THE ENZYMES ASSOCIATED WITH LEAF ABSCISSION IN COTTON

by

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ABSTRACT

The enzymes cellulase and pectinase were examined during ethylene induced leaf abscission in cotton (TAMCOT CAMD-E). Pectinase proved unassayable due to an enzymatic factor that jelled the assay mixture. Cellulase was assayed and the abscission zone was found to have the greatest levels of cellulase, even though it existed throughout the plant. Extraction of the cellulase required high NaCl concentrations in the buffer solution, thereby indicating that the cellulase extracted was 9.5 pI cellulase, the form active in abscission. Further localization studies found the cellulase levels were slightly higher on the proximal side of the abscission zone. A time course comparing per cent leaf abscission and cellulase levels with time in ethylene showed that per cent leaf abscission rose, but cellulase did not. Large amounts of PVP had to be added to the extract preparation.

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INTRODUCTION

Abscission is the process whereby plant parts such as leaves, flowers, and fruits separate from the parent plant. This separation occurs in a special area called the abscission zone. In leaf abscission, this zone is located at the junction of the petiole and stem. Hydrolytic enzymes have been implicated in the abscission process due to the degradation of the cell wall and/or middle lamella that takes place during abscission.

Abscission is an important physiological process in plants, and a better understanding of abscission would be invaluable to agriculture. Manipulation of the abscission process is already widely practiced. Examples of this being: promotion of fruit drop in citrus crops; defoliation in cotton; cluster thinning in grapes; and prevention of preharvest fruit drop in pears, apples, and citrus. However, even though abscission manipulation is widely practiced, problems have been encountered with the selectivity and timing of hormonal control agents, indicating a need for further research.

The purpose of this project was to examine the hydrolytic enzymes associated with leaf abscission in cotton, in an effort to further understand the biochemistry of the abscission process. Cotton was chosen because it is a crop of major agronomic importance in which abscission is a prerequisite to harvesting. Also, most of the work on

abscission in cotton has focused on hormonal control, not enzymology. The enzymes cellulase and pectinase were examined because both are thought to play an important role in abscission (9, 10).

LITERATURE REVIEW

Several hydrolytic enzymes have been implicated in abscission. The two major enzymes are cellulase and pectinase. Cellulase breaks down the cell walls; whereas, pectinase degrades the middle lamella. The destruction of either the cell wall or middle lamella would result in abscission.

Pectinase

The evidence for pectinase as a factor in leaf abscission is two fold: biochemical and morphological. The biochemical evidence is that several researchers have found that increased pectinase levels generally precede abscission (8, 11). Greenberg et al. has found that increased pectinase activity in orange pedicels closely correlates with abscission (3). In addition, the hormones that promote and inhibit abscission also promote and inhibit pectinase. The morphological support for the role of pectinase in abscission was provided by Sexton and Hall (9). They examined the exposed surfaces of abscission zones with an electron mocroscope, and found no evidence of cell wall breakdown, only middle lamella degradation.

Cellulase

Evidence that cellulase is involved in abscission was first provided by Horton and Osborne in 1967 (4). They found increased cellulase activity associated with abscission. Since then many workers have concurred (1, 5). However, conflicting reports on the role of cellulase soon began to appear. The following are some of the points of conflict: first of all, cellulase may be present in relatively high amounts before

abscission is induced; secondly, although cell wall breaksown is confined to two or three layers, cellulase is generally found over a larger area; and thirdly, cellulase may increase without concomitant cell wall breakdown (10). Never-the-less, most of the points of conflict have now been settled. The key to understanding cellulase was the discovery that cellulase exists in more than one form (5). Only one form of cellulase has been implicated in abscission, but the main test for cellulase doesn't distinguish between the various forms (10). Early workers probably measured other cellulases when examining abscission, as well as the proper form.

Cellulase has been shown to exist in three different forms, which are characterized by their isoelectric points: 4.5, 6.1, and 9.5 (6). The 4.5 form of cellulase has a molecular weight of 30,000, and it is under the hormonal control of auxin. It is found throughout the plant and it is associated with highly regulated forms of cell wall change (6). The 6.1 cellulase, which appears to be regulated by auxin, is a tetramer of cellulase 9.5 (6). Its physiological role is unknown.

The 9.5 cellulase is the form that is involved with abscission. It is soluble only in high NaCl fortified buffer (6), and its under the hormonal control of ethylene, which promotes abscission (2). Sexton et al. has shown that a definite strong correlation exists between 9.5 cellulase increases, and decreasing break strength of the abscission zone (10). In addition, he and his associates have shown that 9.5 cellulase activity is primarily confined to the two or three cell layers that the abscission plane will pass through. Finally, they found that if an antibody to the 9.5 cellulase is injected into the abscission zone,

abscission is prevented. The control injection of normal serum, minus the 9.5 antibody, did not prevent absicssion. These discoveries argue conclusively that 9.5 cellulase plays a key role in abscission.

Thus, it seems that abscission can occur by several methods: by pectinase mediated middle lamella degradation; by cellulase 9.5 mediated cell wall destruction; or by a combination of the first two, depending on the plant species.

MATERIALS & METHODS

Plant Material

TAMCOT-CAMD-E seeds were obtained from the Texas A&M University, College Station, Texas. These were planted in a mixture of (1/1) peat moss and vermiculite. They were watered daily, and fertilized weekly with a full strentgh Hoagland's solution. These were grown for 19 to 23 days, treated, and then harvested.

Enzyme Extraction

After treatment, 0.5g of cotyledonary abscission zones were exised and homogenized in a mortor and pestle with 0.5g of PVP and 5.0 ml of 0.02 M potassium phosphate buffer (pH6) fortified with 1 M NaCl. The homogenate was passed through a fine mesh nylon cloth, and the filtrate was then centrifuged at 10,000 rmp for 15 minutes. The supernatant was collected and used in the assays.

Enzyme Assays

Cellulase was determined viscometrically by the method of Lewis and Varner (5) using carboxymethyl cellulase (CMC) and expressed as relative

units. The substrate used was a 1.3% solution of CMC Type 7H3SF (Hercules, Wilmington, Del., USA). The assay mixture consisted of 200 ul of enzyme extract and 400 ul of substrate.

Polygalacturonase (PG) was also assayed by the method of Lewis and Varner (5). The assay mixture consisted of 200 ul of enzyme extract and 400 ul of 2.3% (W/V) Na-polypectate, in 0.04 M Na-acetate buffer, (pH 5.5).

Protein Determination

To determine protein concentration, 0.2 ul of enzyme extract were mixed with 2.0 ml of cold 10% Tricholoroacetic acid (TCA) and set in an ice bath for 30 minutes. The samples were then centrifuged at 10,000 rpm for 10 minutes. After centrifugation, the supernatant was poured off and the method of Lowry (7) was used to determine protein in the pellet. Bovine serum alblumin served as the standard.

RESULTS

Polygalacturonase

PG proved impossible to assay. Anytime the substrate was mixed with the enzyme extract, the resulting solution jelled. When the extract was boiled, the jelling no longer occured. This plus several other experiments indicate that the jelling was due to an enzymatic factor.

Cellulase

Localization. Cellulase was found to exist throughout the plant. The abscission zone contained the most cellulase, almost four times more than the other areas. The stems and petioles contained small amounts,

and the leaves contained very little (Fig. 1). In the abscission zone the proximal side of the zone had cellulase activity of 4.3 (units/mg of protein/hr) as compared to 3.9 (Units/mg of protein/hr) for the distal side of the zone (Table 1).

Extraction. Large amounts of PVP and NaCl fortified buffer were found to be essential for effective enzyme extraction. If salt or PVP was left out of the extraction procedure, very little cellulase could be extracted (Fig. 2)

Time Course. When cotton plants were exposed to ethylene, leaf abscission was induced but no noticable effect on cellulase levels was seen. Leaf abscission was induced within twleve h. and reached its maximum within 24 h., but it was never total. After a maximum of 73% abscission was reached at 24 h. the level of abscission varied between 49 to 71% through the remaining hours of treatment. (Fig. 3).

DISCUSSION

Cellulase levels were highest in the abscission zone of ethylene treated cotton plants, and high salt concentrations were required for the extraction of cellulase. Both of these points are in agreement with the idea that cellulase is a major factor in causing abscission. Sexton et al. has shown that the salt soluble cellulase plays a key role in abscission (10). If cellulase is the key enzyme, this is the form that should be stimulated, and it should rise almost solely in the abscission zone. The fact that cellulase was found throughout the plant does not conflict with the view that cellulase is involved in abscission.

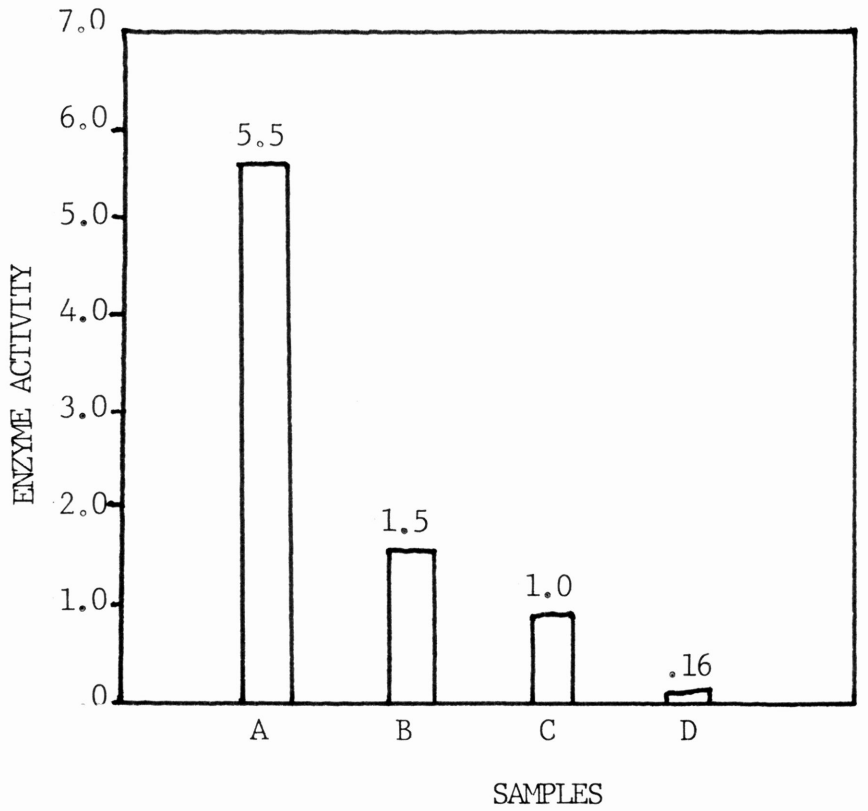

Fig. 1. Cellulase levels in various parts of the cotton plant. Cellulase activity was determined in the abscission zone (A), stems (B), petioles (C), and leaves (D), after 30 hrs. exposure to 25 ppm ethylene.

Table 1. Localization of cellulase in the abscission zone.

Cellulase	Proximal	Distal
Specific Activity (Units/mg protein/hr)	4.3	3.8

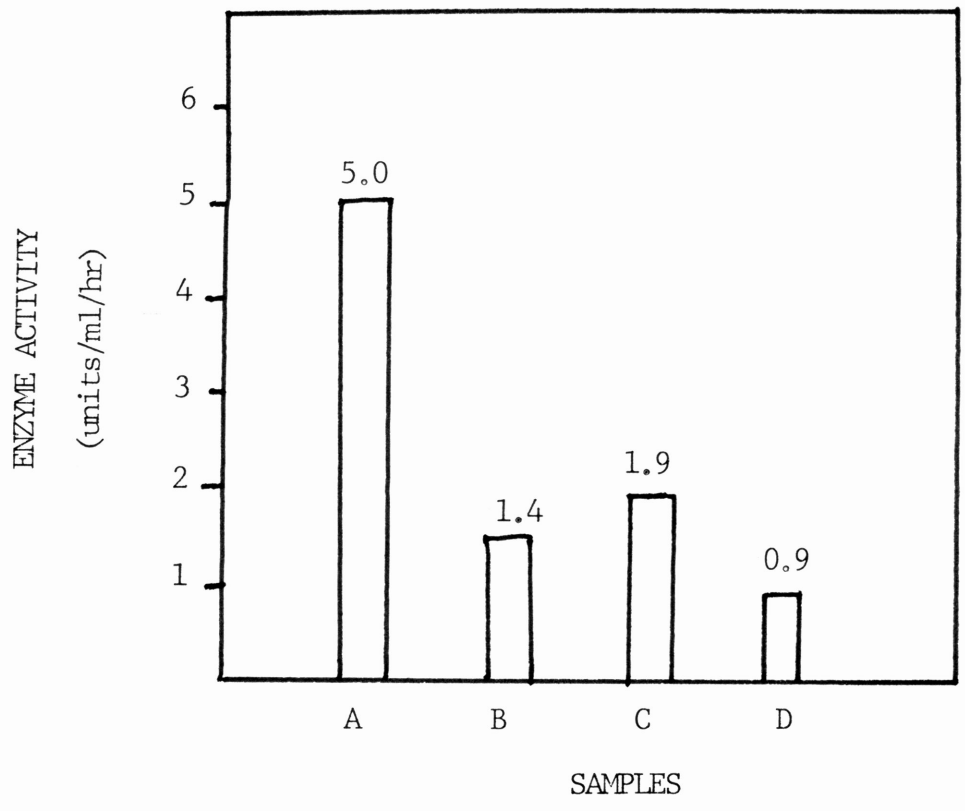

Fig. 2. The effects of NaCl and PVP on enzyme extraction. Abscission zone sapples were ground by four different methods: (A) normal extraction procedure; (B) normal precedure, minus PVP; (C) normal procedure minus 1 M NaCl; and, (D) regrind of pellet from (C) using normal procedure.

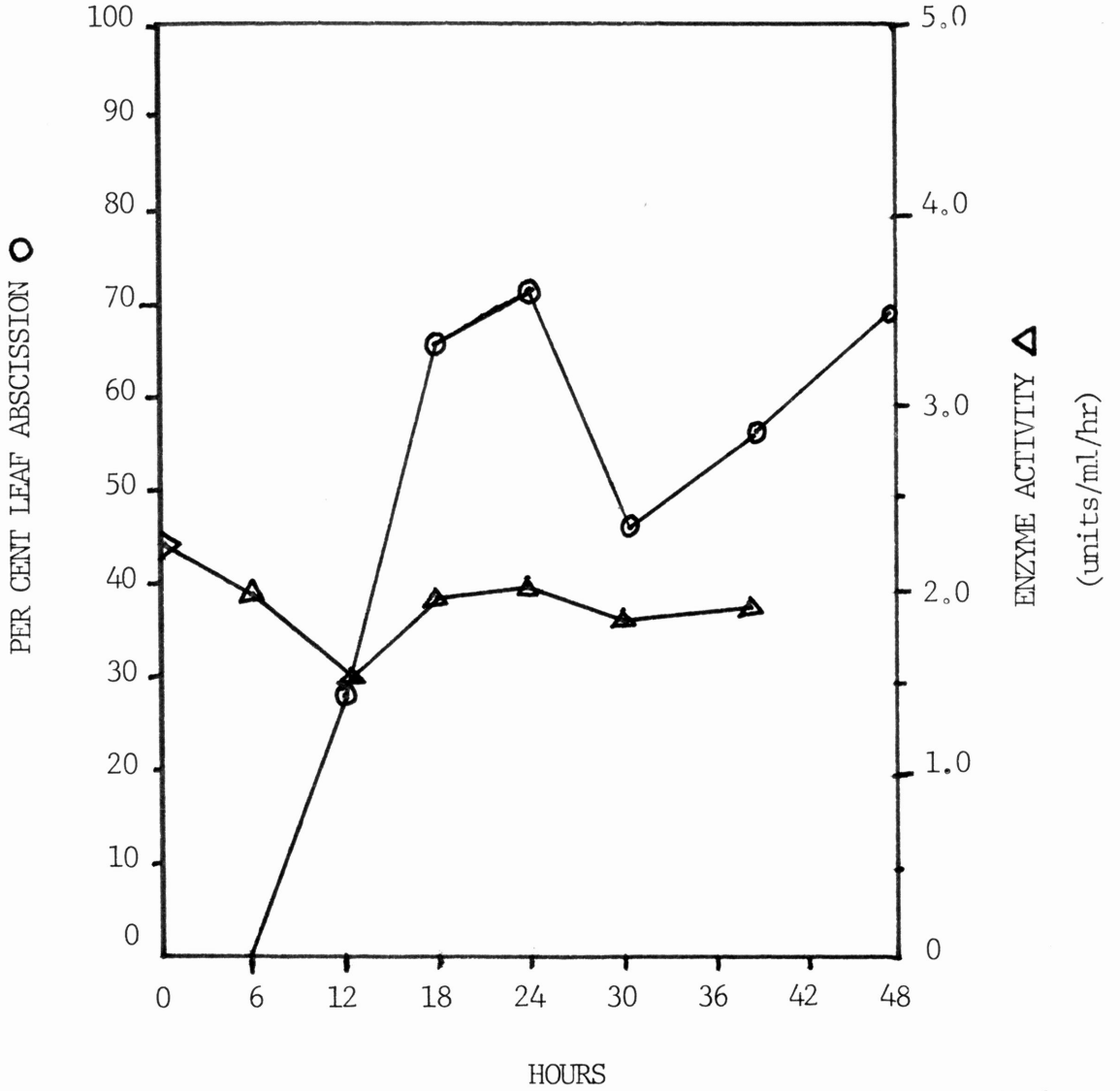

Fig. 3. Effect of ethylene on leaf abscission and cellulase levels.

Cellulase has been shown to exist in more than one form (6). The form that is stimulated by auxin is not associated with abscission and is found throughout the plant (6). However, the time course experiment doesn't point to the involvement of cellulase in abscission. Ethylene promoted abscission, but had little or no effect on cellulase levels. This seems to contradict the present findings in the literature (2, 10). It is possible that the lack of a cellulase increase was due to experimental error. Cotton was difficult to work with, so the physiological state of the plants was often variable. In support of this is the fact that although leaf abscission rose to 73% it never went higher and the percentage varied between 49 and 71% after it reached its peak.

The requirement for large amounts of PVP to effectively extract cellulase indicates that the phenolic compounds in cotton would destroy the activity of cellulase if the PVP weren't present. The PVP is an ion exchange resin that binds-up phenols.

The role of cellulase in cotton is still not known. It is possible that rises in pectinase, with the resulting middle lamella breakdown, are responsible for abscission in cotton. However, problems with the pectinase assay prevented this point from being examined. Some of the research from this project indicates that cellulase could be important to abscission, but due to the inability of a time course experiment to provide support, such eivdence must be considered inconclusive.

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