PRELIMINARY OBSERVATIONS ON POPULUS TREMULOIDES (MICHX.) CLONES AT THE RIDING MOUNTAIN NATIONAL PARK

by

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CONTENTS

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INTRODUCTION

Trembling aspen makes up one tenth of Canada's one million square miles of productive forests, and is one of the most abundant commercial tree species in the prairie provinces. The utilization of the species has increased to the point where it is of concern to pathologists and silviculturist to improve volumetric increments and curtail losses due to pathogenic decay. Future aspen stands can be greatly improved by favoring aspen clones which respond productively to silvicultural treatment. By being able to assess and predict clonal performance, reforestation programs can be undertaken with planting stock of high genetic strain, assuring low mortality and high productivity.

This report presents the results of a preliminary study to identify and separate aspen clones, measure their extent and determine differences in morphology and growth.

LOCATION AND DESCRIPTION OF STUDY AREA

The study area is located eight miles down the Audy Lake Road in the Riding Mountain National Park, which is located in the southeastern extremity of the B18a Forest Section (Rowe 1959).

The soils are primarily grey wooded and have been derived principally from calcareous shaley tills. Soil textures range from clay loam to loam and soil moisture classes (Hills 1952) from fresh to very moist (Waldron 1961).

METHODS

Mapping of clones

A total of 24 aspen clones were selected in 1966 along 12 miles of the Audy Lake Road. In one area, eight clones were identified and intensively sampled while the remaining 16 served primarily as sample areas for lateral bud analysis. In the intensively sampled area the boundaries of the clones were marked, using different coloured tagging tape for each clone. In separating the clones, use was made of any consistent morphological difference between clones, such as bark colour, lenticel pattern, branchiness of trees and angle of branching. However of greatest use in clone separation was the noticeable difference in time of leaf flushing in the spring.

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To obtain an accurate map of the eight individual clones (photo 1), a grid system was set up. The entire area was marked off and broken down into 12 individual quadrats, 2 1/2 x 2 chains. The quadrats were numbered from one to ten. The extremities of the clones were noted and put on graph paper. The areas were then determined by planimeter (see fig. 1, table 1). Audy Lake Road served as the eastern boundary even though some clones could have extended beyond it. No attempt was made to determine the extent of the clones beyond the road.

In Table 1 the size of the various clones which were mapped has been tabulated. Although some intermixing between clones occurred, they were considered to be essentially pure.

Sampling within clones

Apart from differences in tree form, structure and phenology between clones, leaf morphology was used to depict clonal differences. Three trees per clone were sampled. A total of ten leaves per tree were collected. The leaves were obtained about 15 feet above the ground and from the middle of medium length shoots (see table 2, photo 2).

Clonal performance was studied by randomly establishing mil-acre sample plots within the clones. A total of 17 plots were laid out and sampled for height, age and d.b.h. (see table 3).

Increment borings among the clones indicated no unique variations in age. The average age for each clone was 30 to 31 years. Average height data show a range of 14 feet between the shortest and tallest clones. Height differences may be the result of genetic differences between clones, although possible site differences could also have attributed to the variation in tree heights.

The roots of nine trees, which were situated along clonal boundaries, were excavated and mapped (fig. 2, 3, 4, 5, 6). The root maps indicate that the root system of a ramet from a particular clone does not limit itself to the above surface extent of that clone, but that roots of different clones freely intermix. The sharp boundaries that are often seen between clones are therefore most likely the result of different suckering capacities and growth rates between clones followed by suppression of suckers of one clone by those of another.

Suckers develop on the root system of parent trees. Apparently the direction of the nutrient flow as it existed in the roots of the parent tree causes a thickening of these roots at the sucker(s) location on the side distal to the parent tree stump. Use was made of this thickening in attempts to determine the location of the parent tree(s) in relation to that of the present suckers. Attempts were not successful, although root thickenings were still noticeable.

However from the available data obtained it would seem that clonal boundaries could fluctuate quite considerably from one generation to another.

Chemical analysis

Phenological and morphological differences were used to distinguish clones in the field. Attempts were made in the laboratory, using paper and thin layer chromatography techniques to confirm observations made in the field.

Chromatography is a technique by which certain organic compounds contained in the plant tissue can be separated. Presumably tissues from ramets of the same clone will show identical, or at least, similar composition which will differ from that of other clones.

Lateral bud analysis

From the remaining 16 clones (see page 1) lateral buds were sampled. A total of 50 buds were collected at each sampling point.

Paper chromatography was the first method used in the chemical analysis. The information on the method used, was obtained from a thesis by G.M. Blake 1964, University of Minnesota, "Clone Identification and Delineation in the Aspens".

Lateral bud samples weighing 250 mg were macerated in an 80% solution of warm ethyl alcohol. The extracts were then filtered in bottles and stored in a freezer compartment. A 2% solution of hydrated sodium carbonate was prepared and used as the reagent. An attempt was made to use the organic layer of a secondary butyl alcohol, ammonium hydroxide and water solution as the developing solvent. The solution was shaken for ten minutes then put into a separatory funnel for separation. No separation between the organic layer and the rest of the solution occurred. The secondary butyl alcohol, ammonium hydroxide and water seemed to be entirely miscible. As a result of this, the entire solution (Isopropal butyl alcohol and water) was used as the developing solvent in equiliberating the chromatocab for 24 hours. The extracts were then spotted on #1 Whatman filter paper (18.5" x 22.5") by means of a lamda pipette. Five drops per spot were used to give adequate concentrations, 500 ml of isopropal-butyl alcohol and water was placed in the descending tray of the chromatocab. The sheet was hung in the chromatocab for 24 hours until the front reached the bottom. The paper was hung to dry. The paper was then sprayed with a 2% solution of sodium carbonate and dried again. Finally the chromatograms were examined under ultraviolet light. No noticeable separation occurred indicating that either the extracts were not concentrated enough or that isopropal butyl alcohol and water could not be used as the developing solvent.

In the second attempt N-butanol:acetic acid:water was used as the developing solvent (4:1:1.8)V/V. The contents were shaken for five minutes then drained into a reagent bottle. The chromatocab was again equiliberated for 24 hours and the entire procedure repeated. The dried chromatograms were examined under ultraviolet light and again no visible separation occurred. Extreme care was exercised to avoid any contamination of the system, leaving lack of concentration of extracts as a possible conclusion to the fact that no separation occurred or that a stronger reagent was needed.

As a result, the experiment was again repeated using p-anisedine reagent as a detection for sugars. N-butanol:acetic acid:water (4:1:1.8)V/V was used as the developing solvent. The chromatograms were sprayed with p-anisedine reagent, then hung in an oven at 105°C for four minutes. The chromatograms were observed under ultraviolet light. Faint traces of separation were noted, but they were of no use in the separation of clones.

The second technique used in chemical analysis was thin layer chromatography (Kurt Randerath "Thin Layer Chromatography 1963").

Leaf analysis

Thin layer chromatography was performed on lower mature leaves secured from suckers previously started in the greenhouse.

The leaves were macerated in a 80% solution of ethyl alcohol, then filtered into collecting bottles. Ten ml of chloroform was added to each sample and allowed to separate. While separation was occurring, silica gel plates were prepared for spotting. The glass chromatography case was then equiliberated with a solution of proponol-water-ammonia (8:1:1)V/V for one hour. After separation had occurred the top clear layer was extracted by means of a lamda pipette. Five drops per sample were spotted upon the glass plate. The plate was then lowered gently into the cabinet. Two sides of the cabinet were lined with filter paper to avoid atmospheric interference. After the solution had reached the top of the plates they were dried and examined under ultraviolet light. Separation occurred for each sample but no differences were observed between samples.

DISCUSSION AND CONCLUSIONS

Preliminary observations have indicated that morphological and phenological characteristics can be used to differentiate between clones in the field. Chemical analysis of leaf tissues, by means of layer and paper chromatography to confirm field observations has not been successful.

Observations have further indicated that different clones may possess different production potentials. Growth variation found between clones in this study should however not be attributed to the inherent properties of the clones only, since site no doubt has attributed to the observed growth differences. In the present study the role of site was however not assessed.

Observations on root development and spread tend to indicate that clonal boundaries may fluctuate considerably from one generation to another depending on the specific environmental conditions at the time of suckering.

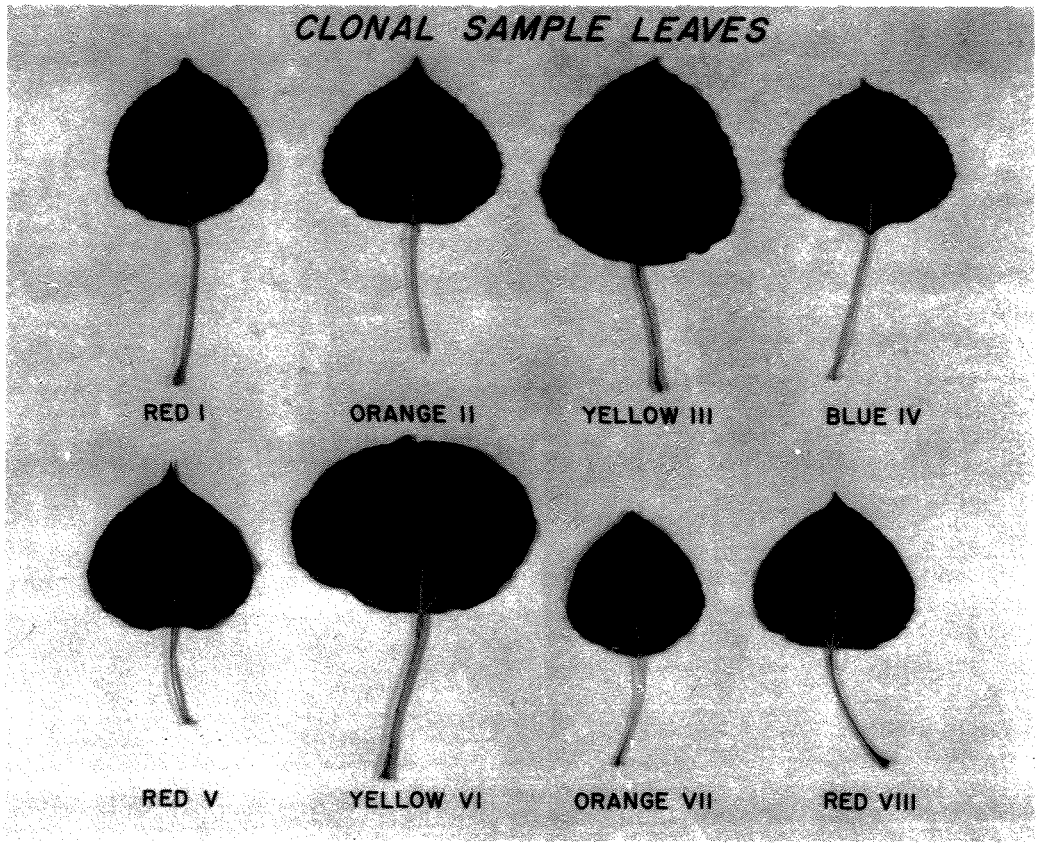
REFERENCES

Hills, G.A. 1952. The classification and evaluation of site for forestry. Ont. Dept. of Lands and Forests. Div. of Res. Dept. 24.

Rowe, J.S. 1959. Forest Regions of Canada. C.D.N.A. and N.R., Bulletin 1: 23.

Waldron, R.M. 1961. Seedbed Preparation for White Spruce Regeneration in the White Spruce-Aspen Stands of Manitoba. Progress Reports MS-124 and MS-156.

Aerial view of aspen clones along the Audy Lake Road, including those shown in fig. 1.



Leaf samples of eight clones mapped along the Audy Lake Road. Note difference in leaf shape such as leaf base, leaf tip, width versis length of leaf blade and serration of leaf edge.

TABLE 1

AREA OF THE AUDY LAKE CLONES AS SHOWN IN FIGURE 1.

Red I	.3996
Orange II	.0888
Yellow III	.0699
Blue IV	.7503
Red V	.6993
Yellow VI	.0810
Orange VII	.1609
Red VIII	.1398
TOTAL	2.3896 or 2.4 acres

TABLE 2

AVERAGE* LEAF DATA PER CLONE

Clone No.	Length of petiole (mm.)	Length of leaf blade (mm.)	Width of leaf blade (mm.)	Max. width of leaf blade (mm.)
1	31.90	45.77	35.87	32.85
2	29.06	45.66	45.10	41.73
3	39.06	34.55	38.00	34.70
4	34.12	53.37	55.27	50.50
5	34.07	49.37	49.67	43.50
6	38.70	54.33	57.43	53.46
7	33.60	45.16	39.30	35.53
8	47.30	52.90	47.80	43.60

* Average values based on 30 observations.

TABLE 3

AVERAGE HEIGHT AND DIAMETER DATA OF THE EIGHT CLONES SAMPLED

Clone No.	Avg. ht.* /clone (ft.)	Avg. dbh (in.)
1	33.1	5.1
2	27.8	5.5
3	41.4	5.4
4	44.2	5.4
5	42.5	5.8
6	47.7	5.6
7	47.1	5.8
8	45.4	6.1

* Clone No. 3 contained one plot and clone No. 4 and 5 contained three plots. Data from other clones are based on two plots.

FIG. 1

Spatial extent of a number of clones along the Audy Lake Road, Riding Mountain National Park

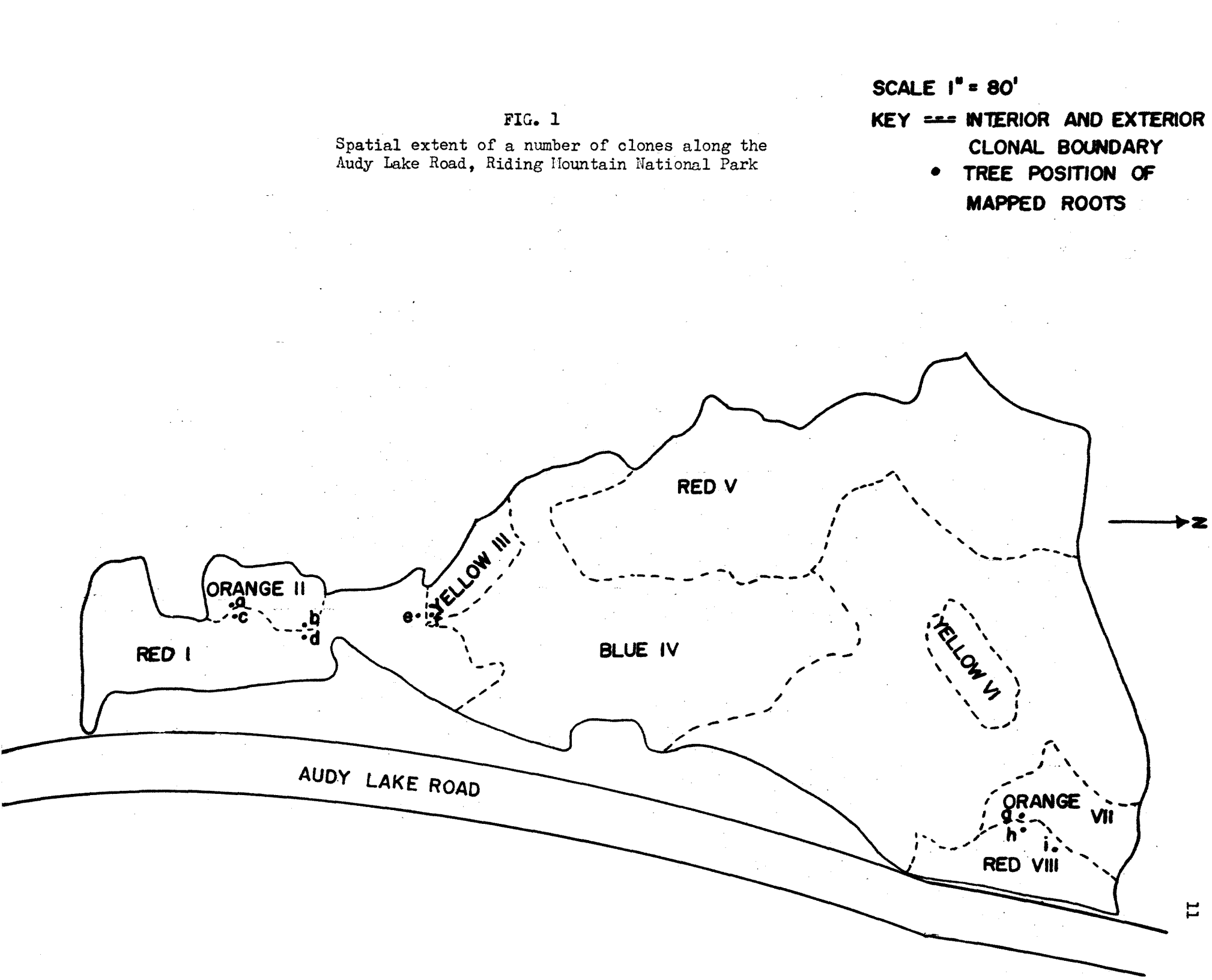
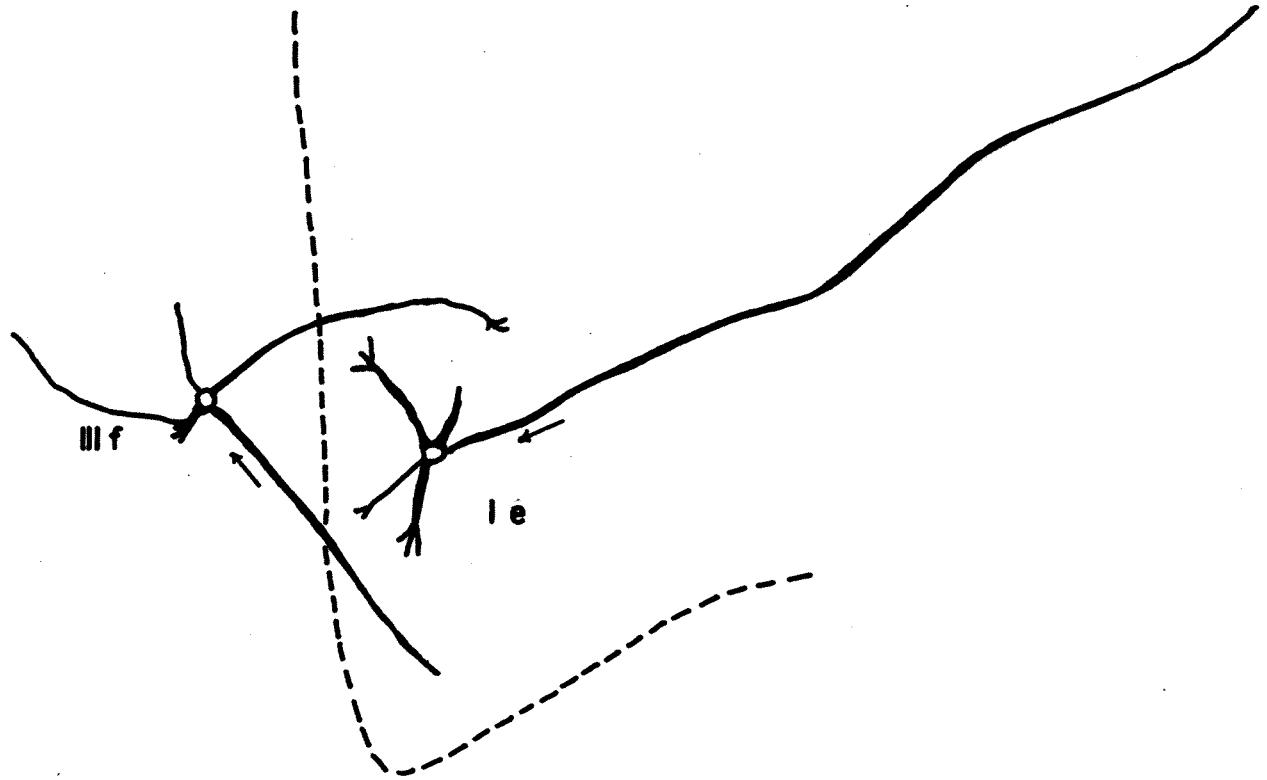


FIG. 2

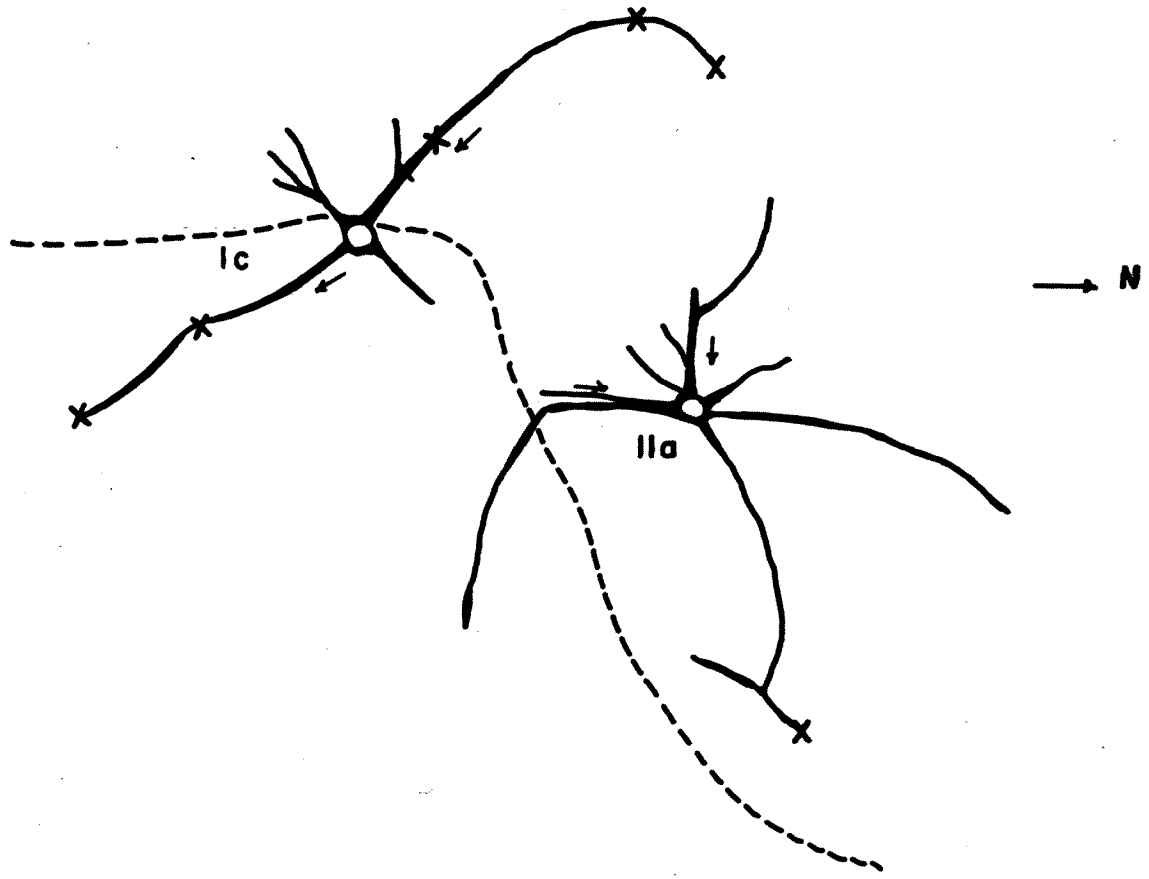
SCALE 1"= 5'
KEY --- CLONAL BOUNDARY
X DEAD STUMP
O LIVE TREE
→ ORIGINAL DIRECTION OF NUTRIENT FLOW



TREE NO. f AND e, CLONE NO. III AND I, 30 YEAR OLD ASPEN, AUDY LAKE ROAD, RIDING MOUNTAIN

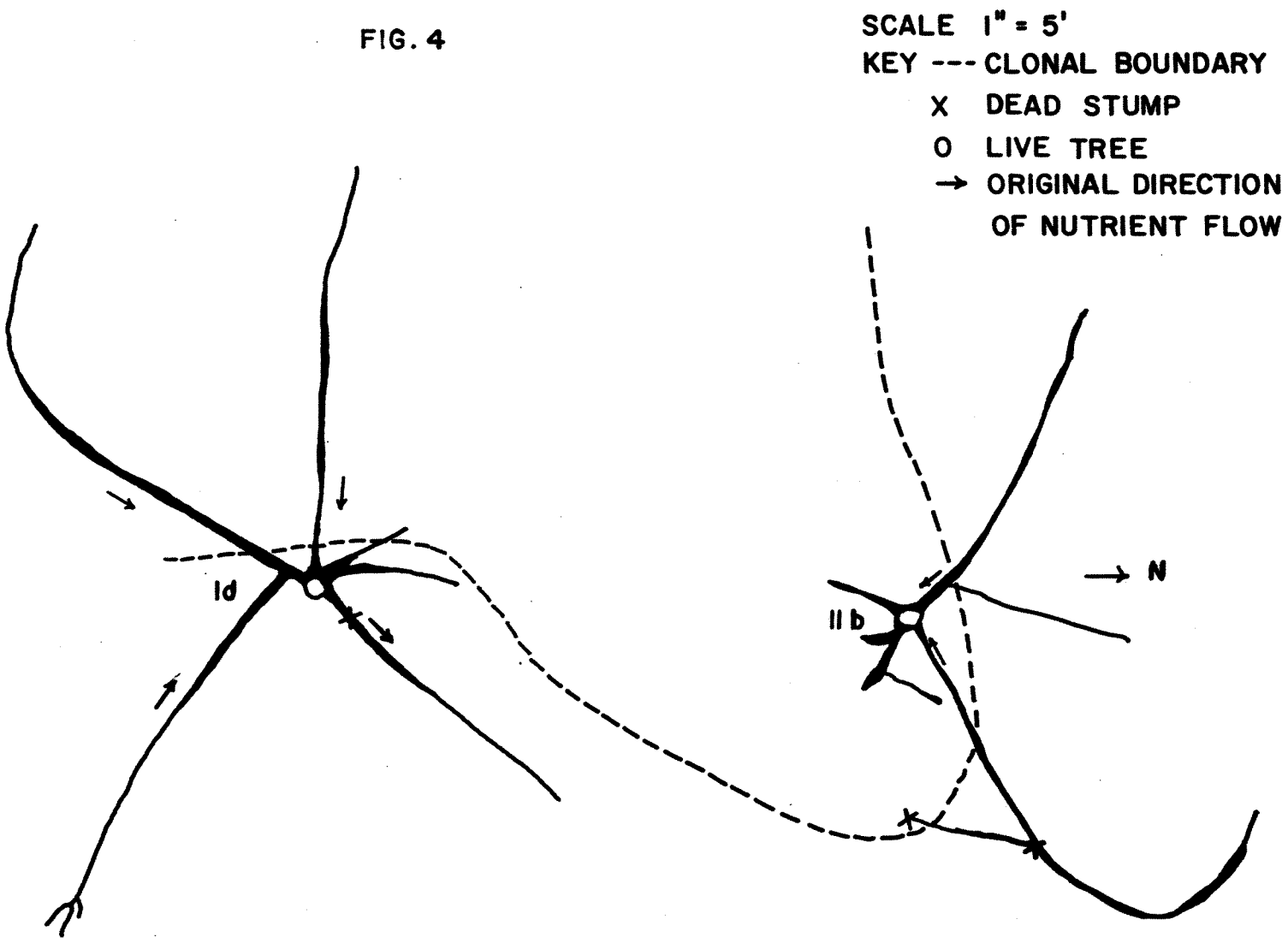
FIG. 3

SCALE 1" = 5'
KEY ---CLONAL BOUNDARY
X DEAD STUMP
O LIVE TREE
→ ORIGINAL DIRECTION OF NUTRIENT FLOW



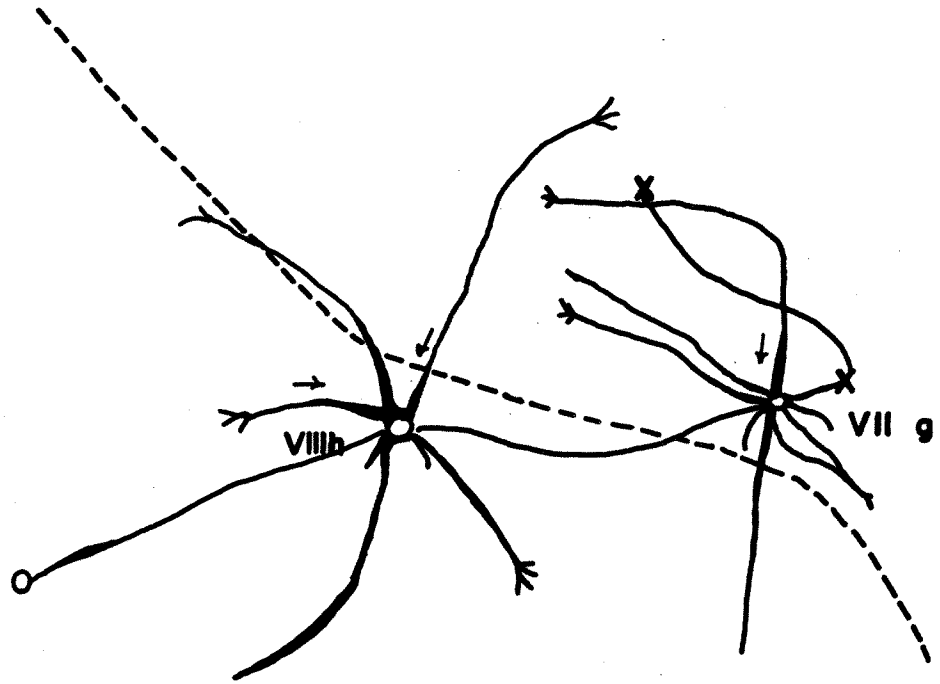
TREE NO. c AND a , CLONE NO. I AND II, 30 YEAR OLD ASPEN, AUDY LAKE ROAD, RIDING MOUNTAIN

FIG. 4



TREE NO. d AND b , CLONE NO. I AND II, 30 YEAR OLD ASPEN, AUDY LAKE ROAD, RIDING MOUNTAIN

FIG. 5



SCALE 1" = 5'

KEY ---CLONAL BOUNDARY

X DEAD STUMP

O LIVE TREE

→ ORIGINAL DIRECTION OF NUTRIENT FLOW

→ N

TREE NO. g AND h , CLONE NO. VII AND VIII, 31 YEAR OLD ASPEN, AUDY LAKE ROAD, RIDING MOUNTAIN

FIG. 6

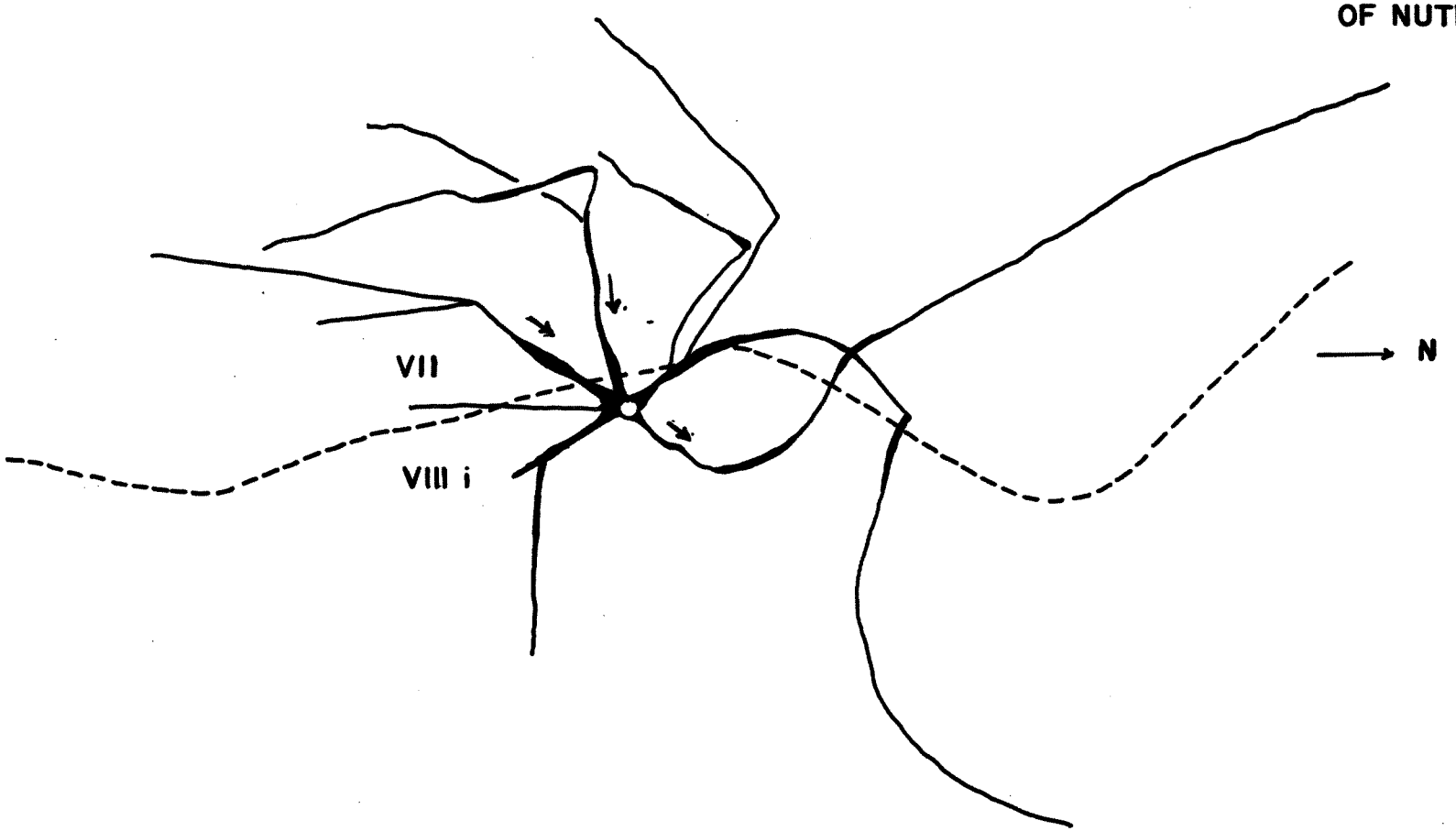
SCALE 1" = 5'

KEY --- CLONAL BOUNDARY

X DEAD STUMP

O LIVE TREE

→ ORIGINAL DIRECTION OF NUTRIENT FLOW



TREE NUMBER i , CLONE NO. VII AND VIII, 30 YEAR OLD ASPEN, AUDY LAKE ROAD, RIDING MOUNTAIN