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TECHNICAL MANUSCRIPT 528

ABCISSION: QUANTITATIVE MEASUREMENT
WITH A RECORDING ABSCISSOR

Lyle E. Craker
Frederick B. Abeles

MAY 1969

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ABSCISSION: QUANTITATIVE MEASUREMENT
WITH A RECORDING ABSCISSOR

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PLANT SCIENCES LABORATORIES

Project 1B562602A061

May 1969

ACKNOWLEDGMENTS

The authors thank Gerald R. Leather and Leonard E. Forrence for assistance during the performance of these experiments. The bean seeds were gifts from the following: W.J. Zaumeyer, USDA, Crops Division, Beltsville, Md.; Ferry Morse Seed Co. Inc., Mountain View, California; R. Jordan, Agway Inc., Buffalo, New York; M. LeBaron, Branch Experimental Station, University of Idaho, Kimberly, Idaho; T. Kiely, Charter Seed Co., Twin Falls, Idaho; and W.R. Fancher, Agway Inc., Canandaigua, New York.

ABSTRACT

The construction, operation, and effectiveness of an abscission-measuring instrument, an abscissor, are described. The device measured the force required for a spring-opposed plunger to shear abscission zone explants and was capable of automatically recording break strength data. Examples of data obtained with the abscissor are presented to demonstrate its capability to rapidly measure significant changes in explant break strength.

CONTENTS

Acknowledgments	2
Abstract	2
I. INTRODUCTION	5
II. MATERIALS AND METHODS	5
A. Plant Materials	5
B. Cellulase Determinations	5
C. Construction and Operation of the Abscissor	6
III. RESULTS AND DISCUSSION	10
IV. CONCLUSIONS	13
Literature Cited	15
Distribution List	17
DD Form 1473	19

FIGURES

1. Construction Details and Schematic of the Recording Abscissor . .	7
2. Calibration Curve Showing Relationship Between Force and Displacement of the Abscissor Plunger as Measured by a Recording Potentiometer	8
3. Methods Used to Apply Force with the Abscissor Plunger Against Bean and Cotton Explants Held in the Explant Vise	9
4. Change in Break Strength and Cellulase Content of Bean Abscission Zone Explants	12

TABLE

1. Break Strength Force for Separation of Explants from Bean Varieties after 30 Hours with or without Ethylene	10
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I. INTRODUCTION*

Two approaches have been used to obtain quantitative data on abscission: (i) using pressure applicators that tell the operator the number of abscission zone explants that will separate at some predetermined force and (ii) measuring the force required to separate plant parts as a result of a pulling or shearing force. Examples of the first group of instruments are the pressure applicators described by Mitchell and Livingston¹ and Addicott et al.² The second group includes the simple but effective laboratory balance,³ the modified Chatillon force measuring device for measuring fruit abscission,^{4,5} and the Instron linear stress-strain analyzer.⁶ The present paper describes the construction and operation of a simple self-recording abscissor that measures the force required for a spring-opposed plunger to shear abscission zone explants.

II. MATERIALS AND METHODS

A. PLANT MATERIALS

The methods used to grow bean (Phaseolus vulgaris L. cv. Red Kidney) and cotton (Gossypium hirsutum L. cv. Acala 4-42) plants and to prepare and store explants have been described earlier.⁷⁻⁹ In the experiment in which the effect of ethylene on the break strength of explants from 18 varieties of P. vulgaris was tested, the plants were grown in the greenhouse during January and February 1968. Supplementary lights (150-watt incandescent bulbs spaced 4 meters apart and 2 meters above the bench) were used to give a 16-hour photoperiod. Seeds were planted in 10-cm-diameter pots filled with soil, and the seedlings were grown for 14 days before explants were excised.

B. CELLULASE DETERMINATIONS

Bean explants were inserted in 1.5% agar in petri plates and placed in a desiccator containing 10 ppm ethylene. Details of the techniques utilized to add ethylene to desiccators have been described earlier.¹⁰ At the times indicated in Figure 4, explants were withdrawn and frozen until analyzed for cellulase. Cellulase was extracted by homogenizing ten separation layers with 4 ml of 0.05 M potassium phosphate buffer,

* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

pH 7, in a Ten Broeck homogenizer. The homogenate was filtered through Miracloth* and centrifuged at 10,000 x g for 10 minutes. A 1-ml portion of the supernatant fluid was analyzed for cellulase by adding it to 1 ml of 1.5% sodium carboxymethyl cellulose (CMC) and measuring the viscosity of 1 ml of the mixture after a 16-hour incubation at 40 C. A model LVT Wells-Brookfield microviscometer** measured the viscosity of the CMC. Details of this method have been presented earlier.¹⁰

C. CONSTRUCTION AND OPERATION OF THE ABCISSOR

A Plexiglas abscissor measured the break strength of abscission zone explants. Details of its construction and a wiring schematic are shown in Figure 1. The resistance of a decade resistance box was set so that the output across the ends of a wire-wound resistor (Ohmite Dividohm vitreous enameled adjustable resistor) was 250 mv. Other values can be used depending on the input of the recorder. The potential across the resistor was measured between one end of the resistor and the brush by a recording potentiometer. The brush was attached to a spring-opposed plunger and the amount of force required to push the plunger a fixed distance was regulated by the resistance of the spring. By inserting springs of different strengths between the plunger and cap of the abscissor, various force ranges were obtained.

Figure 2 shows calibration data indicating a straight line relationship between the force against the plunger and the output of the recorder. This curve was obtained by placing a known force on the face of the plunger held in a vertical position and reading the deflection on the recorder. The curve intercepts the abscissa at 25 g in this example because a finite amount of force is required to overcome the force of the spring holding the plunger against the front adjusting screw. The distance traveled by the steel brush can be set by adjusting screws at each end of the slot in which the brass brush holder moves. Electrical contact to the brush was made by taut copper slide wires attached to these adjusting screws.

An explant-holding vise consisted of two pieces of Plexiglas 0.9 cm wide by 5.1 cm high by 51 cm long held together at their 5.1-cm faces by bolts fitted with wing nuts. Steel guide pins set in the 5.1-cm side of one piece of Plexiglas were inserted into holes in the other piece of Plexiglas to insure proper alignment. Holes centered on the crack between the two pieces of Plexiglas were drilled along the length of the vise. To accommodate explants of different sizes, 2.5 mm, 3.5 mm, or 4 mm holes were used. The explant vise was held with the long axis horizontal by a clamp fastened to the laboratory bench.

* CalBiochem Corp.

** Brookfield Engineering Laboratories, Stoughton, Massachusetts.

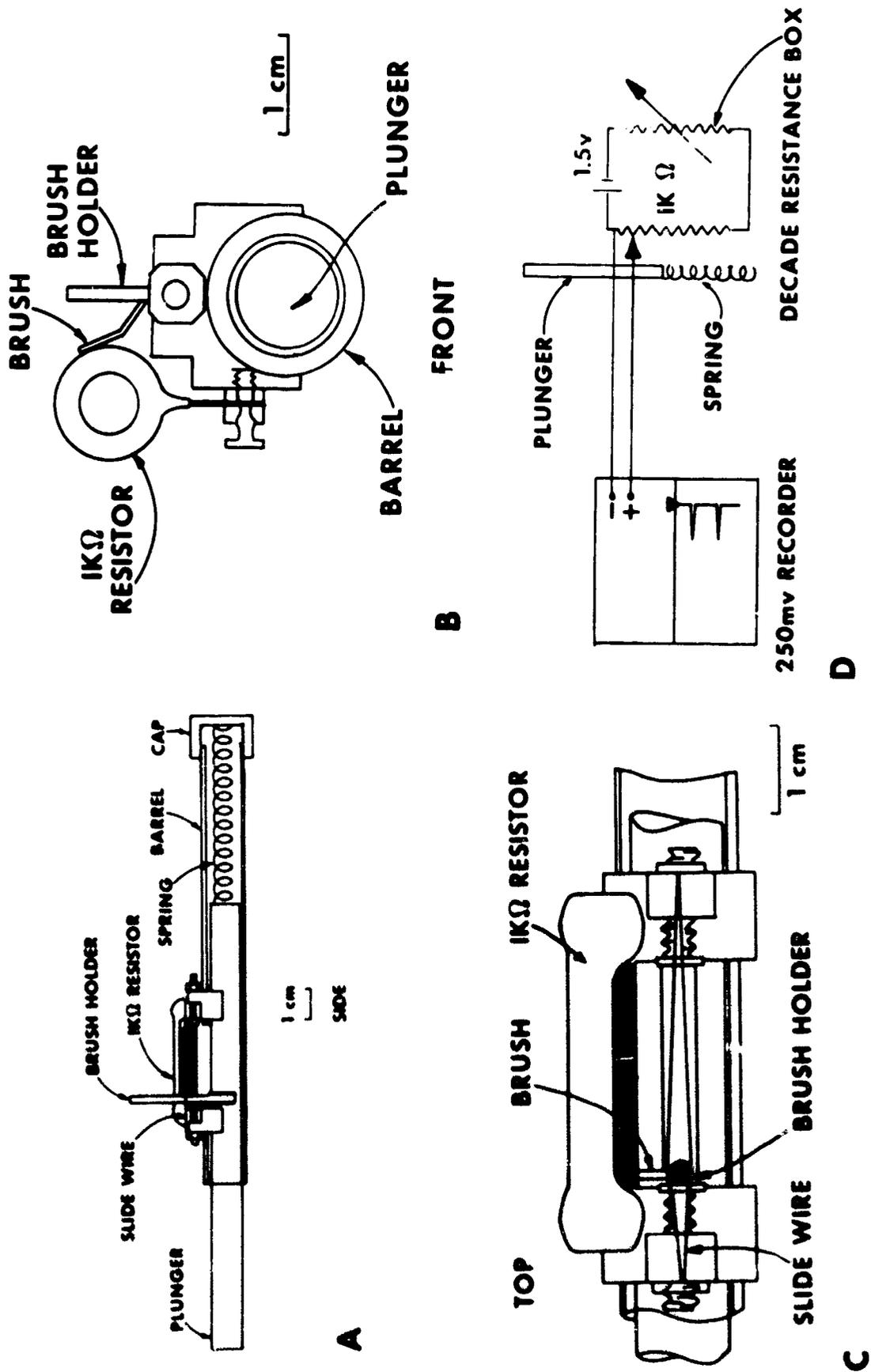


FIGURE 1. Construction Details and Schematic of the Recording Abscissor. The 1-cm line indicates the scale of the drawing. A, Overall view of the abscissor; B, front view of the abscissor; C, top view of the abscissor; D, schematic of the abscissor.

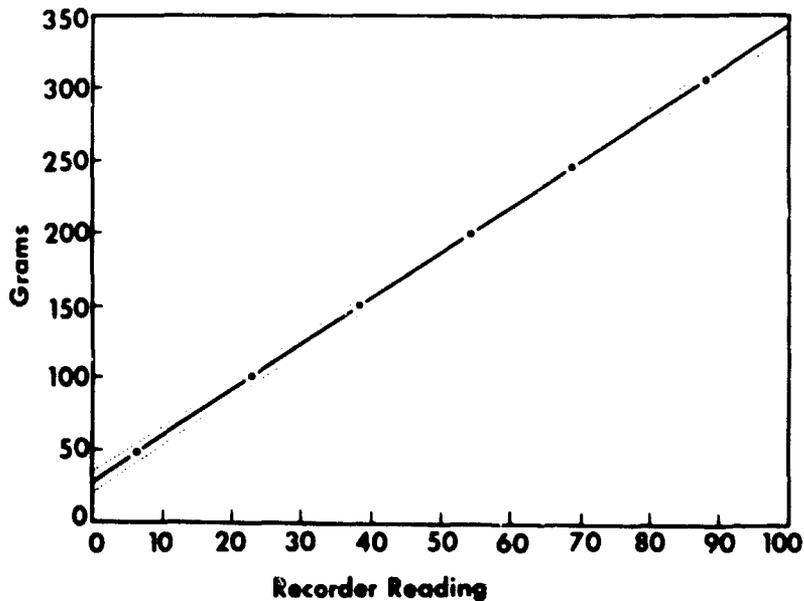


FIGURE 2. Calibration Curve Showing Relationship Between Force and Displacement of the Abscissor Plunger as Measured by a Recording Potentiometer. Dashed lines indicate 5% confidence limits.

The break strength of the explants was measured by placing the abscissor plunger against the explants (Fig. 3) and pushing it slowly and steadily until the explants separated. The speed at which the plunger spring was compressed was limited by the speed at which the recorder pen moved across the chart paper without appreciable lag. The plunger was pushed perpendicularly against the adaxial sides of the pulvinus of bean explants and of the cotyledonary petiole of cotton explants (Fig. 3).

During a break strength determination, the recorder pen moved across the chart paper as the spring was compressed and then returned to the zero base line when the tension was released by separation of the explant. The recorder reading at the peak of this curve was converted to grams by use of graphs similar to Figure 2 and was considered the break strength of the explant.

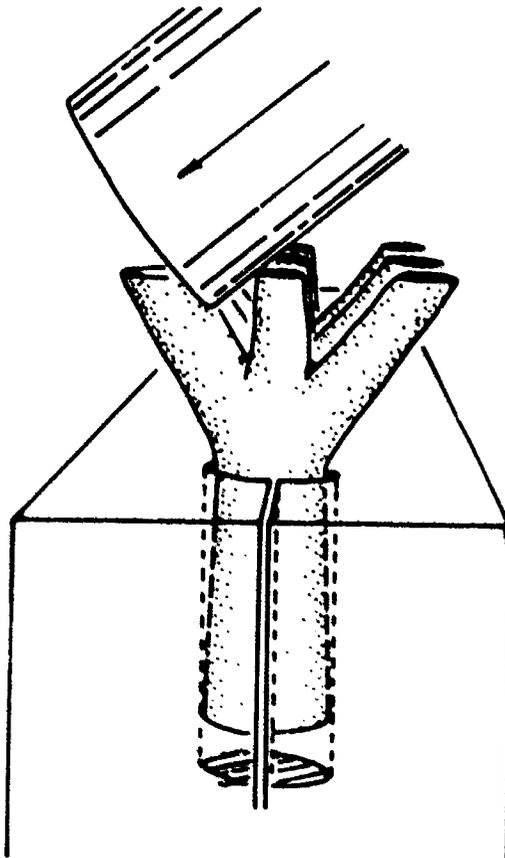
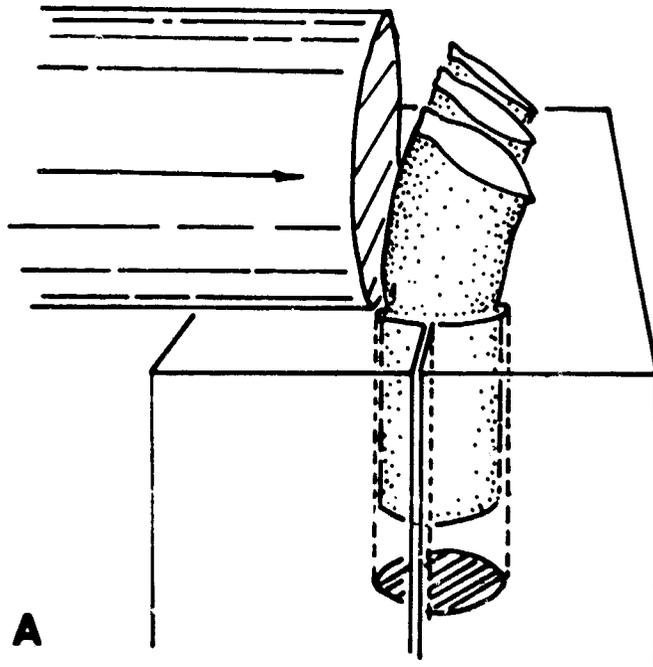


FIGURE 3. Methods Used to Apply Force with the Abscissor Plunger Against (A) Bean and (B) Cotton Explants Held in the Explant Vise.

III. RESULTS AND DISCUSSION

The break strength required for separation of bean explants from 18 varieties of *Phaseolus vulgaris* L. after 30 hours with or without ethylene treatment is shown in Table 1. As observed earlier by Morr e,⁶ the diameter of the explant plays a role in determining the force required for separation; i.e., large explants required more force for separation than small ones. However, the varietal differences shown in Table 1 were only partially due to differences in diameter. The linear correlation between the diameter of the explants from different varieties and the force required for separation was small (0.4). The data in Table 1 show that the abscissor was able to measure varietal differences in break strength and that 10 ppm ethylene reduced the break strength of all varieties. It is interesting to note that the second largest reduction in break strength occurred in Red Kidney, the variety used for most of the experimental work on abscission from this and other laboratories.

TABLE 1. BREAK STRENGTH FORCE FOR SEPARATION OF EXPLANTS
FROM BEAN VARIETIES AFTER 30 HOURS
WITH OR WITHOUT ETHYLENE

Variety	Break Strength Force, g, \pm SD ^a /		
	Control	10 ppm C ₂ H ₄	Difference
Perry Marrow	276 \pm 47	189 \pm 50	87
Lows Champion	253 \pm 35	178 \pm 38	75
Stringless Green Refugee	228 \pm 38	137 \pm 27	91
Dark Red Kidney	219 \pm 44	100 \pm 35	119
Tennessee Green Pod	216 \pm 35	140 \pm 56	76
Red Kidney	210 \pm 38	97 \pm 32	113
Red Mexican 34	199 \pm 47	160 \pm 29	39
Black Valentine	183 \pm 38	79 \pm 44	104
Pinto Univ. of Idaho III	183 \pm 38	152 \pm 44	31
Sanilac	175 \pm 21	94 \pm 27	81
Top Crop	175 \pm 32	68 \pm 21	107
Plentiful	175 \pm 41	126 \pm 50	49
Saginaw	174 \pm 53	148 \pm 50	26
Oregon State Univ. 2065	140 \pm 27	47 \pm 21	93
Tendercrop	137 \pm 41	68 \pm 44	69
Earliwax	134 \pm 38	91 \pm 27	43
Michelite	117 \pm 24	94 \pm 21	23
Slenderwhite	114 \pm 35	97 \pm 38	17

a. Each value represents the average of four experiments each consisting of 50 explants. Explants were isolated from greenhouse-grown plants.

Figure 4 presents data showing a loss in break strength of Red Kidney explants after 14 hours of exposure to 10 ppm ethylene. Cellulase has been proposed as one of the enzymes responsible for cell separation.^{10,11} To check this idea and take advantage of the ability of the abscissor to record the onset of abscission, a set of explants were analyzed for their cellulase content over the same period of time allotted for break strength measurements. In agreement with the idea that cellulase is one of the enzymes responsible for cell separation, we found that an increase in cellulase activity preceded the loss of break strength. A similar experiment was reported earlier by Morr e.⁶ He found that pectinase (as measured by a cucumber pericarp assay) activity rose prior to the separation of bean explants and that increased separation of bean explants was correlated with a decrease in break strength as measured by the Instron. He found that 200 g were required to pull freshly excised explants, which is similar to the lateral shear forces measured by the abscissor.

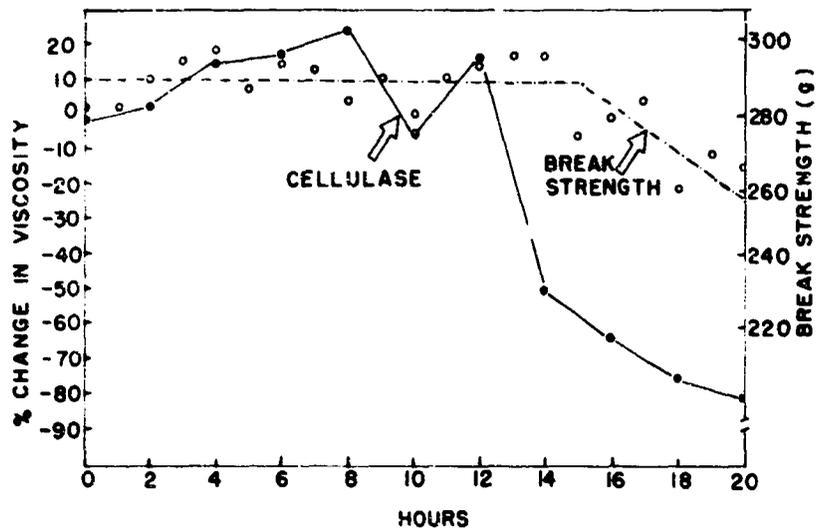


FIGURE 4. Change in Break Strength and Cellulase Content of Bean Abscission Zone Explants. Explants were analyzed for break strength and cellulase content as a function of time after excision from the plant. All explants were exposed to 10 ppm ethylene during the experiment. Each break strength determination is the average of 90 observations and each cellulase determination is the average of two cellulase assays. Increasing cellulase activity is characterized by increased ability of the enzyme to reduce the viscosity of a 1.5% solution of CMC.

IV. CONCLUSIONS

The results obtained with the abscissor indicate that it combines some of the better features of pressure applicators and strain gauges.

It is rapid. A skilled operator can measure the break strength of 100 explants in 20 minutes. This time includes loading explants in the explant vise as well as breaking the explants with the abscissor. The time required to break an explant is about 1 second.

Analogous with the Instron, the recording feature of the abscissor means that data can be examined later at the operator's convenience. However, care must be taken that the rate of pressure application does not exceed the scan speed of the recorder. The abscissor also shares with other techniques³⁻⁶ the ability to measure a range of break strength forces. However, no force less than 10 g can be recorded with the present instrument because this force is required to keep the plunger at the zero position of the abscissor. Because the abscissor measures a range of break strength forces it is able to indicate the onset of change in break strength, which in turn reflects the initial action of cell wall - degrading enzymes. In general practice we have found that a difference of 20 g in break strength was enough to indicate a significant difference between two samples of 10 explants excised from plants grown under controlled conditions. As indicated in Table 1, explants from greenhouse plants were more variable and larger differences in break strength were required for significance.

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Security Classification

DOCUMENT CONTROL DATA - R & D		
<i>(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)</i>		
1. ORIGINATING ACTIVITY (Corporate author)		2a. REPORT SECURITY CLASSIFICATION
Department of the Army Fort Detrick, Frederick, Maryland, 21701		Unclassified
		2b. GROUP
3. REPORT TITLE		
ABSCISSION: QUANTITATIVE MEASUREMENT WITH A RECORDING ABCISSOR		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)		
5. AUTHOR(S) (First name, middle initial, last name)		
Lyle E. Craker Frederick B. Abeles		
6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
May 1969	19	11
8a. CONTRACT OR GRANT NO.		8b. ORIGINATOR'S REPORT NUMBER(S)
a. PROJECT NO. 1B562602A061		Technical Manuscript 528
c.		9a. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.		
10. DISTRIBUTION STATEMENT		
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11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY
		Department of the Army Fort Detrick, Frederick, Maryland, 21701
13. ABSTRACT		
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14. Key Words		
<ul style="list-style-type: none"> *Abscission *Measuring instruments *Breaking strength Cotton plants Beans 		

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