146
070
THS

EFFECT OF CULTIVAR, SEEDLING AGE, AND ETHYLENE TREATMENT ON THE AMOUNT OF LIGNIN-LIKE MATERIAL IN ASPARAGUS (ASPARAGUS OFFICINALIS L.)

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

GEORGINA MARGARET MALLOY WERNER

1977

3 1293 10165 3842

LIBRARY
Michigan State
University

ABSTRACT

EFFECT OF CULTIVAR, SEEDLING AGE, AND ETHYLENE TREATMENT ON THE AMOUNT OF LIGNIN-LIKE MATERIAL IN ASPARAGUS (ASPARAGUS OFFICINALIS L.)

by

Georgina Margaret Malloy Werner

A spectrophotometric procedure was used to determine if genetic differences in lignin content could be detected in young seedlings of asparagus. Seeds of Asparagus officinalis L. (cvs. Mary Washington and U.C. 711) were germinated and the seedlings were grown for 23 or 27 days on a modified Murashige and Skoog media at 27°C with a 16 hour photoperiod. Ethylene (100 ppm) for 4 days was applied to the flasks. Thirty mg samples of tissue were extracted with a strong alkali and the absorbance read at 350 nm. There was no effect of cultivar on lignin content of the control plants at 27 days. However, treatment with ethylene significantly increased lignin-like material of U.C. 711 but not of Mary Washington. Lignin content of plants harvested 31 days after germination were not affected by cultivar or ethylene treatment. The results indicate that ethylene may be used as a possible tool in increasing the amount of lignin-like material in young asparagus seedlings, thus allowing for detection of lignin differences among genetic lines in the seedling stage.

EFFECT OF CULTIVAR, SEEDLING AGE, AND ETHYLENE TREATMENT ON THE AMOUNT OF LIGNIN-LIKE MATERIAL IN ASPARAGUS (*ASPARAGUS OFFICINALIS* L.)

By

Georgina Margaret Malloy Werner

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

1977

ACKNOWLEDGMENTS

I wish to express my appreciation to Dr. Robert C. Herner for his friendship and guidance during the course of my studies. Appreciation is also expressed to the members of my guidance committee, Dr. Peter Carlson, and Dr. Shigemi Honma.

I also wish to express my appreciation to the following people for their encouragement or technical assistance with the thesis problem: Dr. G. Vest, David Cain, Carol Sherman, S. Ketsa, and S. Jedrzejek.

A very special thank you is extended to my parents, Mr. and Mrs. Thomas Malloy, Pia Policcichio Brown, and to Denny whose constant encouragement and help I will always remember.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Edibility of asparagus (Asparagus officinalis L.) is largely dependent upon the fibrousness of the spear. For many years asparagus breeders have characterized breeding lines and commercial cultivars as to whether they were "low", "medium" or "high" in fiber. Fibrousness in asparagus results from the lignification of the cell walls in the pericycle and the vascular bundles of the spear.

The purpose of this study was to evaluate a spectrophotometric procedure for detecting lignin early in the growth of an asparagus seedling. This method of analysis utilizing seedling tissue would aid in the screening of genetic material for lignin and perhaps provide a means of studying lignin formation in seedlings.

It has been well established that differences in the ability to form lignin exists between species (Stafford, 1965, 1967). Cheng and Marsh (1968) found that variations also occurred with species of Pisum sativum L.

Sosa-Coronel et al. (1976) reported that there was differences in fiber content of spears of different asparagus cultivars and also of ten selected crowns from breeding plots at the Sodus Experiment Station of Michigan State University.

Most of the previous fiber research on asparagus has been on investigating environmental factors affecting fiber production, methods for fiber determination, and on altering harvesting practices of the spears from the field. Attempts have been made to avoid the

high amounts of fiber in the spear by snapping the spear at the point at which fiber no longer becomes objectionable rather than the former practice of harvesting at ground level. This would leave the most objectionable fibrous parts in the field.

Several methods for fiber determination have been developed. The blender method by Smith and Kramer (1947) involves the boiling and maceration of the spear. This was used by Sosa-Coronel et al. (1976) to evaluate fiber content in different cultivars of asparagus, but only a gross determination of fiber content may be made using this method and it requires large amounts of material.

Lee (1943) developed the alcohol insoluble solids (A.I.S.) method, which involves the evaluation of insoluble solids in the various segments of a 25 gm sample of a spear. This method would not be very practical because high solids have been found in the tips of spears, which is the tenderest portion of the spear.

Other methods for fiber determination have been used such as instruments for measuring force required to shear the spears (Jenkins and Lee, 1956). The disadvantage of all these methods of analysis is that they all require a large quantity of plant tissue and would only be applicable for detecting large differences in fiber content of mature plants.

Genetic basis for fiber content in asparagus has not been studied. If differences between cultivars do occur the breeder may breed for low fiber content with the other parameters of disease and insect resistance, and yield when selecting lines of asparagus.

REVIEW OF LITERATURE

Lignin, one of the most important cell wall constituents, has been studied extensively but its chemistry is still not fully known or understood. Lignin is extremely difficult to study as a chemical because it cannot be readily extracted without destroying it and some of its subunits; therefore, it has been impossible to determine its exact chemical structure (Kremers, 1959). Much of what is known about lignin structure has been determined by analysis of the intermediates in its synthesis. It is known to be a polymer of high carbon content consisting largely of phenylpropanoid (C_6,C_3) units (Brown, 1961). Lignin is the end metabolic product of a series of reactions originating from carbohydrates via the shikimic acid pathway (Bidwell, 1974). The general outline of the biochemical pathway of phenolic acids and lignin biosynthesis is not well established (Freudenberg and Neish, 1968). Once lignin is formed, it functions primarily as a component of the cell wall and gives strength to upright stems.

Bondi and Meyer (1948) were the first to isolate lignins from herbaceous plant material in a state of comparative purity. Isolation of lignin was accomplished by a series of extractions with an alkali. Once a method was developed that could be readily used for lignin extraction, biosynthetic pathways used by the plants could be studied more closely.

Stafford (1960b, 1962) modified the technique of Bondi and Meyer to study the differences between lignin-like polymers formed by

peroxidation of two lignin precursors, eugenol and ferulic acid of timothy grass (Phleum pratense L.). She concluded that ferulic acid is a closer precursor of true lignin than eugenol in Phleum.

Two of the most important enzymes of the lignin biosynthesis pathway are phenylalanine ammonia-lyase (PAL) and peroxidase. PAL deaminates phenylalanine to trans-cinnamic acid which is then converted to the phenolic precursors of lignin (Neish, 1961). Goldstein (1971) was the first to report the occurence of L-phenylalanine ammonia-lyase in asparagus. The level of PAL activity was highest in the basal sections of freshly harvested spears and decreased toward the tip of the spear. This parallels the known distribution of lignified fibers in asparagus. Similar distribution patterns have been reported by other investigators. Yoshida and Shimokoriyama (1965) found that phenylalanine deaminase activity develops in parallel with lignification in stems of buckwheat (Fagopyrum esculentum Moench.). Higuchi (1966) has also reported similar findings in 2 bamboo species (Phyllostachys pubescens Sieb. et Zucc. and P. reticulata Sieb. et Zucc.). Goldstein (1971) suggested that since there was no extractable tyrosine ammonia-lyase (TAL) activity, PAL presumably may be responsible for the production of cinnamic acid and its derived products leading to lignin biosynthesis.

Tracer studies by Young et al. (1966) have shown that PAL is a major controlling factor in lignification. Higuchi (1966) found a pronounced increase in PAL activity during lignification of bamboo shoots. Yoshida and Shimokoriyama (1965) reported that in studies of buckwheat lignin, incorporation of phenylalanine-C^{14} into lignin was

370 times higher than from tyrosine-C^{14}. A relationship has been reported by Cheng and Marsh (1968) between the activity of PAL and the synthesis of lignin and enhanced production of phenylpropanoid compounds. Rubery and Northcote (1968) reported that PAL activity was highest in preparations made from lignified tissue of sycamore (Acer pseudoplatanus L.).

Haddon and Northcote (1976) found that PAL activity was correlated with the amount of cell differentiation under a variety of growth conditions in bean (Phaseolus vulgaris L.) callus. They also found that in addition to lignin, cinnamic acid was also the precursor of various other polyphenols.

The enzyme peroxidase has been shown to be very important in the formation of lignin. Freudenberg et al. (1952) suggested that peroxidase through the generation of free radicals in phenylpropanoid units such as coniferyl alcohol could catalyze their condensation into the lignin polymer. Studies by Stafford (1965), Nakamura (1967) and Harkin and Obst (1973) lend more credence to the Freudenberg theory. Lignin-like material has been formed in vitro on a artifical matrix by Siegel (1962) from a solution of eugenol, hydrogen peroxide and peroxidase. Helpler et al. (1972) reported peroxidase activity in areas that are known to become lignified in Coleus blumei Benth. Peroxidase activity and lignin were found in the primary wall, in the secondary thickenings and in the corners of the cells of both parenchyma cells and vessel members. These findings agree with the observations of Wardrop and Bland (1958) and Koblitz (1961). Higuchi (1957) has shown that coniferyl aldehyde was formed by autoxidation

of eugenol, and coniferyl aldehyde was condensed to a lignin-like substance with peroxidase and H_2O_2, but purified eugenol was not.

Ethylene, a simple C_2 unsaturated gas, has been used for many years as a regulator of growth and development (Abeles, 1973). Haard et al. (1974) have shown that a one hour application of 100 ppm ethylene to freshly harvested asparagus (cv. Mary Washington) spears stimulated isoperoxidase shifts from the basal portion of the spear to the tip. The development of "fiber" was also accelerated by the exogenous ethylene application.

Goldstein et al. (1972) have reported that in excised tissues from freshly harvested asparagus spears, an increase in PAL activity was found. This increase could be due to the increase in endogenous ethylene due to the excision and incubation of the tissue (Rhodes et al., 1971).

Intact grapefruit (Citrus paradisi Macf. cv. Marsh Seedless) peels have been reported by Riov et al. (1969) to have very low PAL activity in the flavedo tissues. However, excised incubated discs showed an increase in PAL activity as did intact fruit that were treated in an air stream of 300 ppm ethylene. Once the intact fruit was removed from the ethylene environment, the level of PAL activity decreased.

Increased PAL and peroxidase activity in sweet potato (Ipomoea batatas (L.) Poir) root discs has also been shown by Imaseki et al. (1968) to be induced by ethylene.

Extensive research in the area of lignin biosynthesis in response to ethylene was made by Rhodes and Wooltorton (1971, 1973). In studies

on rutabaga (Brassica napus L. (Napobrassica group)), Rhodes and Wooltorton (1971) found a small increase in endogenous ethylene production which resulted from damage due to excision of the discs. Rutabaga discs held in the presence of a very low concentration of ethylene (8 ppm) increased its lignin content 3 fold during a 20 hour ageing period (Rhodes and Wooltorton, 1973). They concluded that the increased level of endogenous ethylene production from excision of the tissue was capable of promoting a small increase in PAL activity and lignin biosynthesis. The addition of exogenous ethylene greater increases in PAL and lignin.

Stafford (1965) has demonstrated repeatedly that the phenolic monomers that are polymerized into lignin products arise from carbohydrates via the shikimic acid pathway. She speculates that since lignin is attached at specific sites on the cell wall, the number and types of these sites may also be controlling factors.

Direct extraction of lignin is difficult due to its complex chemical nature, therefore lignin cannot be measured directly. The method used in this study is based on the technique of lignin extraction developed by Stafford (1960b, 1962). It is the only method known to date for extracting lignin-like substances from herbaceous material. It is characterized by the solubility of lignin-like material in a strong alkali. The determination is based upon a difference spectrum at 350 nm in 0.05 N NaOH (pH 12.3) and in 0.05 M phosphate buffer (pH 7.0) and is expressed as a Δ O.D.

Although it has been demonstrated that there is a correlation between PAL and peroxidase with lignification in asparagus (Goldstein

et al., 1971; Haard et al., 1974) it would not be desirable to extract these enzymes since they are involved in other biosynthetic pathways and increase in activity may not reflect changes in lignin content. It would be preferable to extract the lignin-like material since this would be a much better indication of true fibrousness of the seedling.

MATERIAL AND METHODS

Growth of Plant Material

A modified Murashige and Skoog media (Murashige et al., 1972) was prepared excluding growth regulators, with 20 gm sucrose and 6 gm agar. Thirty five ml of media were placed in 125 ml Erlenmeyer flasks and autoclaved at 15 psi for 20 minutes.

Seeds of two cultivars of asparagus (cvs. Mary Washington and U.C. 711) were surfaced sterilized for 15 minutes in a 1:10 chlorox solution with a few drops of "Tween 20", a surfactant. The seeds were then rinsed 3 times in sterile deionized water.

Transfer of the seeds to the sterile media was done under aseptic conditions. Seven seeds were placed in a flask. Upon replacing the cotton plug in the opening of the flask, a piece of aluminum foil was placed over the cotton plug to prevent dessication of the media. Upon completion of the transfers, the flasks were randomly assigned treatments.

A completely randomized design was used for this experiment. There were two cultivars; Mary Washington (low fiber content), and U.C. 711 (high fiber content) (Sosa-Coronel et al., 1976), 3 harvest dates (22, 27, 31 days after germination) and 0 or 100 ppm ethylene treatment.

There were 25 replicates per treatment combination for the cultivar Mary Washington. Due to the slow growth of U.C. 711 there were only 5 replicates for the second harvest date with 100 ppm ethylene, and 10 replicates for the control. The third harvest date had 15 and

11, respectively. Statistical analysis was done using Fischers F-Test for unequal sample number. Mean separation tests were conducted using the L.S.D. test at the 5% level.

The flasks were randomly placed in a growth room at 27°C with 1000 foot candles of illumination (Sylvania Coolwhite (F72T8-CW) fluorescent tubes and 60 watt incandescent bulbs) with a 16 hour photoperiod.

Day one of germination was considered to be when 50% of the flasks had seeds with radicles emerged. There were 4 to 7 germinated seedlings at harvest.

At 18, 23 and 27 days after germination, treatments were applied to the flasks. The ethylene treated flasks had the cotton plug and aluminum foil removed and the opening closed with a #16 rubber serum stopper. Ethylene was added with a syringe to give a concentration of 100 ppm (v/v). The flasks were not opened during the 4 day treatment. Air samples were taken from the flasks at 24 and 48 hours after ethylene exposure. The air samples were analyzed in a flame ionization gas chromatagraph. The levels of CO_2 and O_2 at 48 hours after exposure were 3.55% and 17.8%, respectively.

Lignin-like Polyphenol Accumulation

Spectrophotometric assay. -- Lignin extraction and determinations were done using a modified method of Stafford (1965). After the appropriate treatments the plants were removed from the flasks, and the roots were dissected from the shoots. The tissues were weighed, oven dried at 70°C and then ground to a fine powder in a mortar and

pestle. A 30 mg sample was placed in the bottom of the 12 ml centrifuge tube. The sample was extracted 3 times with 5 ml aliquots of diethyl ether at 20°C for 5 minutes followed by low centrifugation. The supernatant was discarded. The sample was then extracted 2 times with 5 ml aliquots of deionized water followed by centrifugation. The supernatant was also decanted and discarded. The residue was extracted with 2.5 ml of 0.5 N NaOH at 70°C for 16 hours then centrifuged. The supernatant was decanted and saved. The residue was reextracted with an additional 2.5 ml 0.5 N NaOH for 2 hours then centrifuged. The supernatant were combined. All solutions were brought to a 10 ml volume with distilled water. They were then divided into two equal aliquots. One was diluted to 10 ml with 0.05 N NaOH (pH 12.3) and in 0.05 M phosphate buffer (pH 7.0) and was expressed as a Δ O.D. Optical density readings were made using a Beckman DB Spectrophotometer at intervals of 10 nm from 230 to 450 nm. Each ml of solution in the cuvette contained an aliquot of a lignin extract equivalent to 1.5 mg dry weight of the original material.

Histological Test for Lignin

After the treatments were completed, one seedling per flask was removed for histological study. The basal sections of the seedlings were sectioned using a rotary cryostat. Sections 15 μ thick were affixed to glass slides. The sections were stained with phloroglucionol:HCl (Jensen, 1962) to test for the presence of lignin.

Histological Test for Peroxidase

Sections 15-20 μ thick were prepared on a rotary cryostat. The sections of tissue were affixed to a glass slide and stained for peroxidase activity with 1% H_2O_2 and 70% ethanol solution of 0.1 M benzidine (Poovaiah and Rasmussen, 1973).

RESULTS AND DISCUSSION

Effects of Ethylene on the Formation of Lignin-like Polyphenols

Spectrophotometric Determination

These lignin-like polyphenols extracted from the asparagus shoots are characterized by their solubility in 0.5 N NaOH and by a difference spectrum with a relatively high peak in the 350 nm region and small ones at 320 and 260 nm, and a negative minimum in the 280 nm region (Figure 1, 2). Stafford (1960a) has shown that the peaks at 260 and 320 nm in the difference spectrum can be attributed to ionizable groups in simple phenols, while the peak around 350 nm is due to large conjugated phenols which are probably breakdown products of lignin (Figure 1, 2).

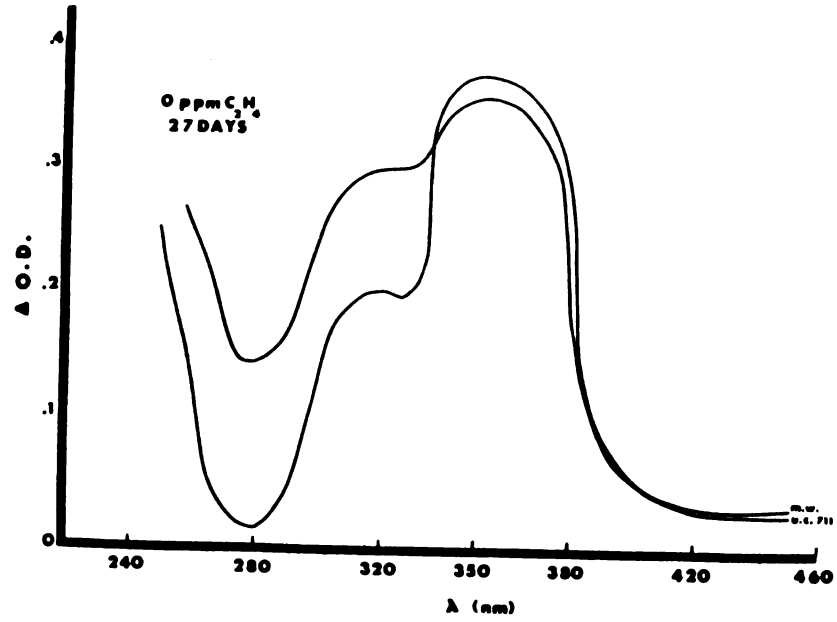
The first harvest date did not yield sufficient dry material for analysis. The second and third harvest dates (27, 31 days) did yield adequate dry matter and were analyzed for lignin-like substances at 350 nm.

Extracts from the two control cultivars after 27 days of growth did not show significant differences in Δ O.D. at 350 nm (Table 1). However, treatment of the asparagus plants for 4 days with ethylene caused a significant increase in Δ O.D. of extracts of U.C. 711 but not of extracts from Mary Washington (Table 1, Figure 1).

Extracts of plants harvested 31 days after germination did not show any differences between cultivars or between controls or ethylene treatment (Table 1, Figure 2).

Figure 1. (Top) Δ O.D. of alkali extracts (260 to 450 nm) of 'Mary Washington' and 'U.C. 711' from 27 day old seedlings treated with 0 ppm ethylene.

(Bottom) Δ O.D. of similar extracts from seedlings treated 4 days with 100 ppm ethylene.


0 ppm C_2H_4
27 DAYS
Δ O.D.
.4
.3
.2
.1
0
240
280
320
350
380
420
460
λ (nm)

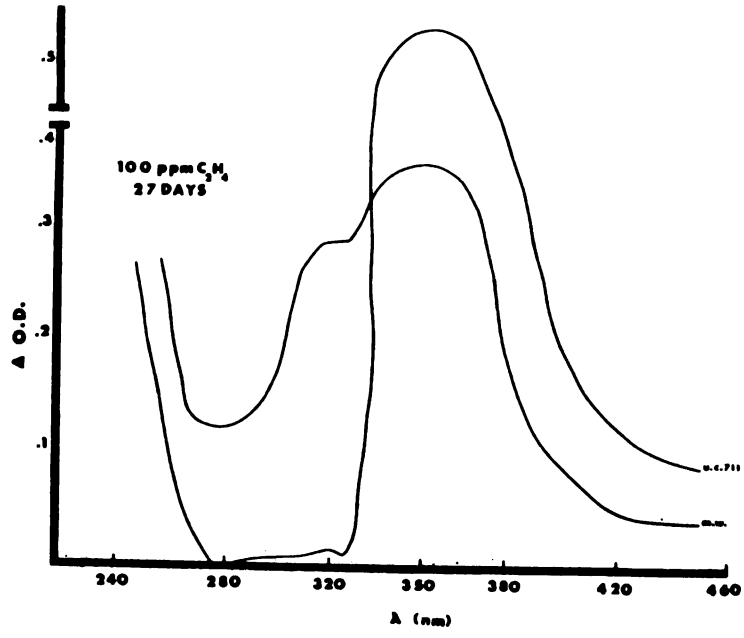
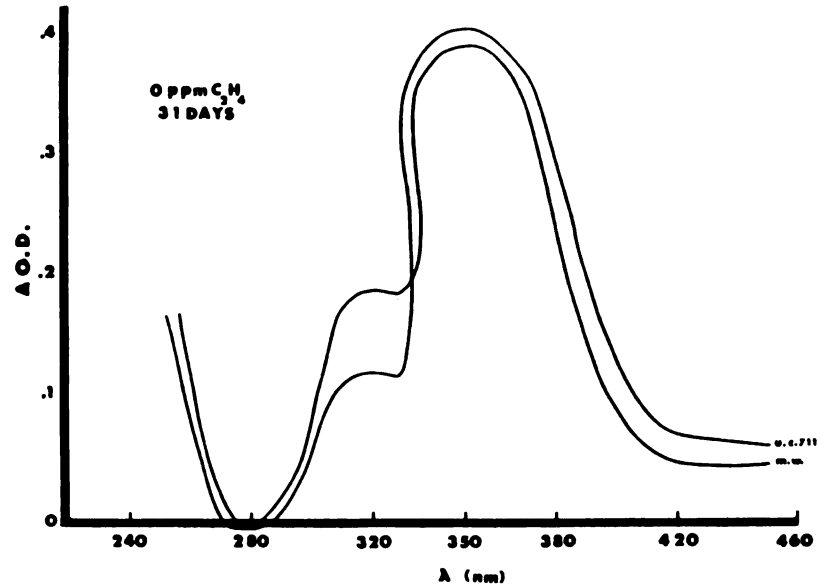

100 ppm C_2H_4
27 DAYS
Δ O.D.
.5
.4
.3
.2
.1
240
280
320
350
380
420
460
λ (nm)

Figure 2. (Top) Δ O.D. of alkali extracts (260 to 450 nm) of 'Mary Washington' and 'U.C. 711' from 31 day old seedlings treated with 0 ppm ethylene.

(Bottom) Δ O.D. of similar extracts from seedlings treated 4 days with 100 ppm ethylene.


0 ppm C_2H_4
31 DAYS
Δ O.D.
.4
.3
.2
.1
0
240
280
320
350
380
420
460
λ (nm)

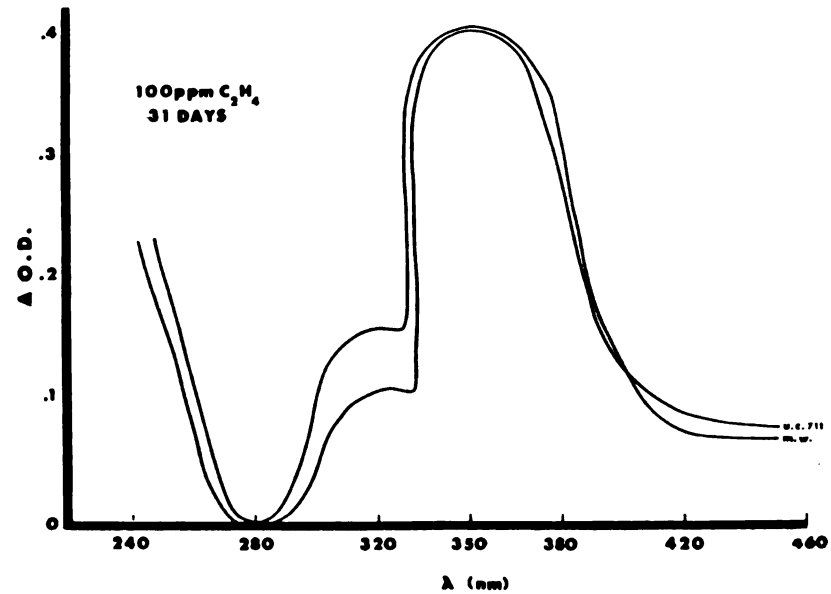

100 ppm C_2H_4
31 DAYS
Δ O.D.
.4
.3
.2
.1
0
240
280
320
350
380
420
460
λ (nm)

Table 1. Mean Δ OD (350 nm) of alkali extracts of 27 and 31 day old asparagus seedlings treated with 0 and 100 ppm ethylene (C_2H_4) 4 days prior to harvest.

Cultivar	27 day harvest		L.S.D.	31 day harvest		L.S.D.
	0 ppm C_2H_4	100 ppm C_2H_4		0 ppm C_2H_4	100 ppm C_2H_4	
Mary Washington	0.3804	0.3666	n.s.	0.3823	0.4098	n.s.
U.C. 711	0.3660	0.5616	0.174	0.4089	0.4046	n.s.
L.S.D.	n.s.	0.1550		n.s.	n.s.	

Ethylene, therefore, caused an increase in lignin-like substances in the asparagus cultivar U.C. 711 at 27 days after germination. It therefore appears to accentuate the difference between the cultivars so that they are significantly different at the 5% level. It was observed during the cutting of the tissue at both harvest dates that seedlings of both cultivars treated with ethylene appeared brittle as compared to the succulent nature of the controls. No controlled or precise measurements were made, however.

Reaction of Ethylene Treated Tissue with Phloroglucinol:HCl

Mounted sections of untreated asparagus shoots of both cultivars (27, 31 days) showed red coloration in the epidermal layer and the vascular bundles when stained with phloroglucinol:HCl. However, the red coloration was more extensive throughout the ethylene treated tissue. There were also masses of red coloration throughout the treated tissues. Differences could not be detected between the two cultivars.

Reaction of Ethylene Treated Tissue for Peroxidase Activity

Mounted sections of untreated and treated sections of the asparagus shoots harvested at 27 and 31 days after germination reacted positive to the 1% H_2O_2 and 70% ethanol solution of 0.1 M benezidine (Poovaiah and Rausmussen, 1973).

SUMMARY AND CONCLUSIONS

Spectrophotometric assay of alkaline extracts of asparagus shoots suggests that 100 ppm ethylene for 4 days induced a significantly higher amount of lignin-like polyphenols in asparagus plants of U.C. 711 than Mary Washington that were harvested at 27 days after germination. However, at 31 days after germination, the results were dissimilar. There was no significant cultivar difference within and between treatments at 31 days.

This phenomenon could possibly be explained by the decrease in the proportion of younger cells to the total between the two harvest dates. Older cells would not be expected to respond to ethylene by producing more lignin since the cells have undergone a greater degree of differentiation.

The 100 ppm ethylene treatment at 27 days after germination increased the amount of lignin-like substances in the cultivar U.C. 711 (high fiber). It appears that the two cultivars responded differently to the ethylene treatment and that the differences between Mary Washington (low fiber) and U.C. 711 (high fiber) is significant at the 5% level. This relationship agrees with conclusions reached by Sosa-Coronel et al. (1976), who analyzed fiber content of marketable spears of these cultivars. The differences in the response of the cultivars to ethylene suggests that they differ genetically in their ability to synthesize lignin at this stage of development. Since the environmental conditions under which both cultivars were

grown was identical, the premise that true genetic differences for lignin content exist in the two cultivars is further supported.

Tissue sections stained with phloroglucinol:HCl did exhibit differences in the intensity and quantity of staining. Ethylene treated tissues showed more secondary thickening in the xylem vessels than the controls. Differences in staining quality could be detected between the controls and ethylene treated tissues; but not between cultivars. This type of histochemical staining procedure could not be used to distinguish between the two cultivars at this early stage of development.

No differences were found in the sites of peroxidase localization between control and ethylene treated plants. Goff (1975) found that there were moderate fluctuations in the level of peroxidase between different tissues or within the same tissue due to the effect of reagent penetration on the intensity of the reaction. The pattern of peroxidase activity was determined in this study, however, no differences were detected between the control and ethylene treated plants of the two cultivars.

It may also be possible that the material extracted from the asparagus shoots and measured may not be a true representation of the amount of lignin due to its complex chemical nature, however, this method does hold some promise if further testing is done.

The results of this study indicate that it may be feasible to detect lignin-like substances very early in the development of an asparagus seedling. Through the use of ethylene it appears that seedlings of U.C. 711 (high fiber content) can be induced at a very young age to produce a higher amount than a cultivar (Mary Washington) that is low in fiber.

Further studies should include a larger number of cultivars to validate the results of this study. Possibly the cultivars that are ranked from low to high in fiber by breeders may respond similarly using this technique and ethylene application.

It appears that this analysis should also be done with seedlings of both younger and older ages than used in these experiments. As stated earlier the seedlings at 31 days after germination may have lost their ability to respond to ethylene. Future studies with various cultivars should be made to learn whether similar results are repeated.

If an analytical technique could be developed for screening large populations of seedlings it would be a very valuable tool for the plant breeder and physiologist studying lignin formation in asparagus.

LITERATURE CITED

1. Abeles, F. B. 1973. Ethylene In Plant Biology. Academic Press. New York. 302 pp.

2. Bidwell, R. G. S. 1974. Plant Physiology. Macmillan Publishing Co., New York. 643 pp.

3. Bondi, A. and H. Meyer. 1948. Lignins in young plants. Biochem. J. 43: 248-256.

4. Brown, S. A. 1961. Chemistry of lignification. Sci. 134: 305-313.

5. Cheng, C. K. C. and H. V. Marsh, Jr. 1968. Gibberellic acid-promoted lignification and phenylalanine ammonia-lyase activity in a dwarf pea (Pisum sativum). Plant Physiol. 43: 1755-1759.

6. Freudenberg, K., H. Reznek, and D. Rosenach. 1952. Das ander Verholzung beteiligte Ferment-system. Chem. Ber. 85: 641-647.

7. Freudenberg, K. and A. C. Neish. 1968. Constitution And Biosynthesis of Lignin. Berlin-Heidelberg. New York: Springer. 128 pp.

8. Goff, C. W. 1975. A light and electron microscopic study of peroxidase localization in the onion root tip. Am. J. Bot. 62: 280-291.

9. Goldstein, L. D., P. H. Jennings, and H. V. Marsh, Jr. 1971. A preliminary investigation of L-phenylalanine ammonia lyase activity in asparagus: Distribution and response to storage, excision and incubation. Plant Cell Physiol. 12: 657-661.

10. Goldstein, L. D., P. H. Jennings, and H. V. Marsh, Jr. 1972. L-Phenylalanine ammonia lyase activity in asparagus spears: Effects of excision and incubation. Plant Cell Physiol. 13: 783-793.

11. Haard, N. F., S. C. Sharma, R. Wolfe, and C. Frenkel. 1974. Ethylene induced isoperoxidase changes during fiber formation in postharvest asparagus. J. Food Sci. 39: 452-456.

12. Haddon, L. E. and D. H. Northcote. 1976. Correlation of the induction of various enzymes concerned with phenylpropanoid and lignin synthesis during differentation of bean callus (Phaseolus vulgaris L.). Planta 128: 255-262.

13. Harkin, J. M. and J. R. Obst. 1973. Lignification in trees. Indication of exclusive peroxidase participation. Sci. 180: 296-298.

14. Hepler, P. K., R. M. Rice, and W. A. Terranova. 1972. Cytochemical localization of peroxidase activity in wound vessel members in Coleus. Can. J. Bot. 50: 971-983.

15. Higuchi, T. 1957. Biochemical studies of lignin formation. I. Physiol. Plant. 10: 633-648.

16. Higuchi, T. 1966. Role of phenylalanine deaminase and tyrase in the lignification of bamboo. Agr. Biol. Chem. 30: 667-673.

17. Imaseki, H., M. Uchiyama, and I. Uritani. 1968. Effect of ethylene on the inductive increase in metabolic activities in sliced sweet potato roots. Agr. Biol. Chem. 32: 387-389.

18. Jenkins, R. R. and F. A. Lee. 1940. Tenderometer readings as an index of quality of fresh asparagus. Food Res. 5: 161-166.

19. Jensen, W. A. 1962. Botanical Histochemistry. Freeman Publishing Co. San Francisco. 408 pp.

20. Koblitz, H. 1961. Einige Bemerkungen zum histochemischen Nachweis von an der Verholzung beteiligten Fermenten. Ber. Dtsch. Bot. Ges. 74: 274-287.

21. Kremers, R. E. 1959. The lignins. Ann. Rev. Plant Physiol. 10: 185-196.

22. Lee, F. A. 1943. Determination of toughness of frozen asparagus. Food Res. 249-253.

23. Murashige, T., M. N. Shabde, P. M. Hasegawa, F. H. Takatori, and J. B. James. 1972. Propagation of asparagus through shoot apex culture. I. Nutrient medium for formation of plantlets. J. Am. Soc. Hort. Sci. 97: 158-161.

24. Nakamura, W. 1967. Studies on the biosynthesis of lignin. I. Disproof against the catalytic activity of laccase in the oxidation of coniferyl alcohol. J. Biochem. (Toyko) 62: 54-61.

25. Neisch, A. C. 1961. Formation of m- and p-coumaric acids by enzymatic deamination of corresponding isomers of tyrosine. Phytochem. 1: 1-24.

26. Poovaiah, B. W. and H. P. Rasmussen. 1973. Peroxidase activity in the abscission zone of bean leaves during abscission. Plant Physiol. 52: 263-267.

27. Rhodes, M. J. C. and L. S. C. Wooltorton. 1971. The effect of ethylene on the respiration and on the activity of phenylalanine ammonia lyase in swede and parsnip root tissue. Phytochem. 10: 1989-1997.

28. Rhodes, M. J. C. and L. S. C. Wooltorton. 1973. Changes in phenolic acid and lignin biosynthesis in response to treatment of root tissue of the Swedish Turnip (Brassica napo-brassica) with ethylene. Qual. Plant. 23: 145-155.

29. Riov, J., S. P. Monselise, and R. S. Kahan. 1969. Ethylene-controlled induction of phenylalanine ammonia-lyase in citrus fruit peel. Plant Physiol. 44: 631-635.

30. Rubery, P. H. and D. H. Northcote. 1968. Site of phenylalanine ammonia lyase activity and synthesis of lignin during xylem differentiation. Nature 219: 1230-1234.

31. Siegel, S. M. 1962. The Plant Cell Wall. Pergamon Press. New York 123 pp.

32. Smith, H. R. and A. Kramer. 1947. The fiber content of canned green asparagus. Canner 104: 15-16.

33. Sosa-Coronel, J., G. Vest, and R. C. Herner. 1976. Distribution of fiber content in asparagus cultivars. HortSci. 11: 149-151.

34. Stafford, H. A. 1960a. Comparison of lignin-like polymers produced peroxidatively by cinnamic acid derivatives in leaf sections of Phleum. Plant Physiol. 35: 612-618.

35. Stafford, H. A. 1960b. Differences between lignin-like polymers formed by peroxidation of Eugenol and Ferulic Acid in leaf sections of Phleum. Plant Physiol. 35: 108-114.

36. Stafford, H. A. 1962. Histochemical and biochemical differences between lignin-like materials in Phleum pratense L. Plant Physiol. 37: 643-649.

37. Stafford, H. A. 1965. Factors controlling the synthesis of natural and induced lignin in Phleum and Elodea. Plant Physiol. 40: 844-851.

38. Stafford, H. A. 1967. Biosynthesis of phenolic compounds in first internodes of sorghum: Lignin and related products. Plant Physiol. 42: 450-455.

39. Wardrop, A. B. and D. E. Bland. 1958. The process of lignification in woody plants. IN: Proc. 4th Int. Congr. Biochem. Vol. 2. Ed. K. Kratzl and G. Billek Publishers pp 92-116.

40. Yashida, S. and M. Shimokoriyama. 1965. Studies on phenylalanine deaminase in buckwheat plant. Bot. Mag. 78: 14-19.

41. Young, M. A., G. H. N. Towers, and A. C. Neisch. 1966. Taxamonic distribution of ammonia-lyases for L-phenylalanine and L-tyrosine in relation to lignification. Can. J. Bot. 44: 341-349.

MICHIGAN STATE UNIV. LIBRARIES
31293101653842