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THE ELECTRICAL RESISTANCE PROPERTIES OF TREE TISSUES IN CANKERS INCITED BY ENDOTHIA PARASITICA AND NECTRIA GALLIGENA

A Thesis Presented

By

David Martin Sylvia

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125

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August 1977

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ABSTRACT

Electrical resistance (ER) measurements were taken with a Shigometer across tree cankers incited by *Endothia parasitica* and *Nectria galligena*. Measurements taken along the vertical axis of the stem were at 2 cm intervals, from 8 cm above the canker margin to 8 cm below the canker. Three zones of distinct electrical character were distinguished. The canker margin exhibited decreased ER as compared to healthy tissue, whereas ER increased substantially in the canker face. Moisture content, mobile cation concentration, and the path of current flow were all found to contribute to ER. Multiple regression analysis indicate fundamental changes in the variables contributing to ER in summer and winter samples. Strong, inverse relationships were found between ER and total ash and cation concentrations in September. Conversely, February samples exhibited a strong, inverse correlation between ER and moisture content. These data indicate that moisture is the limiting factor in ER measurements of stem tissue in the winter. Attempts to monitor canker progression on seedlings through the use of permanent electrodes yielded mixed results. The inconclusiveness of these data were attributed to the small sample size and discoloration noted around the electrodes.

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INTRODUCTION

Canker diseases have played a major role in shaping the character of the forest in the northeastern United States. Chestnut blight caused by Endothia parasitica (Murr.) Anderson, and Nectria canker caused by Nectria galligena Bres. are among the most important diseases of this type. Both these canker diseases are characterized by the killing of the bark and cambial tissues. Chestnut blight involves a rapid killing of host tissue which results in a diffuse canker, soon girdling the tree (French and Cowling 1972). Nectria canker, however, is characterized by the slow killing of well-defined, localized areas of bark and cambium resulting in a target shaped canker (Lortie 1964).

The use of electronic instrumentation has proven to be valuable in the study of plant diseases (Tattar and Blanchard 1976). Electrical measurements of trees have been used to detect discoloration and decay of woody stems and roots (Miller-Jones et al. 1977; Tattar et al. 1972; Shigo and Berry 1975) and vascular wilt diseases (Tattar 1976), as well as to study physiological activity of the cambial region (Zhurauleva 1972) and response to various stresses (Newbanks and Tattar 1977; Wargo and Skutt 1975; Polozhentsev and Zolatov 1970). The advantage of these studies are that they have combined the potential for rapid detection with a relatively nondestructive technique.

Most injury to plant tissue involves the disruption of the plasma membrane with subsequent release of electrolytes resulting in a large, local increase in ion concentration (Wheeler and Hanchey 1968). The passage of an electrical current through a dilute solution, such as is found in the apoplast, is known to depend on the movement of mobile ions (Bull 1971). This increase in ion concentration in the apoplast upon wounding or pathogen invasion results in decreased resistance to an applied current, thereby providing a basis for meaningful electrical measurements.

The development of a sensitive technique to distinguish the healthy/infected interface in the canker margin could be useful in the control and study of canker diseases. Bark excision has been recommended for the control of many canker diseases (Nichols 1974). However, ample marginal tissue must be removed to insure eradication (Matthews _et_ _al_. 1976). The larger the excision the longer the time of healing and the greater the likelihood for reinfection. Accurate delineation of the margin of infected tissue by electrical techniques could prove useful in excision work. Electrical measurements may also be applied to the study of canker growth. For example, recent investigations with _E_. _parasitica_ on American chestnut, (_Castanea_ _dentata_ [Marsh.] Borkh), have indicated slowed canker development with hypovirulent strains of the fungus (Van Alfen _et_ _al_. 1975). A sensitive method to monitor canker development could facilitate the evaluation of the several hypovirulent strains, in the effort to provide a control measure for chestnut blight.

The objectives of this study were to determine the electrical resistance properties of tree tissues in naturally occurring cankers, and to investigate the factors contributing to these measurements. This preliminary study is prerequisite to the future application of ER measurements to tree canker research suggested above. In addition, we attempted to monitor canker development over an extended period after inoculation on chestnut seedlings in the greenhouse.

METHODS AND MATERIALS

Electrical Resistance Characteristics of Established Cankers

Electrical resistance (ER) measurements were taken across cankers found on five American chestnut sprouts and four birch (Betula lenta L. and B. populifolia Marsh.) saplings. Cankers were incited by Endothia parasitica and Nectria galligena respectively. Measurements were taken on September 9, 1976, on trees located in Cadwell State Forest, Pelham, Massachusetts, using a field ohmmeter (Shigometer model 7950, Northeast Electronics Co., Concord, NH.) set on the 50 kilohm (K ohm) scale. Electrodes were uninsulated stainless steel pins, 1 cm in length, set 1.5 cm apart in a plastic handle (Delmhorst Instrument Co., Boonton, NJ.). Measurement taken along the vertical axis were at 2 cm intervals, from 8 cm above the canker margin to 8 cm below the canker (Figure 1). The trees were then cut and bolts including the cankered segments were transported back to the laboratory for further analysis.

A second sample was collected February 1, 1977 from nine American chestnuts and seven black birch. Cankered segments were cut allowing at least 30 cm of healthy tissue on either side of the cankers in order to prevent dissication of the measurement area. The bolts were transported to the laboratory where they were allowed to reach room temperature before ER readings were taken. To accomodate higher ER readings the Shigometer was set on the 500 K ohm scale.

Moisture and Ion Analysis

Modifications of techniques described by Tattar et al. (1972) were used to determine moisture, total ash, and concentrations of potassium, calcium, and magnesium. Three blocks 2 x 1 x 1 cm, were excised from each canker at points corresponding to normal, low, and high ER. These blocks were immediately weighed and oven-dried at 110°C for 24h and weighed again where upon percent moisture was determined. Samples were then ashed in a muffle furnace at 450°C for 24h and percent ash calculated. Twenty ml of 1:1:8, $HCl:HNO_3:H_2O$ solution was added to each beaker containing ash and heated to boiling for 3 min. The solutions were brought to 100 ml in volumetric flasks with distilled, deionized H_2O. Concentrations of potassium, calcium, and magnesium were determined using a Perkin-Elmer 214 Atomic Absorption Spectrophotometer.

Contribution of Bark and Xylem to Total ER

To understand the interaction between xylem and bark (periderm, phloem, and cambium) tissue in relationship to ER, measurements were taken on six healthy stems (3 American chestnut and 3 black birch) ranging in diameter from 5 to 10.5 cm. The first set of ER readings were taken by inserting electrodes to a depth of 1 cm into the intact stem. A patch of bark, 3 x 6 cm, was then removed from each stem and ER determined separately for the bark and xylem.

Monitoring Canker Progression

Canker progression was monitored on nine American chestnut seedlings obtained from the Connecticut Agricultural Experiment Station, New Haven, CT. Stainless steel electrodes (insect pins) were inserted to a depth of 0.3 cm at points 15 and 20 cm above the soil on five of the stems, and 15 and 16.5 cm above the soil on the remaining four seedlings. Each pin was sealed with grafting wax.

As a preliminary step, one seedling (having 5.0 cm electrode separation) was used to observe the effects of wounding on ER. Bark patches 0.5 x 0.5 cm were removed consecutively beginning midway between the electrodes, and ER measurements recorded by attaching leads from a Shigometer to the electrodes. The final wound extended 1 cm beyond each electrode.

The remaining 8 seedlings were inoculated with cultures of <u>E</u>. <u>parasitica</u> that were either virulent and hypovirulent (EP 43, obtained from the Connecticut Agricultural Experiment Station). Inoculations were made midway between the probes using a 2 cm cork borer (Figure 2) and were immediately covered with masking tape to prevent drying. Two seedlings (one having 5.0 cm and the second 1.5 cm electrode separation) were inoculated with the virulent strain, two with the hypovirulent strain, two with virulent plus hypovirulent, and two with sterile potato dextrose agar as a control. ER readings were taken periodically for several months. At the conclusion of this period ER readings were taken across the resulting cankers at 1 cm intervals, beginning 5.0 cm above the canker margin and continuing to 5 cm below the canker. Finally, seedlings were dissected longitudinally to determine if discoloration occurred in response to the electrodes.

RESULTS

Electrical Resistance Characteristics of Established Cankers

Three zones of distinct electrical character were observed in the cankered region of each stem sampled (Figure 3). The canker margin exhibited decrease ER as compared to healthy tissue, whereas ER increased substantially in the canker face. The February sample gave higher ER measurements than did the September sample. No significant differences were observed in the patterns of electrical response between American chestnut and the two birch species sampled.

Moisture and Ion Analysis

The results of moisture and ion analyses are summarized in Table 1. Percent moisture in the canker face differed significantly (Duncan's multiple range test, P=0. 05) from moisture levels in the healthy tissue and in the canker margin. Moisture was consistently found to increase in the canker margin and then drop rapidly in the canker face.

As compared to healthy tissue, total ash and concentrations of potassium, calcium, and magnesium were higher in the canker margin for the September sample. Total ash and potassium concentrations were the highest, followed by calcium and magnesium. Similar trends were noted in total ash and potassium concentrations for the February sample, but to a lesser extent. However, concentrations of calcium and magnessium were lower in this second sample.

No apparent trends were observed in the changes in total ash or cation concentrations in the canker face.

Multiple regression analysis indicate fundamental changes in the variables influencing ER on the two sample dates. Strong, inverse relationships were found between ER and total ash and cation concentrations in September (Table 2). In healthy tissue, moisture explained less of the variance then did total ash. In the canker margin and canker face, total ash and potassium each accounted for a greater amount of variability then did moisture. Conversely, samples taken in February (Table 3) exhibited a strong, inverse relationship between ER and moisture. For all tissue types in this sample, percent moisture contributed most to the explained variance.

Contribution of Bark and Xylem to Total Electrical Resistance

The ER of the intact, healthy stem was found to be less then either the xylem or bark component alone (Figure 4). Bark tissue exhibited the highest and most variable resistance whereas xylem tissue was considerably lower in resistance. Results obtained for the intact stem approximate the value expected if the bark and xylem tissue are considered to be resistors in parallel. If two resistors are connected in parallel the total effective resistance is given by:

$$R_{Total} = \frac{R_B\, R_X}{R_B + R_X}$$

where R_B = bark tissue and R_X = xylem (Simpson, 1974).

Attempt to Monitor Canker Progression

Increasing wound size resulted in a corresponding rise in ER during the preliminary study (Table 4). Based on these data, we intended to correlate the rate of canker development with ER.

The first set of measurements (Figure 5A) were taken on stems with 5 cm electrode separation. Electrical resistance measurements for the hypovirulent treatment closely parallel the control. However, the control exhibited no increase in necrotic tissue, whereas the hypovirulent canker had expanded 3.5 cm and extended approximately halfway around the stem by the conclusion of the experimental period. The virulent and virulent-hypovirulent combined treatments also followed a parallel pattern. In these two treatments the cankers extended beyond the electrodes at the conclusion of the experiment and the stems were girdled. Both cankers grew at approximately equal rates with the canker margins reaching the electrodes at day 70. At this point a decrease of 15 k ohms in the ER of the combined treatment was noted, yet no corresponding decrease in the virulent treatment was observed. There was a steady increase in ER for these two treatments following the passage of the canker margin through the electrodes. Daily fluctuations in ER which paralleled the control treatment were attributed to variations in greenhouse temperature.

A second series of measurements were taken on stems having 1.5 cm electrode separation (Figure 5B). In this case all treatments paralleled the control which exhibited a steady increase in ER. Only the virulent treatment produced a canker which spread past the embedded electrodes. The passage of the canker margin through the electrodes coincided with a decrease in ER on day 26.

ER measurements taken across the resulting cankers on these seedlings yielded data similar to measurements on established cankers in the field. The canker margin showed a decrease in ER when compared to healthy tissue, whereas ER rose rapidly in the canker face (Table 5).

Dissection of the stem revealed discoloration around the electrodes in all cases. Discolored wood extended 1.0 to 5.0 mm from the electrode (Figure 6).

DISCUSSION

Cankers were found to change the ER properties of woody stems. Decreases in ER were correlated with the canker margins of chestnut blight and Nectria canker. Increases in both moisture content and mobile cation concentrations were found to contribute to this response. Seasonal variations in the physiology of the tree were reflected in the tissue analysis for moisture and cations. For the summer sample (September), moisture levels and cation concentration were generally higher then for the winter (February), thereby accounting for lower ER readings during the summer.

Moisture is known to be the medium for ion movement Tattar _et_ _al_. (1972) have reported that moisture levels in the xylem only affect resistance when it reaches the point where free water is limiting, ie. near the fiber saturation point. However, our findings indicate that moisture also plays an important role in ER at substantially higher levels. Multiple regression analysis of the variables contributing to ER suggest that moisture became the limiting factor during the winter, yet most samples had moisture levels considerably greater then 30%. Moisture also appears to be the most significant variable in determining ER in the canker face. In this case also, moisture levels were greater than fiber saturation for most samples. Bier (1964) has reported a close correlation between the development of bark cankers and the moisture content of living bark. Our results indicate that the ER of woody stems during the winter months is greatly influenced by moisture. Further research relating

ER of dormant trees to the incidence of cankers may provide a new technique to evaluate disease susceptibility.

When moisture is not a limiting factor, the concentration of mobile ions can be readily correlated with ER, which is in agreement with a previous investigation (Tattar et al. 1972). Presumbly, the activities of fungal mycelium in the canker margin kills living cells, resulting in the release of electrolytes into apoplastic fluids. In the summer sample, concentrations of total ash, potassium, and calcium increased substantially in the canker margin. Magnesium was excluded from the regression analysis because this cation exists in a hydrated form which is too large to carry any significant current (Bull 1971).

The path of current flow, long with moisture content and mobile ion concentration, will contribute to ER. Glerum and Krenciglowa (1970) reported that healthy stem tissue could be modeled by a simplified circuit which considered the bark and xylem components as resistors connected in parallel. Considering the case of tree cankers, viable bark tissue is found in healthy and margin tissue and therefore, the standard model for parallel resistors (Figure 7A) may be applied. This implies that the total resistance will be less than either component individually. The canker face, however, no longer has viable bark tissue and one would expect the ER to rise to that inherent in the bark alone (Figure 7B).

Vary degrees of difficulty were encountered in taking ER measurements due to the development of callus tissue. Chestnut blight produces a rapidly growing canker which prevents callus development therefore allowing ER to be measured easily. In contrast, Nectria canker is a slow growing canker providing the host opportunity to produce a distinct callus ridge. This callus tissue interfered with ER measurements in the canker margin. However, removal of excessive callus tissue with a hatchet allowed measurement of the cambial zone in the canker margin. The physical properties of the canker face in Nectria canker also presented difficulties to ER measurements. This tissue was very resistant to electrode penetration and therefore, readings in this zone were taken without full insertion (1 cm) of electrodes.

Attempts to monitor canker progression on seedlings through the use of permanent electrodes yielded mixed results. We had expected to observe a steady rise in ER as the cankers expanded, with a slight decrease when the canker margins passed by the electrodes. The inconclusiveness of the data may be partially attributed to the sample size as only a small number of seedlings were available for this study. Another factor involved was the discoloration noted around the electrodes. This response may have well masked the expected patterns.

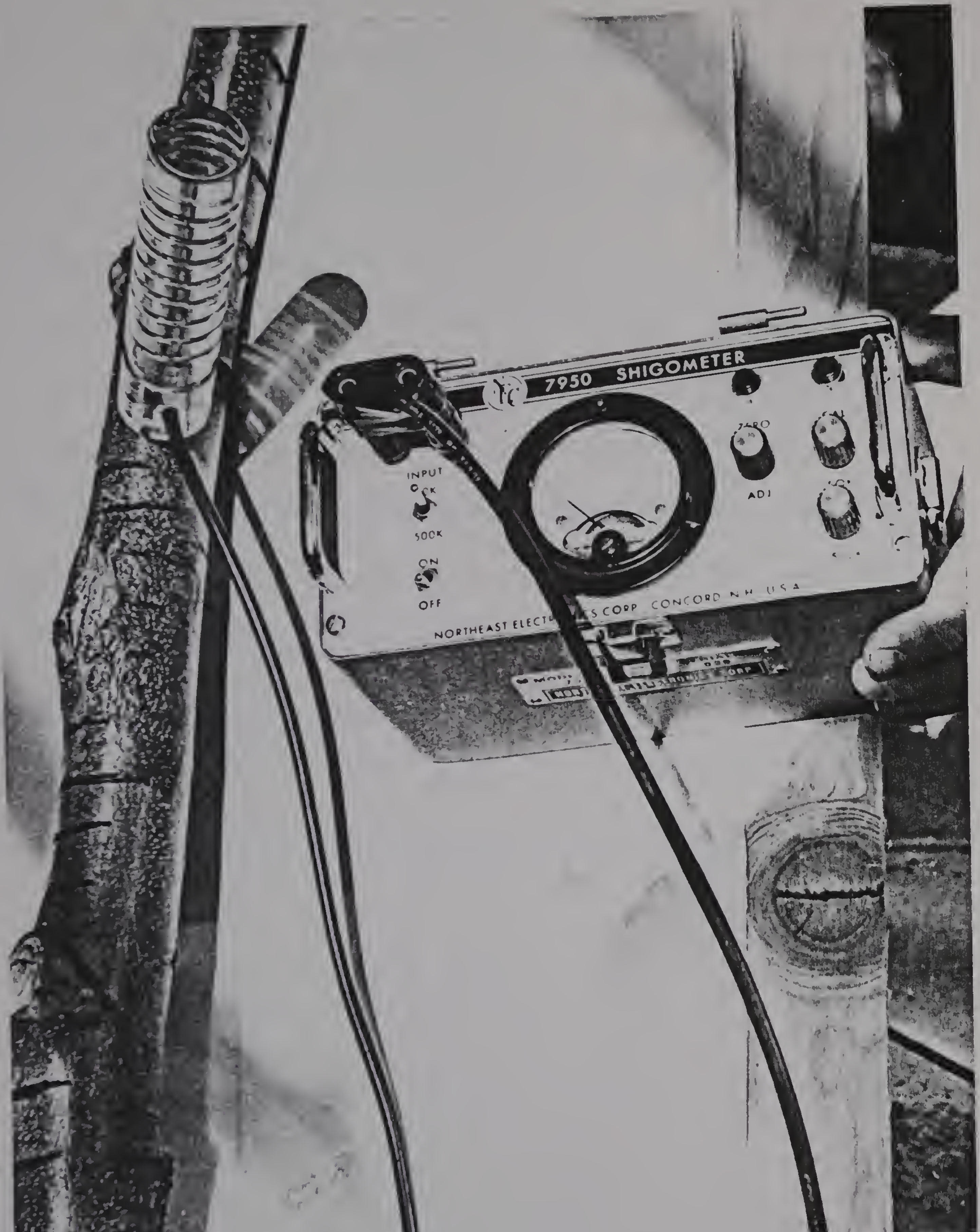

7950 SHIGOMETER
INPUT
500K
ON
OFF
ZERO
ADJ
CAL
NORTHEAST ELECTR S CORP. CONCORD N.H. USA

Figure 1. Cankered American chestnut stem with Shigometer and electrodes, indicating technique used to obtain electrical resistance measurements on established cankers.


DS
HH
4A

Figure 2. Preparation of American chestnut seedling for canker progression study. This seedling was inoculated with a hypovirulent strain of *Endothia parasitica*.

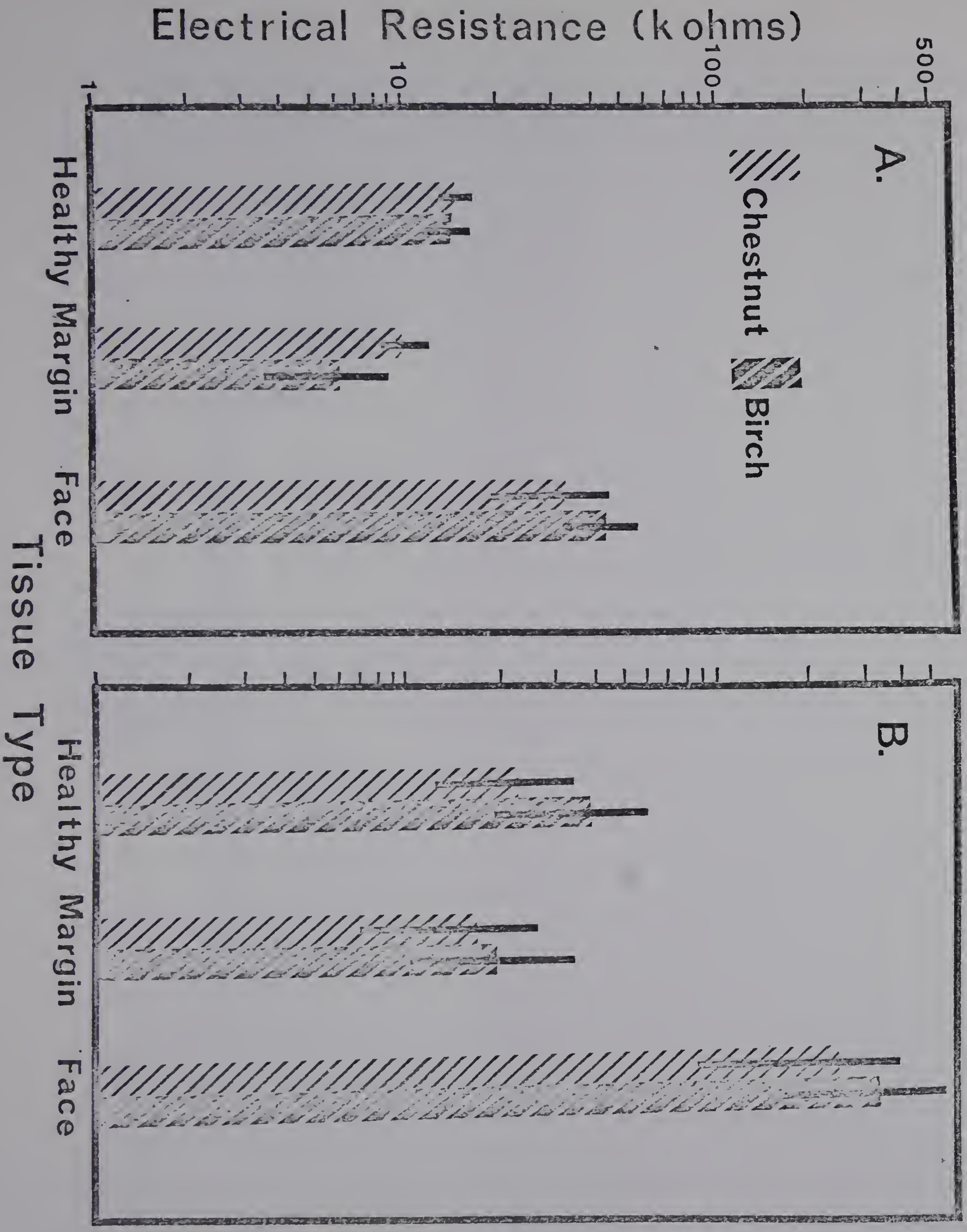

Electrical Resistance (kohms)
1
10
100
500
A.
Chestnut
Birch
Healthy
Margin
Face
B.
Healthy
Margin
Face
Tissue Type

Figure 3. Mean electrical resistance of healthy, margin, and face tissue in the cankered region of woody stems for (A) September and (B) February. Means are based on at least 4 measurements. The 95% confidence limits are reported.

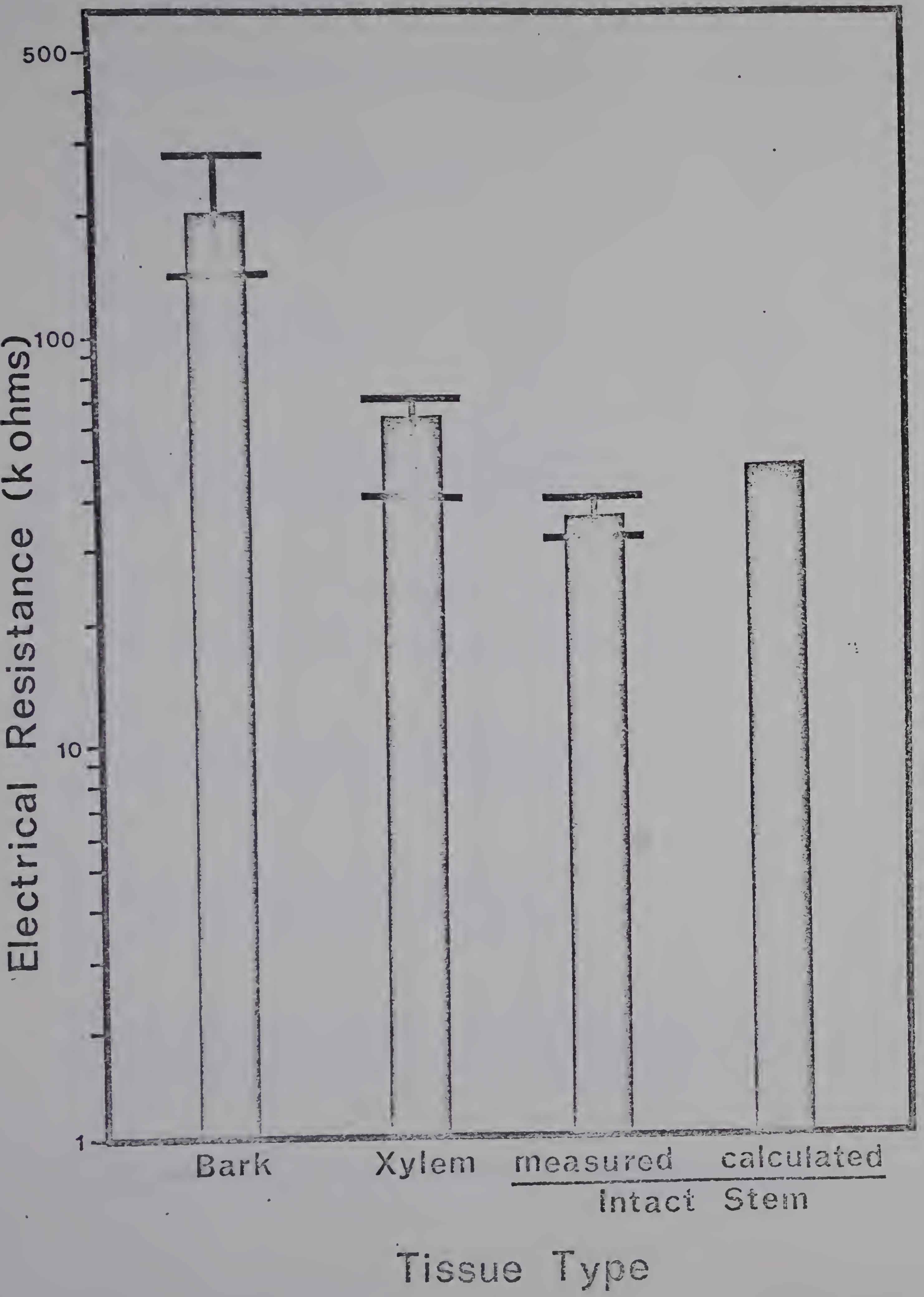

500
100
10
1
Electrical Resistance (k ohms)
Bark
Xylem
measured
calculated
Intact Stem
Tissue Type

Figure 4. Mean electrical resistance of the healthy bark, xylem, and intact stem. Calculated value for the electrical resistance of the intact stem obtained by treating bark and xylem components as parallel resistors. Means based on 6 measurements. The 95% confidence limits are reported.

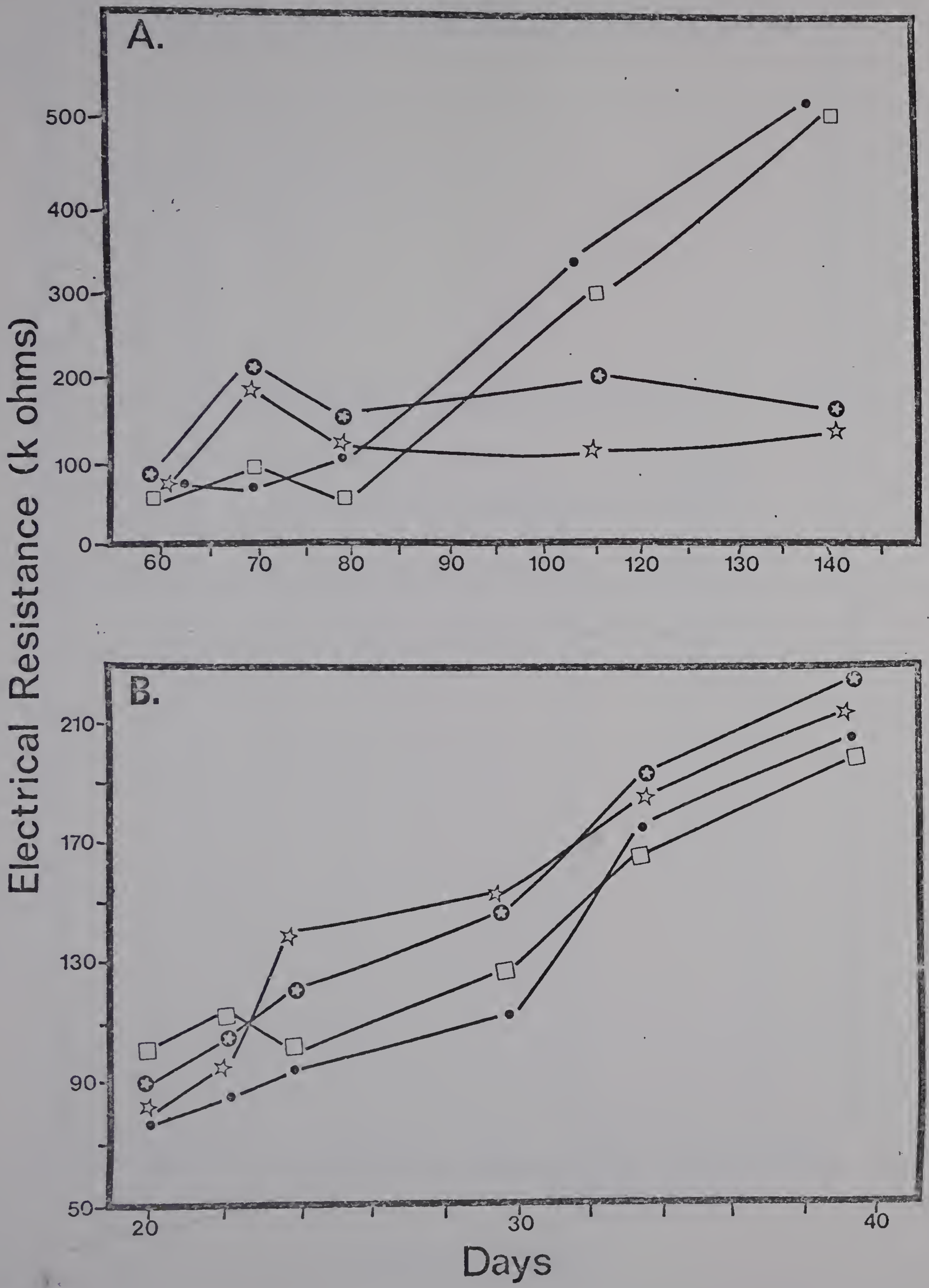
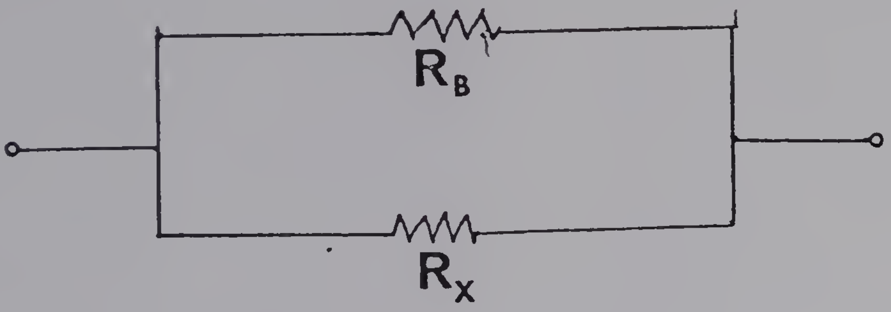

A.
B.
Electrical Resistance (k ohms)
0
100
200
300
400
500
60
70
80
90
100
120
130
140
50
90
130
170
210
20
30
40
Days

Figure 5. Progression of electrical resistance across inoculations on American chestnut seedlings having (A) 5.0 cm and (B) 1.5 cm electrode separation. Treatments were control (✪), virulent (□), hypovirulent (☆), and virulent-hypovirulent combined (●).

Figure 6. Longitudinal section through seedling showing internal discoloration around permanent, stainless steel electrode wound. Electrodes were embedded in the stem up to 140 days before dissection.

A.

HEALTHY + MARGIN TISSUE



$$\frac{1}{R_T} = \frac{1}{R_B} + \frac{1}{R_X}$$

B.

CANKER FACE

$$R_T \simeq R_X$$

Figure 7. Electrical models indicating the path of current flow in (A) healthy and margin tissue and (B) in the canker face. R_B= ER of bark (periderm, phloem, and cambium), R_X=ER of xylem, and R_T= total ER.

Table 1. Relationship of electrical resistance to moisture, total ash, and concentrations of potassium, calcium, and magnessium in healthy, margin, and face tissue of cankered stems

A) September, 1976 sample*

Species	Tissue type	Resistance (k ohms)	Percent				
			moisture	total ash	K	Ca	Mg
Chestnut	Healthy	14.0	96.9	1.84	0.182	0.512	0.049
	Margin	10.1	99.0	1.96	0.374	0.548	0.050
	Face	32.6	63.8	2.17	0.374	0.450	0.040
Birch	Healthy	13.9	55.2	2.52	0.108	0.710	0.035
	Margin	6.0	63.1	4.30	0.792	0.965	0.075
	Face	42.5	38.4	4.40	0.915	0.775	0.185
B) Feburary, 1977 sample[t]							
Chestnut	Healthy	22.4	94.8	1.26	0.151	0.870	0.049
	Margin	16.3	93.6	1.30	0.166	0.839	0.047
	Face	237.5	48.3	1.18	0.137	0.497	0.049
Birch	Healthy	38.4	50.7	2.40	0.063	1.370	0.042
	Margin	18.8	56.7	2.61	0.186	1.160	0.059
	Face	324.3	30.1	1.89	0.340	0.646	0.129

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* Means based on atleast 4 observations.

[t] Means based on atleast 7 observations.

Table 2. Multiple regression showing the relationship between electrical resistance and percent moisture, total ash, potassium and calcium by tissue type. The order of inclusion is determined by the respective contribution of each variable to the explained variance. September, 1976 sample.

Tissue Type	Variable	Multiple R	R Squared	Change in R Squared	Simple R
Healthy	ash	.769	.591	.591	-.767
	moisture	.883	.780	.189	.161
	K	.895	.802	.021	-.564
	Ca	.896	.803	.001	-.327
Margin	Ash	.806	.649	.649	-.805
	K	.869	.754	.105	-.796
	moisture	.901	.812	.058	.674
	Ca	.962	.925	.112	-.295
Face	K	.513	.263	.263	.513
	ash	.647	.418	.155	.094
	moisture	.677	.458	.040	-.512

Table 3. Multiple regression showing the relationship between electrical resistance and percent moisture, total ash, potassium and calcium by tissue type. The order of inclusion is determined by the respective contribution of each variable to the explained variance. Feburary, 1977 sample.

Tissue Type	Variable	Multiple R	R Squared	Change in R Squared	Simple R
Healthy	moisture	.722	.522	.522	-.723
	Ca	.789	.622	.100	.032
	K	.828	.685	.062	-.318
	ash	.840	.705	.020	.180
Margin	moisture	.406	.165	.165	-.406
	ash	.498	.248	.084	.054
	Ca	.553	.306	.057	.375
	K	.583	.340	.034	-.007
Face	moisture	.756	.571	.571	-.756
	Ca	.833	.693	.122	.549
	K	.856	.732	.038	.510
	ash	.879	.773	.041	.292

Table 4. Change in electrical resistance with increasing wound size on an American chestnut seedling.

Wound size (cm)	Resistance (k ohms)
0	115
0.5	110
1.0	115
1.5	120
2.0	130
2.5	135
3.0	150
3.5	165
4.0	165
4.5	170
5.0	170
5.5	195
6.0	220
6.5	240
7.0	240

Table 5. Mean electrical resistance of healthy, margin, and face tissue of cankered American chestnut seedlings.[*]

Tissue type	Resistance (k ohms)
Healthy	19.1 ± 2.6
Margin	13.2 ± 3.5
Face	>50.0 [t]

[*] Means based on five samples. The 95% confidence limits are reported.

[t] All readings exceed the 50 k ohm maximum of the scale used.

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