was the oblong form of the epidermal cell (Figure 11A). As in all of the specific epidermal characters, the cell form was best demonstrated when the epidermis occurred as a single layer of cells. The single layer of epidermal cells was most frequently observed at the periphery of the plant particles which were at least partially composed of epidermal material.

One of the most unique characters found on the eleven species of browse analyzed was the stellate hairs which were found on basswood (Figure 11B). Simple hairs were also observed, however, stellate hairs were observed most frequently. Another relatively obvious character of basswood was the cross-hatched-appearing vessels (Figure 11C). The cross-hatched appearance was due to the presence of spiral thickenings and bordered pits on the same vessel.

Figure 1A. Some of the materials used for the *in vitro* fermentation including the CO_2 tank and regulator, manifold, fermentation bottle stoppers, pH meter, water bath, and wide mouth pipette.

Figure 1B. The tame, rumen fistulated deer used as a source of inoculant.

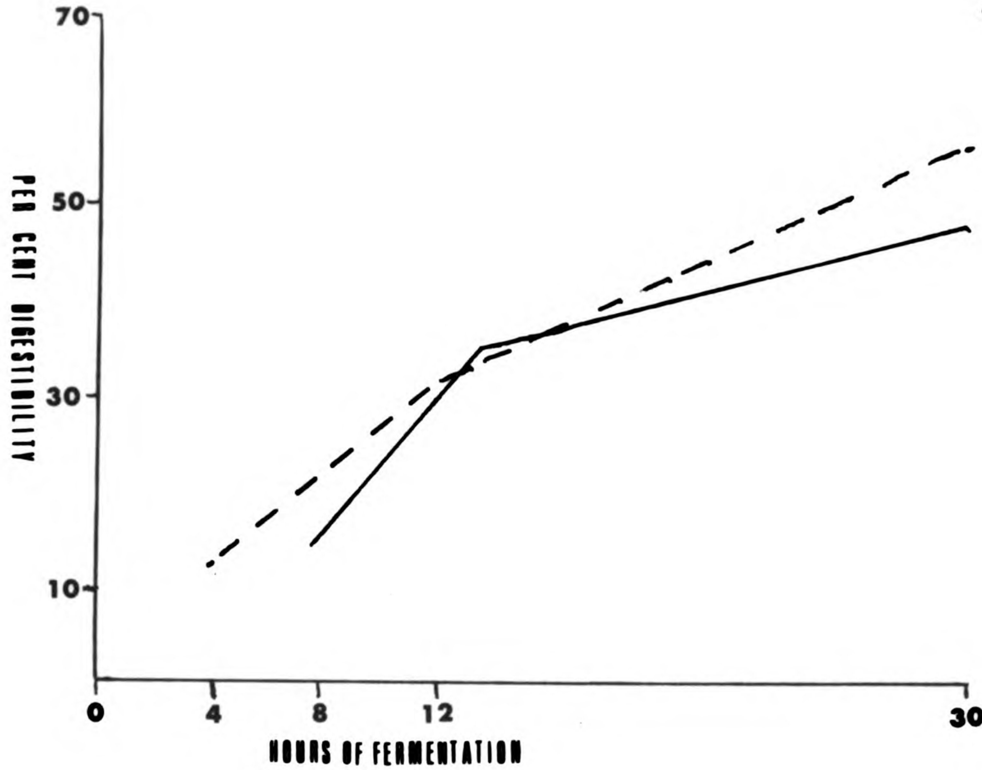
A

B

Figure 2A. Graph showing the digestibility of cellulose using sheep inoculant (solid line) and deer inoculant (broken line).

Figure 2B. Equipment used for dry-grinding winter browse --Wiley mill and collection vials, sieve series used to separate particles for analysis, and one of the microscopes used for analysis.

A

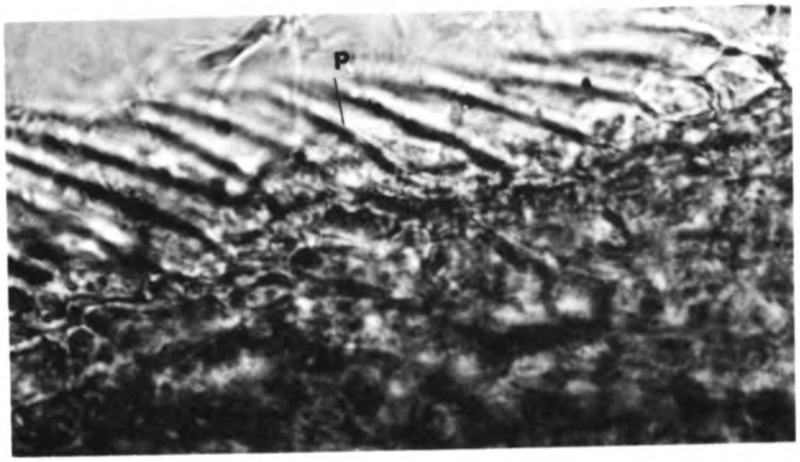

70
50
30
10
PER CENT DIGESTIBILITY
0
4
8
12
30
HOURS OF FERMENTATION

B

Figure 3A. The papillate epidermal cells (p) of white cedar that appear as almost transparent wedge-shaped structures (500 X).

Figure 3B. White cedar stomates having cell wall thickenings at their conjuncture which frequently appear bone-shaped (c) (500 X).

A



B

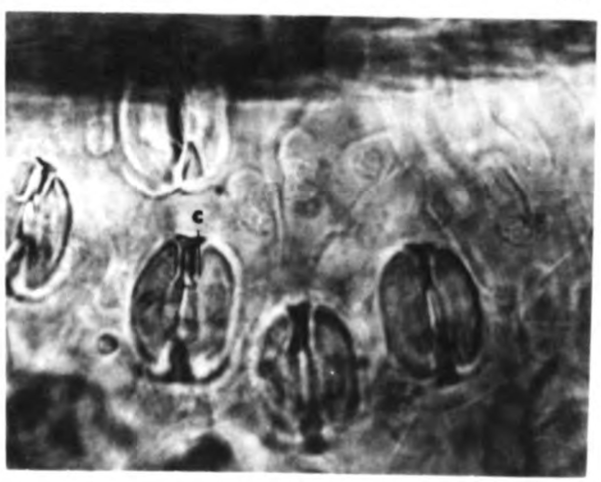
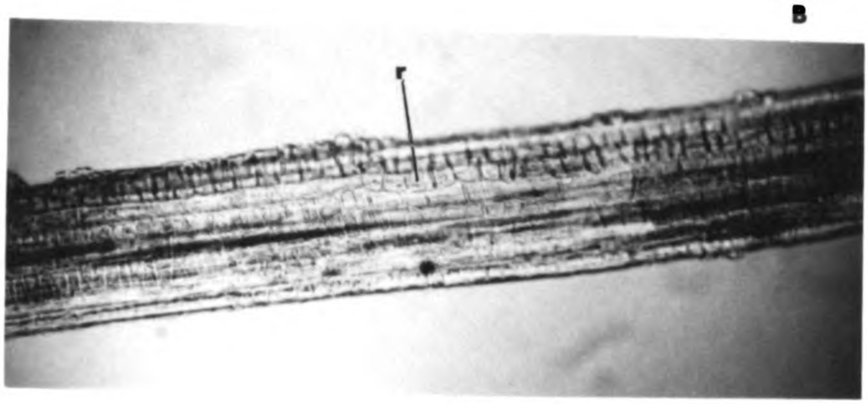


Figure 4A. Alternately arranged bordered pits (p) on the vessels of trembling aspen (500 X).

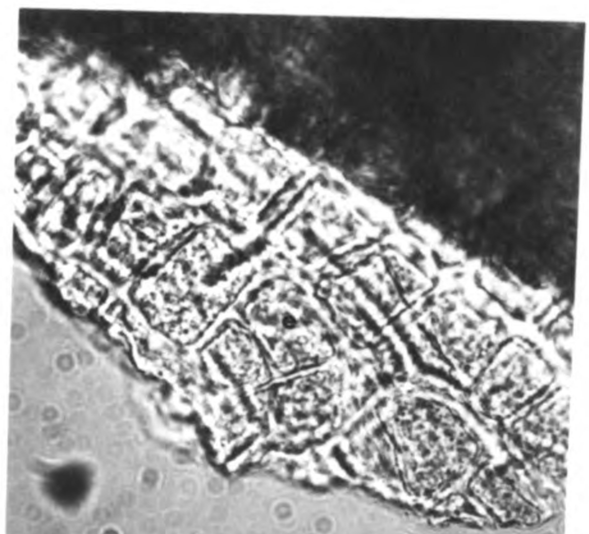
Figure 4B. Rows of rectangular cortical parenchyma (r) which were frequently found associated with fragments of trembling aspen vascular material (500 X).

Figure 4C. A single layer of square-appearing aspen epidermal cells (e) (500 X).

A



B



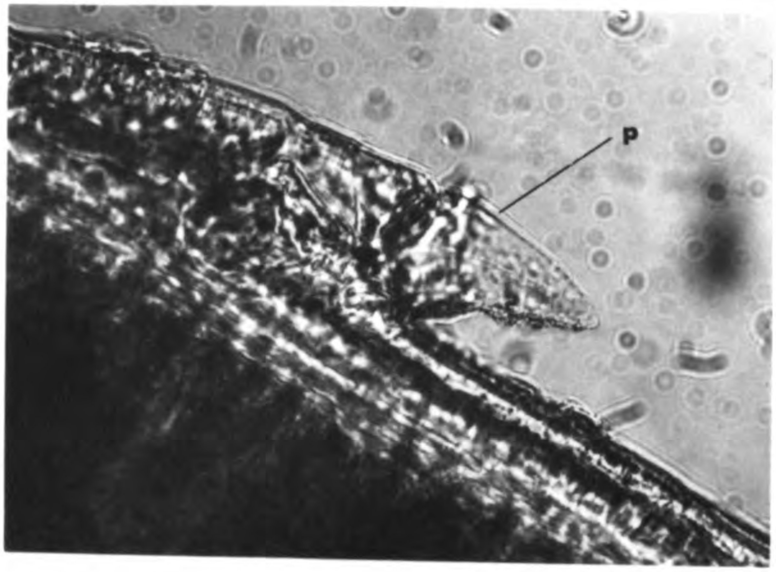
C

Figure 5A. The barb-like, almost transparent prong cell (p) appeared at the margin of the white pine needle (500 X).

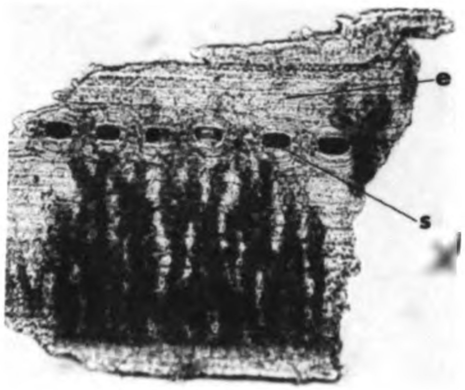
Figure 5B. The dark, square-appearing white pine stomatal aperature (s) and the column of thick walled epidermal cells (e) (125 X).

Figure 5C. A single layer of flowering dogwood epidermal cells (e) (500 X).

A



B



C

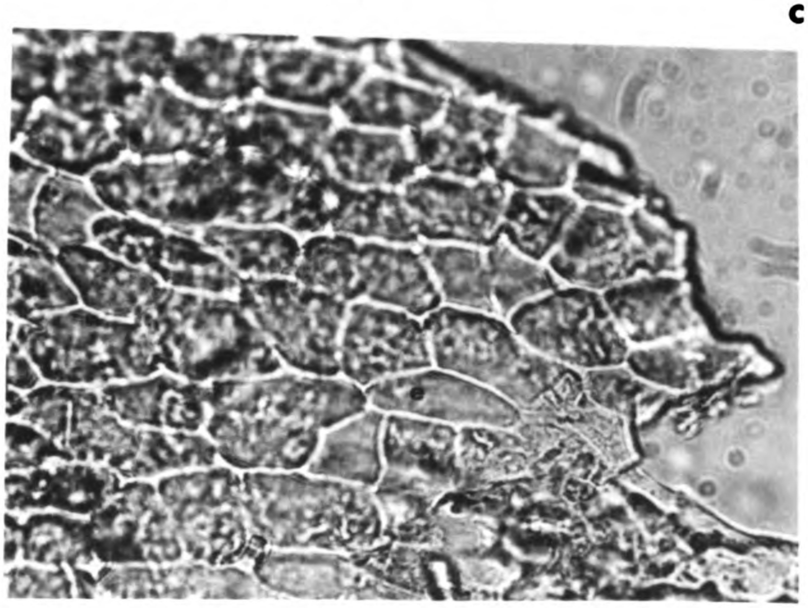
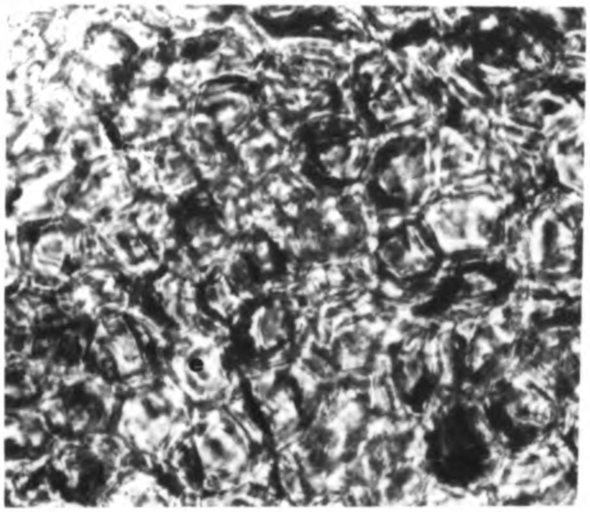


Figure 6A. Flowering dogwood epidermis (e) associated with several other layers of cells (500 X).

Figure 6B. Linearly arranged bordered pits (p) on the vessels of flowering dogwood (500 X).

A



B

Figure 7A. The large, oval and thin walled cortical parenchyma cells (cp) of smooth sumac (500 X).

Figure 7B. The scalariform bordered pits (s) found on the vessels of smooth sumac (500 X).

Figure 7C. The hair (h) covered epidermis of staghorn sumac (500 X).

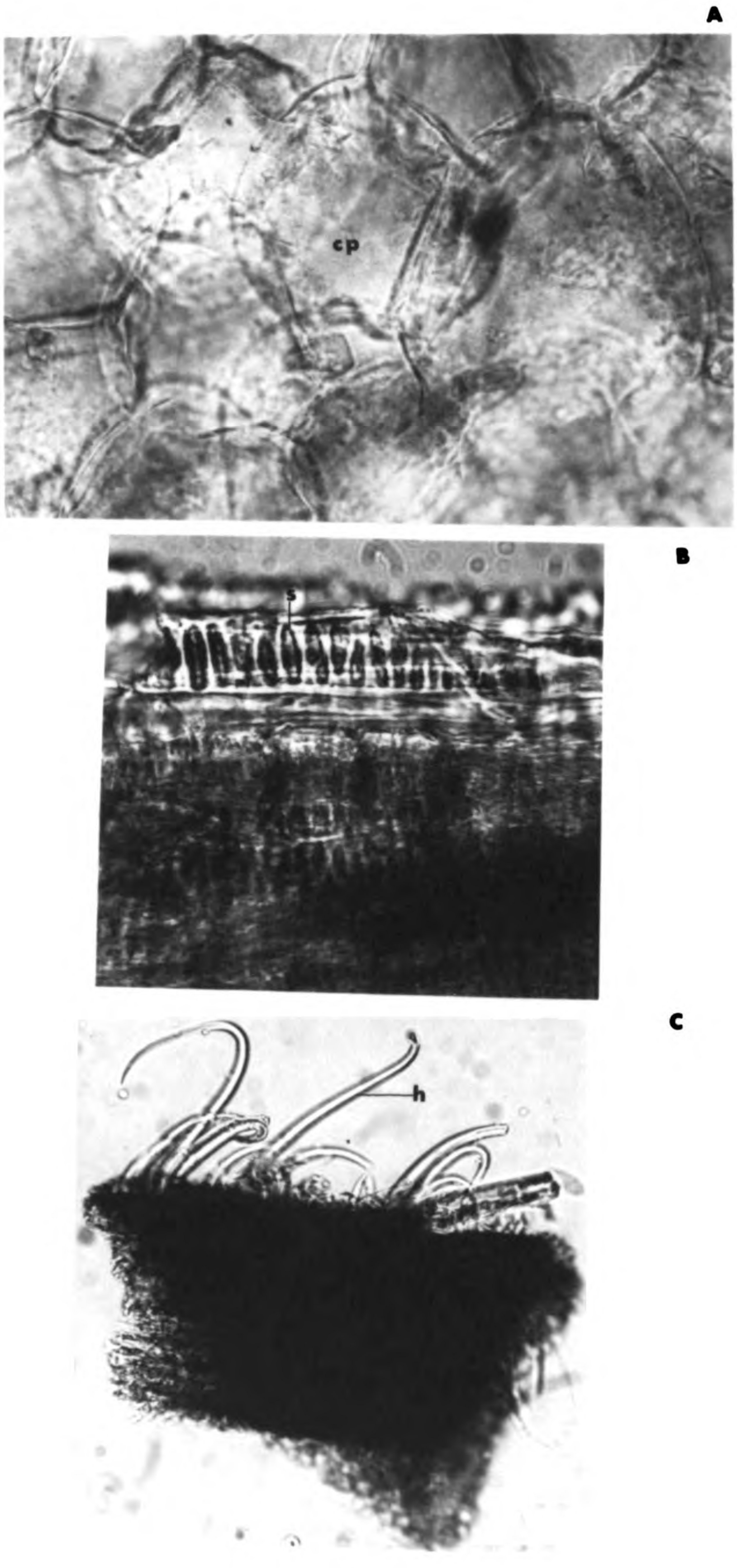
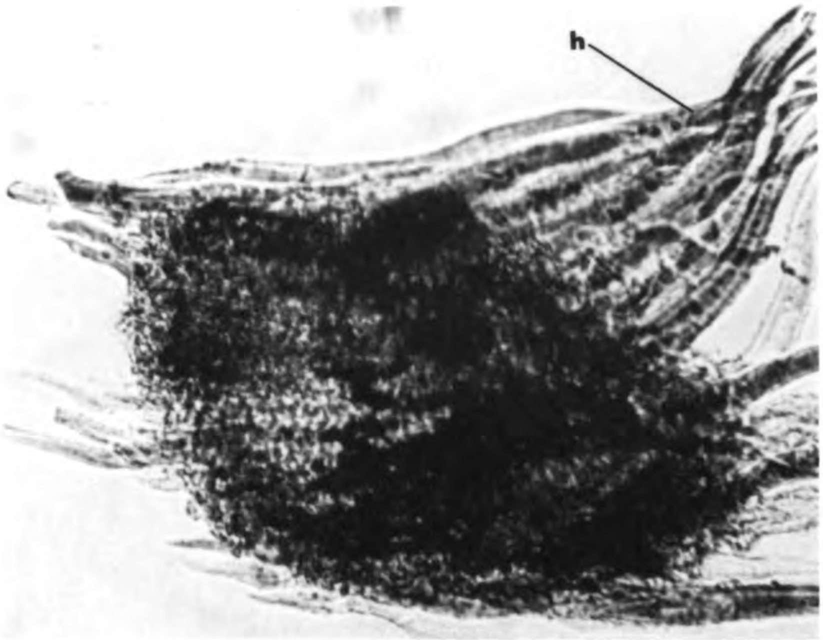

A
cp
B
s
C
h

Figure 8A. The dense bud scale hairs (h) of red osier dogwood (500 X).

Figure 8B. A single layer of red osier dogwood epidermal cells (e) (500 X).

A



B

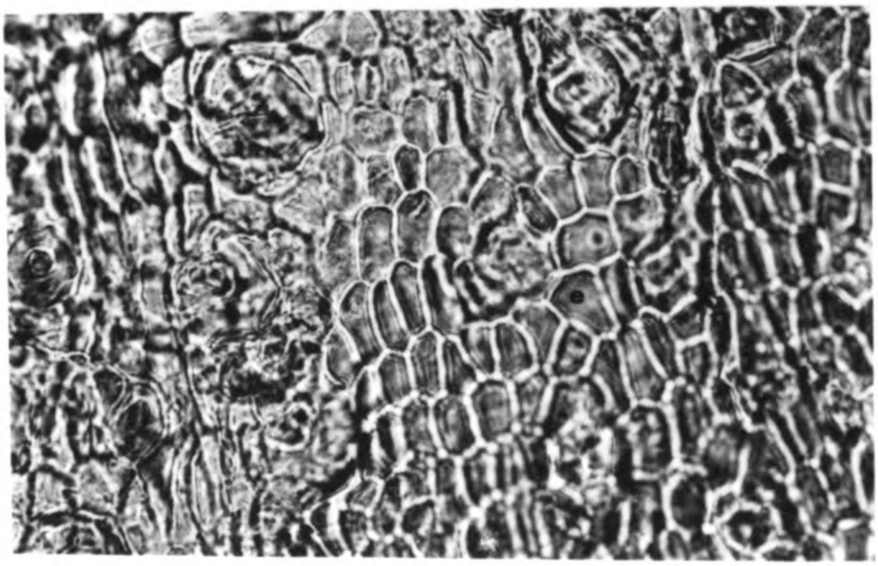


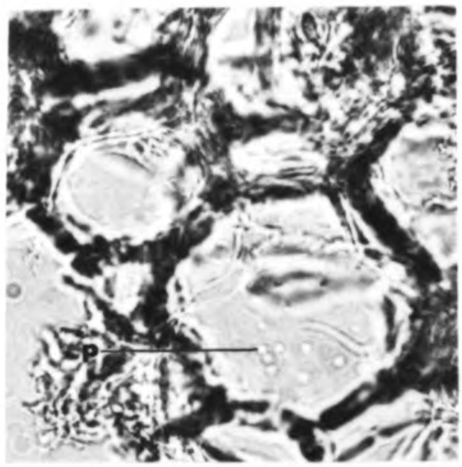
Figure 9A. The rigid lance-like bud scale hairs (h) of gray dogwood (500 X).

Figure 9B. Simple pits (p) which occur on the cortical parenchymal cells of gray dogwood (500 X).

Figure 9C. The epidermal cells (c) of gray dogwood (125 X).

A

B



C

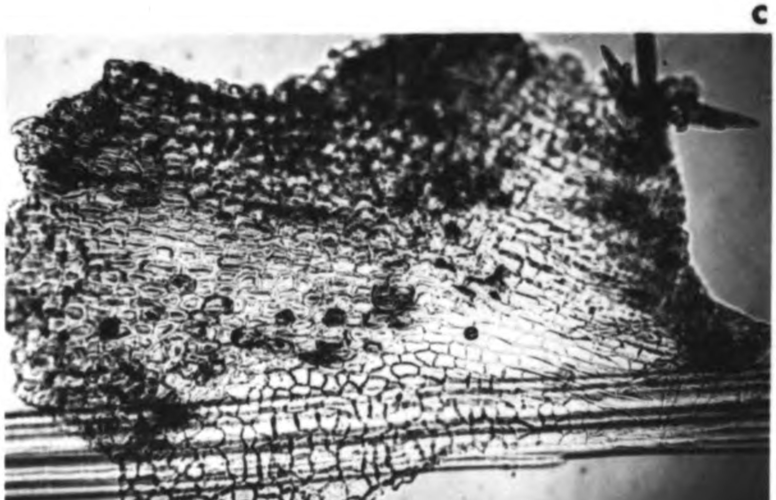
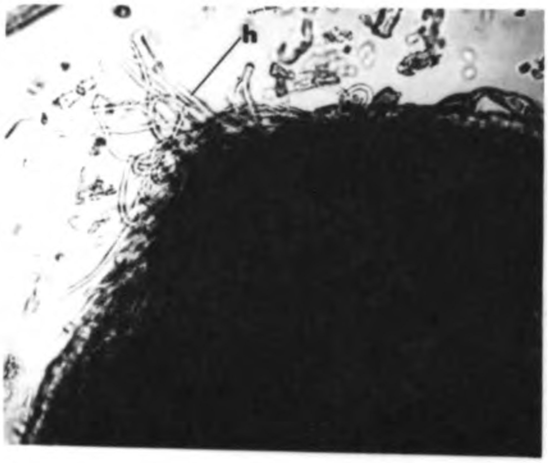


Figure 10A. The oval bud scale of red maple which had hairs (h) at the apex (500 X).

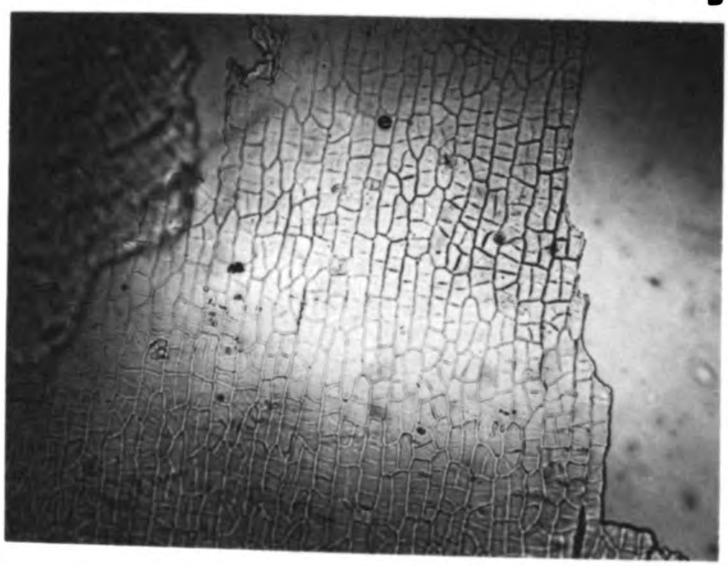
Figure 10B. A single layer of red maple epidermal cells (e) (500 X).

Figure 10C. The long, dense, and flimsy appearing red oak bud scale hairs (h) (500 X).

A



B



C

Figure 11A. A single layer of oblong-appearing red oak epidermal cells (e).

Figure 11B. Stellate hairs (s) found on basswood (500 X).

Figure 11C. The cross-hatched appearing vessels (cu) of basswood (500 X).

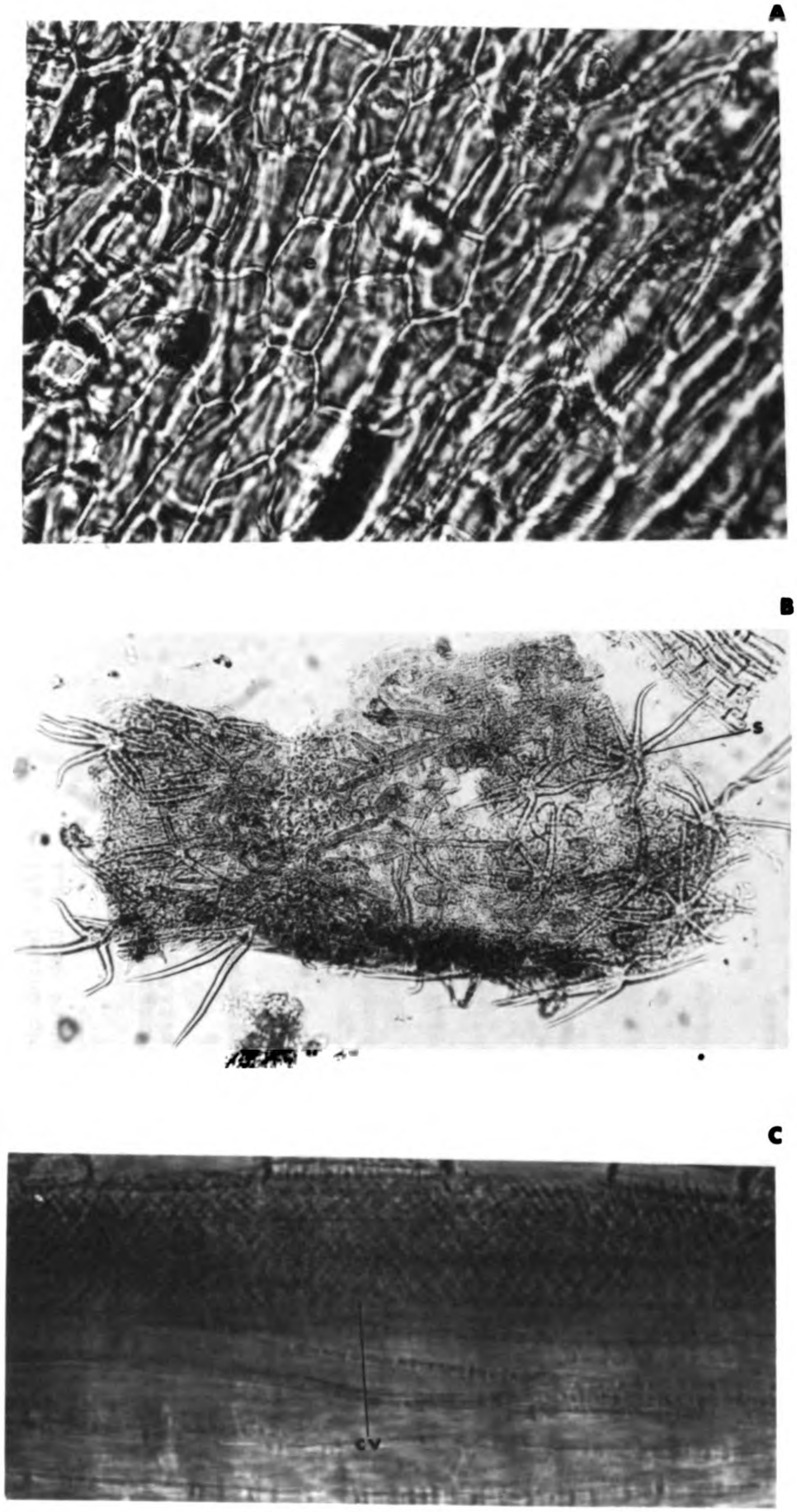

A
e
B
s
C
cv

TABLE 1. A list of species characteristics with the technique used to produce the small particles observed.

Species	Characteristic	Method of Browse Particulation		
		Dry-grinding	*In Vitro*	*In Vivo*
White cedar	Papillate epidermal cells	X*	X	X
	Stomatal guard cell wall thickenings	X	X	X
Trembling aspen	Epidermis	X	X	X
	Cortical parenchyma	X	X	X
	Alternate pitting on vessels	X	X	X
White pine	Prong cells	X	X	X
	Stomate arrangement	X	X	X
	Epidermis	X	X	X
Flowering dogwood	Epidermis and linear pits on vessels	X	X	X
Smooth sumac	Cortical parenchyma	X	X	X
	Sclariform bordered pits on vessels	X	X	X
Staghorn sumac	Epidermis with hairs	X	X	O**
Red osier dogwood	Bud scale hairs	X	X	O
	Epidermis	X	X	O
Gray dogwood	Bud scale hairs	X	X	O
	Simple pitted cortical parenchyma	X	X	O
	Epidermis	X	X	O
Red maple	Bud scale hairs	X	X	O
	Epidermis	X	X	O
Red oak	Bud scale hairs	X	X	O
	Epidermis	X	X	O
Basswood	Stellate hairs	X	X	O
	Vessel with cross-hatched appearance	X	X	O

*The characteristic was observed in particles that were produced by the stated technique.

**This species was not given this treatment.

DISCUSSION

Of the eleven species analyzed, white pine, white cedar, gray dogwood and basswood had either more observed specific characters or more obvious characters than the other species examined. One possible reason for the discrepancy in specific characters observed for the various species microscopically analyzed was that the observed particles were difficult to orientate. That is, one usually could not determine if the particle viewed illustrated a transverse, radial or tangential surface which is usually the reference used in specific identification at the particle size level examined (Esau 1965 and Panshin and De Zeeuw 1964).

The specific structural characters found in this study could, in my opinion (based on the observations made) be used to identify rumen material which held all eleven or any part of the eleven species analyzed. If similar techniques were applied to the analysis of the rumen contents from wild deer it would be essential that the reference collection, analyzed to determine the specific characters used for identification, include all browse species available to the deer in the area from which it was collected.

A single species characteristic may not be sufficient to permit the observer to make a positive identification, especially when species of the same genera are mixed together. When all of the observed species characteristics are used as a basis for identification, positive identification is possible. Many of the species characters, such as the stellate hairs found on basswood, are relatively obvious to the untrained observer. Even this characteristic, however, would require prior examination of reference material by the observer before he could make conclusive species identification of material taken from the rumen. This study has shown that material previously termed unidentifiable is in fact identifiable and that one now has a basis for assuming that species of winter browse, air-dried and ground to a desired range of sizes, can illustrate to the observer those specific structures that one would find in the rumen of a deer that has fed on the same species of browse.

The techniques used were not intended to be exhaustive, but because of the absence of similar studies on finely digested winter browse, they could be termed exploratory. Several possible improvements in technique were noted at the conclusion of the study.

The *in vivo* fermentation procedure would be improved if sufficient quantities, 4-6 pounds, of each species of winter browse fed to the rumen fistulated deer were collected and frozen prior to the initiation of the test feeding program. This procedure would permit the deer to be fed one species ad libitum for 24-30 hr followed by a collection of rumen material and presentation of a new browse species. The chance of observing test species in subsequent rumen samples and in varying degrees of breakdown would be increased. An example would be to feed the deer white cedar for 25 hr after which a rumen sample would be taken, containing white cedar, primarily, along with particles of food previously eaten. Aspen might then be fed immediately and after another 25 hr a rumen sample containing aspen as well as white cedar might be taken. The increased observations of white cedar would make the observer more aware of true specific species characteristics.

The same specific species characteristics would probably be found using the in vitro method, however, this required considerable time and equipment. The dry-grinding treatments resulted in particles having characteristics identical to those found after in vivo and in vitro treatment, but required very little time and equipment.

SUMMARY

Eleven species of winter browse were given two or three treatments, *in vivo* digestion, *in vitro* digestion and dry-grinding, to produce relatively small plant particles that could pass sieve mesh openings of 2.380 mm, but be held by a sieve with mesh openings of .250 mm. These particles were then given several histological treatments prior to analysis for specific structural characteristics. Of the histological treatments used, those found to be most beneficial for observation of specific characteristics were treatments with either a macerating solution, 10 per cent acetic and 10 per cent chromic acid, and mounting in glycerin-jelly or only mounting in glycerin-jelly. Species characteristics, which differentiated one species from the other 10 species, were determined and found to be identical, regardless of the technique used to produce the small plant particles examined. It was concluded that specific structural characteristics are evident in rumen material and that these characteristics may be determined by microscopic analysis of dry-ground material.

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