POSSIBLE ROLE OF PECTIC ENZYMES IN ABSCISSION[1]

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Abscission has been of interest to botanists since Mohl described it as early as 1860 (20). Many of the early workers assumed that enzymes are involved in the dissolution of the middle lamella as abscission occurs (11, 12, 16, 17). As Addicott and Lynch indicate in their review of the physiology of abscission, however, little has been done with the identification of specific enzymes which may be involved in the dissolution reactions (2). Much of the current research concerned with abscission deals with chemical substances which affect the process with little attention to specific chemical reactions that may be involved.

Osborne has shown recently that the activity of pectin methylesterase (PME) decreases when the time for abscission of leaves in Phaseolus vulgaris occurs normally or when abscission is induced by chemical factors (23). Since the activity of PME is affected by growth substances (4, 21, 26) which also affect abscission (1, 25, 27), a role for PME in the reactions involved in abscission is suggested.

The middle lamella is generally considered to be pectic in nature (6, 13, 18). The middle lamella of the abscission zone dissolves as floral abscission occurs in Nicotiana (12, 28). In 1950 Facey reported the results of experiments with Fraxinus cuttings which identified water soluble pectin as the end product of a series of reactions which occur as the calcium pectate of the middle lamella is dissolved during abscission (10). As early as 1918 Kendall reported the appearance of pectin as abscission occurred in tobacco flowers (12). The problem arises as to whether the shifts from pectate to pectin as the middle lamella dissolves is simply caused by a decrease in PME activity or whether additional pectic enzymes are involved.

The literature connected with pectic enzymes other than PME is conflicting, including their actual occurrence in higher plants. Pectin-polygalacturonase is the best known of these other enzymes. Kertesz is doubtful of its occurrence in higher plants although he cites several reports of its occurrence (15). Demain and Phaff recently reported that instead of a single pectin-polygalacturonase, there are several exo-polygalacturonases specific for pectins of different degrees of esterification and size of polymer and a single endo-galacturonase (7) (8). Schubert (24), Dingle et al (9), and Ayres et al (3) have reported that endo-polygalacturonase is a complex of enzymes. This complexity in the identification of specific pectin-polygalacturonases may explain some of the conflicting reports. McClendon and Somers have reported the occurrence of two pectin-glycosidases with different conditions required for activity (19). Kertesz has postulated the occurrence of additional pectic enzymes in general and describes one that he has named pectic acid depolymerase (15). Although there is general lack of information concerning pectic enzymes, it is probable that there are one or more enzymes present in abscission tissue which could act to dissolve the cementing middle lamella.

The purpose of this investigation was to examine the occurrence of PME in the abscission zone of tobacco flowers and to note effects that various substances known to affect abscission might have upon its activity (22, 29, 30). In addition, attempts were made to duplicate in vitro the macerating effects presumably caused by pectic enzymes as abscission occurs normally. These same substances which affect abscission were studied as to their effect upon the macerating activity of certain pectic enzymes.

METHODS

PME ANALYSIS Little Turkish and Lizard's Tail varieties of Nicotiana tabacum were used throughout the investigation in order that any varietal differences associated with self and cross pollination mechanisms could be observed. To determine the activity of PME, a cross section of tissue 1 mm thick was removed from the abscission zone of approximately 60 pedicels which abscised at touch. The PME activity in the abscission tissue was compared to the activity in tissue from the leaf, stem, petiole, and upper pedicel. The fresh weights of these tissues were recorded after which they were ground in a mortar with acid-washed white quartz sand. This preparation was added to a 1 % pectin solution containing 0.1 N NaCl. This mixture was agitated for 30 min while 0.1 N NaOH was added to maintain a constant pH 6.8 as measured by a glass electrode.

The relative activity was computed using the method proposed by Kertesz (14). The number of milligrams of methoxyl groups that are split off during the 30 min reaction period is obtained by multiplying the volume of alkali required by 3.1, the equivalent molecular weight of the methoxyl group. The resulting unit of relative activity is termed the PME unit per gram of fresh tissue. Effects of indoleacetic acid (IAA) and methionine upon activity of tissue from the abscission zone were determined.

SEPARATION ACTIVITY Thin free-hand longitudinal sections measuring 5 mm in length were removed from several pedicels so that the cells of the abscission region could be observed with a microscope and compared to surrounding cells. These sections were added to solutions of various pectic enzymes. Thirty sections were incubated with 10 ml of enzyme preparation in a Petri culture at 25° C. Several sections of tissue were removed from the cultures and mounted for microscopic examination periodically. Following such examination the tissue was discarded. Visible separation of the cells was noted and the time for complete separation was compared to the time required when a different enzyme concentration, buffer, or pH level was used and when IAA and methionie were added to the media. Each experiment was duplicated ten times.

PME and a crude preparation called pectinase are available commercially and were secured from the Nutritional Biochemicals Corporation. McClendon and Somers of the University of Delaware have generously provided the two pectin-glycosidases which they secured from Rome Beauty apples that were rotted by *Botryosphaeria ribis*. The author refers to these two pectin-glycosidases as enzymes A and B. [Enzyme A is a concentrate from the pressed juice (pH 3.5) while enzyme B is a concentrate of an extract from the pressed cake after removal of the juice (pH 4.5). Enzyme A has a pectin-polygalacturonase activity (measured viscometrically with pectate pH 4) about two times that of enzyme B while the maceration activity upon tissues from tomato leaves and potato slices is ten times more active in B than A (expressed on a dry weight basis). These preparations can hydrolyze polygalacturonic acid, araban, galactan, galactomannan, xylan, and pectin at varying

TABLE I

PECTIN-METHYLESTERASE ACTIVITY OF VARIOUS TISSUE MACERATES

TISSUE FROM	ACTIVITY/g FRESH TISSUE	
	LIZARD'S TAIL	LITTLE TURKISH
Abscission Zone	12.12	13.95
Upper Pedicel	10.72	11.78
Petiole	7.00	6.27
Stem	7.01	6.87
Leaf*	9.83	9.64

* Gel formation often interfered with the reaction before the 30 minute time interval was complete.

rates. The pectinase preparation (from NBC) is much more potent in all of these activities.][2]

RESULTS

PME ANALYSIS The PME activity in the tissue of the abscission zone of the pedicel is greater than in any other tissue tested with the possible exception of leaf tissue (See table I). In this instance gel formation interfered with agitation during the 30 min interval in which 0.1 N sodium hydroxide was added. When IAA is added, the activity rates are altered as tabulated in table II. Differences between the effects caused by adding IAA are statistically significant as indicated in table III. Duplicate tests each with half the ground tissue produced nearly identical values for enzyme activity.

Adding 5 ppm IAA decreases PME activity. Adding 100 and 1,000 ppm IAA increases PME activity. Tests with still higher concentrations of IAA produce only slight increases in PME activity over that observed when 1,000 ppm IAA is added.

Methionine decreases PME activity when added to the culture media (see table II). This difference in PME activity between the control and the addition of 0.01 M methionine is significant at the 1 % level (see table III). Adding 1,000 ppm IAA in addition to the methionine, however, causes the disappearance of the decreased PME activity that was observed

[2] Information received through personal communication with Dr. John H. McClendon, University of Delaware.

TABLE II

EFFECT OF IAA AND METHIONINE UPON ACTIVITY OF PME OF MACERATES OF TISSUE FROM ABSCISSION ZONE

ADDITION TO TISSUE	NUMBER OF TRIALS	MEAN ACTIVITY/G FRESH TISSUE ($\pm$ 1 STANDARD ERROR)	
		LIZARD'S TAIL	LITTLE TURKISH
0	15	12.21 ± 0.33	13.16 ± 0.12
5 ppm IAA	5	11.61 ± 0.95	12.92 ± 0.54
100 ppm IAA	5	13.20 ± 0.59	14.55 ± 0.77
1,000 ppm IAA	10	16.71 ± 0.27	16.97 ± 0.38
0.01 M Methionine	5	9.54 ± 1.00	10.65 ± 1.13
1,000 ppm IAA + 0.01 M Methionine	5	15.32 ± 0.63	15.31 ± 0.68

TABLE III

DETERMINATION OF SIGNIFICANCE BETWEEN EFFECTS
OF IAA AND METHIONINE UPON ACTIVITY
RATE OF PME FROM TISSUE OF ABSCISSION ZONE

DIFFERENCES BETWEEN ADDITIONS OF	[t] VALUES	
	LIZARD'S TAIL	LITTLE TURKISH
1. 0 and 5 ppm IAA	0.67	0.60
2. 0 and 100 ppm IAA	1.33	2.67*
3. 0 and 1,000 ppm IAA	8.33***	11.20***
4. 0 and 0.01 M Methionine	2.93**	3.53**
5. 0 and 1,000 ppm IAA + 0.01 M methionine	3.74**	4.88***
6. 5 ppm IAA and 100 ppm IAA	1.28	1.27
7. 100 ppm IAA and 1,000 ppm IAA	5.83***	2.91**
8. 1,000 ppm IAA and 0.01 M methionine	8.74***	6.22***
9. 1,000 ppm IAA + 0.01 M methionine and 0.01 M methionine	6.21***	2.74*
10. 1,000 ppm IAA and 1,000 ppm IAA + 0.01 M methionine	2.28*	2.21*

* Significant at the 5 % level
** Significant at the 1 % level
*** Significant at the 0.1 % level

when methionine was added singly. Nearly the same increase in PME activity caused by the 1,000 ppm IAA alone is observed. Additions of 5 ppm IAA and the methionine produced no significant difference in PME activity than when the methionine was present alone.

ESTERIFICATION REACTIONS Other experiments were performed in the same manner except that 0.1 N hydrochloric acid was added to maintain a constant ph 4.5 during the 30 min reaction time. This presumably would be a direct test for the esterification reaction which would oppose the activity of PME.

The activity of such an esterification enzyme was confined to the first seven or eight minutes of the usual 30 min reaction time. Since the time of reaction was only one-fourth that in the other experiments, activity values were considerably smaller and there was less overall variation in activity when IAA and methionine were added. Averages of several such experiments produced figures which indicated that addition of methionine increased esterification. Additions of 1,000 ppm IAA completely inhibited esterification in Little Turkish and nearly so in Lizard's Tail tissue. Variations were slight in all of the experiments, however.

SEPARATION ACTIVITY Pectinase and enzyme B cause dissolution of the middle lamella and separation of cells of the abscission zone as well as other cells of the tissue slice through the pedicel. The dissolution of the middle lamella with these enzymes resembles the dissolution which normally occurs during abscission (28). The chief difference in this induced dissolution from that which normally occurs is the normal restriction of activity to certain tiers of cells. Since many tiers of cells are involved in the actual separation of cells in Lizard's Tail variety as compared to relatively few in Little Turkish, the pattern of separation of cells caused by pectinase and enzyme B is more like that which occurs in Lizard's Tail. The results of the incubation of tissue slices with the four pectic enzymes are tabulated in table IV. Where separation occurred (and hence dissolution of the middle lamella), no difference could be observed between the action of pectinase and enzyme B.

The action of the enzymes could be observed with a microscope. Cells near the periphery were acted upon first. Incubation was continued until the cells
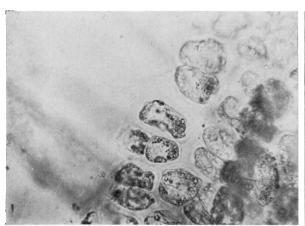

FIG. 1 (left). Separation of cells of the pedicel of Lizard's Tail tobacco as a result of treatment with pectinase. (x 820)

FIG. 2 (right). Separation of cells of the pedicel of Little Turkish tobacco as a result of treatment with pectinase. (x 820)

were separated by the weight of a cover slip when the section was mounted for microscopic examination. Figures 1 and 2 represent samples of separation of cells which were termed complete separation. The values recorded in tables IV to VII indicate the amount of time required for the enzymes to cause such separation. In several duplicated experiments variation in time did not exceed 1 hr except in a few cultures where fungal contamination occurred.

The kind of buffer and the pH affect the separation activity of pectinase and enzyme B (see tables IV and V). Separation occurs more rapidly at pH 3.5. Resulting discoloration of tissue slices, however, indicates adverse physiological effects upon the cells. No such effects are observed at pH 4.5. Higher pH levels result in sharp decreases of activity. No separation is observed at pH 6.2. The maleate buffer increases the activity of enzyme B slightly. The citrate buffer increases the activity of pectinase slightly at pH 4.5 while enzyme B is affected considerably at pH 3.5 (see table V).

Low concentrations of IAA (5 ppm) and methionine increase the activity of pectinase and enzyme B considerably (see table VI). When 5 ppm and 0.01 M methionine are added to the substrate in combination, only a slight increased activity is observed by pectinase and none by enzyme B (see table VII). By adding 1,000 ppm IAA to the substrate the action of pectinase is inhibited completely during the time

limit of 56 hrs for these experiments. This inhibitory effect of 1,000 ppm IAA is still apparent when 0.01 M methionine is added as well. The effects of methionine upon rate of separation is proportional to the concentration added (see table VI).

DISCUSSION

There is evidence that PME acts to retard or to prevent abscission. Methionine and low concentrations of IAA accelerate abscission; high concentrations of IAA retard or prevent abscission (30). Methionine and low concentrations of IAA decrease PME activity; high concentrations of IAA increase PME activity. In addition, if pectin is the end product of the abscission reactions as reported earlier (10, 12), an enzyme which tends to de-esterify pectin would act to prevent abscission. Osborne's data which show a decrease in PME activity as abscission approaches in the leaves of *Phaseolus vulgaris* is added evidence that PME acts to retard or prevent abscission. Since the action of PME is irreversible (15), it cannot act under certain conditions to produce pectin and hence cause abscission.

The existence of other pectic enzymes active in abscission seems likely. A transmethylase to provide methyl groups for the shift from pectic acid to pectin is a probable enzyme. The action of one or more pectin-polygalacturonase would certainly increase the

TABLE IV

EFFECT OF VARIOUS PECTIC ENZYMES UPON SEPARATION
OF CELLS OF TISSUE SLICES FROM
ABSCISSION ZONE

ENZYME (0.025 %)	HRS REQUIRED FOR SEPARATION*		
	pH 3.5**	pH 4.5	pH 6.2
No Enzyme	No separation	No separation	No separation
Pectinase (from NBC)	6	20	No separation
Pectin-methylesterase (from NBC)	No separation	No separation	No separation
Enzyme A*** (from McClendon and Somers)	No separation	No separation	No separation
Enzyme B*** (from McClendon and Somers)	56	No separation	No separation
Enzyme (0.1 %)			
No enzyme	No separation	No separation	No separation
Pectinase (from NBC)	4	8	No separation
Pectin-methylesterase (from NBC)	No separation	No separation	No separation
Enzyme A*** (from McClendon and Somers)	56	No separation	No separation
Enzyme B*** (from McClendon and Somers)	18	24	No separation

* Experiments observed for 56 hrs
** Tissue discolored in 6 to 8 hrs
*** 0.05 M Citrate buffer used

TABLE V

EFFECT OF BUFFER UPON SEPARATION ACTIVITY OF
VARIOUS PECTIC ENZYME PREPARATIONS

ENZYME	pH	ENZYME CONC	HRS REQUIRED FOR SEPARATION*		
			NONE	MALEATE	CITRATE
				BUFFER	
No Enzyme	4.5	0.00 %	No separation	No separation	No separation
Pectinase	4.5	0.025 %	20	20	16
Pectin-methylesterase	4.5	0.025 %	No separation	No separation	No separation
Enzyme A	4.5	0.025 %	No separation	No separation	No separation
Enzyme B	4.5	0.025 %	No separation	No separation	No separation
No enzyme	3.5	0.00 %	No separation	No separation	No separation
Pectinase	3.5	0.1 %	4	4	4
Pectin-methylesterase	3.5	0.1 %	No separation	No separation	No separation
Enzyme A	3.5	0.1 %	No separation	No separation	56
Enzyme B	3.5	0.1 %	30	28	18

* Experiments observed for 56 hrs

rate of dissolution of the middle lamella and separation of the cells of the abscission zone. Such enzymes are conceivably present in the pectinase and enzyme B preparations used in the experiments. Evidence that enzymes effective in abscission are present in these preparations is found in the effect of IAA and methionine upon their action in causing dissolution of the middle lamella. Just as methionine and low concentration of IAA accelerate abscission, they also increase the dissolution activity of pectinase and enzyme B. Also, as high concentrations of IAA retard or prevent abscission, they prevent the action of pectinase and enzyme B in causing separation of cells.

Type of buffer salt and pH are two additional factors important in interpreting the data. Facey reported the necessity of low pH to accomplish the change from calcium pectate to pectic acid. The separation activities exemplified by enzyme B and pectinase can be illustrated only in the range of 3.5 or 4.5. No activity occurs at pH 6.2. Definite buffer effects are illustrated in table V. Carr and Ng have recently reported that several organic acids (including citric and maleic) which are commonly used as buffers, form complexes with calcium, thus removing it from the cell wall (5). This could explain the increase in separation activity of enzyme B and pectinase in the presence of the maleate and citrate buffers since the shift from calcium pectate to pectic acid would be promoted by the presence of the buffer.

The results of this investigation suggest possible roles for pectic enzymes in abscission. Factors which prevent or retard the action of PME could cause abscission by the accumulation of pectin which is soluble in water and hence ineffective as a cementing layer. Other enzymes could be involved as indicated by the in vitro separation caused by the pectinase and enzyme

TABLE VII

EFFECTS OF IAA AND METHIONINE UPON SEPARATION
OF CELLS BY PECTINASE AND
ENZYME B PREPARATIONS

CONDITION	HRS REQUIRED FOR SEPARATION	
	PECTINASE*	ENZYME B**
No Enzyme	No separation	No separation
Enzyme	20	18
Enzyme & 0.01 M methionine	12	10
Enzyme & 5 ppm IAA	12	12
Enzyme & 0.01 M methionine and 5 ppm IAA	10	10
Enzyme & 1,000 ppm IAA***	No separation	48
Enzyme & 0.01 M methionine and 1,000 ppm IAA***	No separation in 48	48

* 0.025 % solution at pH 4.5 with 0.0025 M maleate buffer
** 0.1 % solution at pH 3.5 with 0.05 M citrate buffer
*** Discolored a few hours following treatment

TABLE VI

EFFECTS OF VARIOUS CONCENTRATIONS OF IAA
AND METHIONINE UPON SEPARATION OF CELLS
OF ABSCISSION ZONE CAUSED BY PECTINASE*

CONC OF METHIONINE (M)	HRS REQUIRED FOR SEPARATION	CONC OF IAA (PPM)	HRS REQUIRED FOR SEPARATION
0.0	20	0	20
0.001	18	5	12
0.01	14	100	48
0.05	11	1,000**	No separation in 48
0.1	8		

* 0.025 % enzme solution with maleate buffer at pH 4.5
** Discolored a few hours following treatment

B preparations. A transmethylase is a probable enzyme important in yielding methyl groups for the pectic acid to pectin change. This and other enzymes which could affect the polygalacturonic chains are possibly present in the preparations used in the experiments as well as in the abscission tissue normally.

SUMMARY

PME activity has been found to be high in the abscission region of tobacco pedicels. Its activity is affected by IAA. High concentrations increase PME activity. Methionine decreases PME activity. IAA can overcome this inhibition of activity caused by methionine.

It has been possible to duplicate the separation of cells observed normally as abscission occurs by incubating tissue from the abscission zone with various pectic enzymes. The conditions which affect abscission on the plant also affect the rates of cell separation caused by these enzyme preparations.

Since IAA and methionine (which have been shown to affect abscission on intact plants) affect PME activity as well as rates of cellular separation caused by other pectic enzymes, it is possible that similar enzymes are active in the usual abscission reactions.

Two possible roles of pectic enzymes in abscission are suggested. First, conditions which prevent PME activity may cause abscission by allowing for the accumulation of pectin which is soluble in water. Or secondly, other pectic enzymes may be involved in the production of pectin or by some other chemical pathway which ultimately causes the dissolution of the middle lamella. Various pectic enzymes were shown to cause this dissolution in vitro.

LITERATURE CITED

1. ADDICOTT, F. T. and R. LYNCH 1951. Acceleration and retardation of abscission by indole-acetic acid. Science 114: 688–689.
2. ADDICOTT, F. T. and R. LYNCH 1955. Physiology of abscission. Ann. Rev. Plant Physiol. 6: 211–238.
3. AYRES, A., J. DINGLE, A. PHIPPS, W. W. REID, and G. L. SOLOMONS 1952. Enzymic degradation of pectic acid and the complex nature of polygalacturonase. Nature 170: 834–836.
4. BRYAN, W. H. and E. H. NEWCOMB 1954. Stimulation of pectinmethylesterase activity of cultured tobacco pith by indoleacetic acid. Physiol. Plantarum 7: 290–297.
5. CARR, D. J. and E. K. NG 1959. The sequestration of calcium from preparations of wheat coleoptile cell walls. Physiol. Plantarum 12: 264–274.
6. CORMACK, R. G. H. 1955. Action of pectic enzymes on surface cells of living Brassica roots. Science 122: 1019–1020.
7. DEMAIN, A. L. and H. J. PHAFF 1954. Hydrolysis of the oligogalacturonides and pectic acid by yeast polygalacturonase. Jour. Biol. Chem. 210: 381–393.
8. DEMAIN, A. L. and H. J. PHAFF 1957. Recent advances in the enzymatic hydrolysis of pectic substances. Wallerstein Lab. Communs. 20: 119–140.
9. DINGLE, J., W. W. REID, and G. L. SOLOMONS 1953. The enzymic degradation of pectin and other polysaccharides. II. Application of the "cup-plate" assay to the estimation of enzymes. Jour. Sci. Food Agr. 4: 149–155.
10. FACEY, V. 1950. Abscission of leaves in Fraxinus americana L. New Phytologist 49: 103–116.
11. GOODSPEED, T. H. and J. N. KENDALL 1916. On the partial sterility of Nicotiana hybrids made with N. sylvestris as a parent. Univ. Calif. (Berkeley) Publs. Botany. 5: 293–299.
12. KENDALL, J. 1918. Abscission of flowers and fruits in the Solanaceae, with special reference to Nicotiana. Univ. Calif. (Berkeley) Publs. Botany 5: 347–428.
13. KERR, T. and I. BAILEY 1934. The cambium and its derivative tissues. X. Structure, optical properties, and chemical composition of the so-called middle lamella. Jour. Arnold Arboretum 15: 327–334.
14. KERTESZ, Z. I. 1937. Determination of pectinmethoxylase activity. Jour. Biol. Chem. 121: 589–597.
15. KERTESZ, Z. I. 1951. The Pectic Substances. Part 3. The Biochemistry of Pectic Substances. Pp. 333–398.
16. KUBART, B. 1906. Dei organische Abloesung der Korollen nebst Bemerkung über die Nolsche Tennungschichte. S.-B. Akad. Wien, Math-nat. Kl. 115.1: 1491.
17. LLOYD, F. E. 1916. Abscission of flower buds and fruit in Gossypium and its relation to environmental changes. Trans. Roy. Soc. Can. 10: 55–62.
18. MANGIN, L. 1889. Sur la présence des composés pectiques dans les végétaux. Compt. rend. acad. sci., Paris. 109: 579–584.
19. MCCLENDON, J. H. and G. F. SOMERS 1956. Identification of protopectinase. Plant Physiol. 31 suppl.: xliii.
20. MOHL, H. VON. 1860. Ueber den Abloesungsprozess saftiger Pflanzenorgane. Bot. Zeitung. 18: 273–274.
21. NEELY, W. B., C. D. BALL, C. L. HAMNER, and H. M. SELL 1950. Effect of 2,4-dicholorophenoxyacetic acid on the invertase, prosphorylase, and pectinmethoxylase activity in the stems and leaves of red kidney bean plants. Plant Physiol. 25: 525–528.
22. OSBORNE, D. J. 1955. Acceleration of abscission by a factor produced in senescent leaves. Nature 176: 1161–1163.
23. OSBORNE, D. J. 1958. Changes in the distribution of pectin methylesterase across leaf abscission zones of Phaseolus vulgaris. Jour. Exp. Bot. 9: 446–457.
24. SCHUBERT, E. 1952. Differentiation of the polygalacturonases of Aspergillus niger. Nature 169: 931–932.
25. SHOJI, K., F. T. ADDICOTT, and W. A. SWETS 1951. Auxin in relation to leaf blade abscission. Plant Physiol. 26: 189–191.
26. WAYGOOD, E. R., L. KAPICA, and J. MOZIE 1954. The effect of plant growth regulating substances on the production of pectin esterase by Cladosporium herbarum (Pers) Link. Proc. Can. Phytopath. Soc. 21: 19–24.
27. WETMORE, R. and W. JACOBS 1953. Studies on abscission: The inhibiting effect of auxin. Amer. Jour. Botany 40: 272–276.
28. YAGER, R. E. Abscission zone anatomy, development, and separation in Nicotiana tabacum. Proc. Iowa Acad. Sci. (In Press).
29. YAGER, R. E. and R. M. MUIR 1958. Amino acid factor in control of abscission. Science 127: 82–83.
30. YAGER, R. E. and R. M. MUIR 1958. Interaction of methionine and indoleacetic acid in the control of abscission in Nicotiana. Proc. Soc. Exp. Biol. Med. 99: 321–323.