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LOSS OF FERULIC ACID FROM DECOMPOSING LEAVES OF CELTIS LAEVIGATA AND SUBSEQUENT ISOLATION OF ORGANISMS ABLE TO UTILIZE IT AS THE SOLE CARBON SOURCE

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LOSS OF FERULIC ACID FROM DECOMPOSING LEAVES OF CELTIS LAEVIGATA AND SUBSEQUENT ISOLATION OF ORGANISMS ABLE TO UTILIZE IT AS THE SOLE CARBON SOURCE

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ABSTRACT

The suppression of plant growth by different phenolic acids is well known. This work was designed to determine if ferulic acid, a known phenolic inhibitor of plant growth, accumulates in the soil and if soil microorganisms could be isolated that metabolize it. Over 99% of the extractable ferulic acid was lost from decaying hackberry leaves in 300 days. During this time the amount in the soil remained fairly constant at about 30 ppm except for the March sample which was significantly higher than the rest. Addition of ferulic acid to soil caused an increase in CO_2 evolution and in numbers of a select group of microorganisms. Rhodotorula rubra and Cephalosporium curtipes that actively metabolize ferulic acid were isolated but the metabolic pathway employed appears to be different than the reported one. The reported pathway for ferulic acid breakdown is ferulic acid to vanillic acid to protocatechuic acid to beta-keto-adipic acid. Rhodotorula rubra was found to convert ferulic acid to vanillic acid, but no evidence was found for utilization of the rest of the pathway. Cephalosporium curtipes appears to use a different pathway because no phenolic compounds were found during the breakdown of ferulic acid. The

presence in the soil of microorganisms that metabolize ferulic acid and other phenolic acids is ecologically significant because such organisms prevent accumulations of these substances, which are toxic to many other microorganisms and higher plants.

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CHAPTER I

INTRODUCTION

Ferulic, synapic and p-coumaric acids are important in the synthesis of lignin (Freudenberg and Neish 1968). These same acids have been shown to be products of lignin degradation. The resistance of lignin to decomposition has been reviewed by Norman (1936), Alexander (1969) and Hurst and Burges (1967). These authors attribute this resistance to the chemicals of which it is composed and the complex bonding of the lignin molecule. Many different organisms have been found to be involved in the decomposition of lignin (Gottlieb and Pelczar 1951).

The products of lignin decomposition have been shown to suppress plant growth (Hennequin and Juste 1967, Wang, Yang, Chuang 1967). Ferulic acid and p-coumaric acid have been shown to retard ion uptake and protein synthesis in plants (Croak 1972), inhibit seed germination (Rasmussen

and Rice 1971, Wilson and Rice 1968), and suppress plant growth (Börner 1960, Muller 1966, Guenzi and McCalla 1966a, Rice 1967, Kefeli and Kadyov 1971). Degradation products have also been shown to retard growth of plant pathogens (Davey and Papavizas 1959, Lingappa and Lockwood 1962, Whitehead 1963). Rice (1964, 1965a, 1968) demonstrated that phenolic acids retard the growth of several organisms involved in the nitrogen cycle.

Many authors have shown that organic material on the soil surface is usually high in phenolic acids and especially high in acids involved in lignin synthesis. Therefore, it might be expected that these compounds would accumulate to high concentrations in the soil. This does not occur, however, because the amount in the soil is generally very low (Guenzi and McCalla 1966b, Wang, Cheng, Chuang 1967).

I hypothesized that lack of accumulation of high concentrations of phenolic acids in soil is due to the rapid decomposition of these acids upon entry into the soil. I also hypothesized, however, that if leaves are allowed to decompose on the surface, any loss in phenolic acids from them will cause at least a temporary increase in the amounts in the soil. Experiments were designed, (1) to test these hypotheses, (2) to determine if addition of ferulic acid to soil reduces the number and activities of microorganisms, (3) to attempt to isolate microorganisms from soil capable of utilizing ferulic acid as their sole carbon source, and

(4) to determine metabolic pathways employed in the metabolism of ferulic acid.

CHAPTER II

MATERIALS AND METHODS

Disappearance of Ferulic Acid from Decomposing Leaves

In order to follow the loss of ferulic acid from a natural source, leaves of Celtis laevigata (hackberry) were collected immediately after leaf drop in the fall of 1971. This species was chosen because of the high amounts of ferulic acid found in its leaves (Lodhi and Rice 1971). The leaves were oven dried at 60 C for 24 hours and then sown into nylon net bags which were weighed and numbered. The bags were returned to the collection area and placed on the soil surface in five rings spaced 0.75 m apart around a hackberry tree. Each ring consisted of 12 bags to permit removal of one bag from each ring every month for a year. When the bags were collected, 300 g of soil were removed from the 0-15 cm layer beneath each one and the ferulic acid concentration of this soil was determined.

The soil and leaf samples were oven dried at 60 C for 24 hours. All visible roots and debris were removed from the soil, and soil particles were removed from the leaf surfaces before soil and leaves were analyzed for ferulic acid.

Ferulic acid extractions were done by the procedure of Guenzi and McCalla (1966b). The samples were autoclaved in 2N NaOH for 45 minutes at 15 psi, the debris was removed by filtration, and the pH of the filtrate was adjusted to 2 with concentrated HCL which precipitated the humic acid fraction. This fraction was then removed by centrifugation at 12,000 x g for 15 minutes. The resulting supernatant was extracted three times with one-third volume of anhydrous ethyl ether. The ether portions were evaporated to dryness, and the residue taken up in a known volume of 95% ethanol. A portion of the extract was then spotted on Whatman 3 MM chromatographic paper and developed by the descending technique in n-butanol-acetic acid-water (63-10-27), (BAW), in the first dimension and 6% aqueous acetic acid, (6% AA), in the second dimension. The developed papers were viewed under short (2537 A) and long (3360 A) UV light to reveal the bright blue ferulic acid spot. Other phenolics were identified but no attempt was made to quantify them. The ferulic acid spots were eluted from the paper with 40% ethanol, and the optical density (O.D.) of the eluate was determined at 285nm with a Beckman DBG Spectrophotometer. The concentration of ferulic acid was determined from a standard curve made by adding known amounts of the acid to Whatman 3 MM paper and following the same procedure as with the unknowns. The ferulic acid in the soil was also extracted and its concentration determined by the above procedure.

Influence of Ferulic Acid on Microbial Activity

Prairie soil was air dried, passed through a 2mm sieve, and amended with 500 ug/g of ferulic acid. One hundred grams of this soil were added to a 500 ml Erlenmeyer flask with a side tube containing KOH (Bartha and Pramer 1965). The moisture content was brought to 30% with distilled water. Carbon dioxide was trapped in 0.08N KOH and titrated with 0.08N HCl. The control soil was treated the same as the test except it did not contain ferulic acid.

In connection with the above study, the number of microorganisms capable of utilizing ferulic acid was determined. A serial dilution was performed on a sample of this soil and 0.5ml of each dilution was spread on agar plates containing M-9FA. M-9FA is a chemically defined medium consisting of 1g NH_3Cl, 0.13g of $MgSO_4$, 3.0g of KH_2PO_4, and 6.0g of Na_2HPO_4 per liter of water with ferulic acid (500 ppm) as the carbon source. These plates were incubated at 30 C for 48 hours before counting.

In addition to ferulic acid (3 methoxy-4-hydroxy-cinnamic acid), vanillic (3 methoxy-4-hydroxybenzoic acid) and cinnamic acid (trans-benzenepropenoic acid) were tested to determine their influence on the soil. Vanillic acid was chosen because it is structurally similar to ferulic acid and is one of the intermediate products of the decomposition of ferulic acid (Henderson and Farmer 1955, Henderson 1956, 1963, Tom and Wood 1970b). Cinnamic acid

was chosen in an attempt to determine the role, if any, that the methoxy group plays in decomposition. Most authors feel that the methoxy group is metabolized immediately before ring cleavage. It was assumed that cinnamic acid without a methoxy group should be metabolized faster. The evolution of CO_2 was used as an indicator of microbial activity.

Isolation and Identification of Organisms

Two methods were used to isolate organisms that decompose ferulic acid. The first was to take soil from areas with the potential for large amounts of ferulic acid, do a serial dilution on the soil samples, and plate each dilution out on the previously described medium (M-9 plus 500 ppm ferulic acid). The organisms that appeared in the highest dilution were streaked on slants of M-9FA. If the organism continued to grow, it was saved; if not, it was discarded. The second method was a soil perfusion procedure (Goswami and Green 1971) in which ferulic acid at 1000 ppm in water was allowed to circulate through a column of soil for two weeks. Then a portion of the soil was removed, diluted, and plated out on M-9FA. These two methods plus the organisms isolated from the respiration study yielded eighteen different isolates. Each isolate was inoculated into M-9FA liquid medium and placed on a reciprocating shaker for 24 hours to determine which organisms utilize ferulic acid as the sole source of carbon. The amount of

growth was determined by the density of the medium. Two cultures grew significantly better in a 24-hour period and these were identified as Cephalosporium curtipes and Rhodotorula rubra.

Metabolic Studies

The optimum pH for growth in ferulic acid of these two organisms was determined by culturing them at different pH's and then determining the utilization of ferulic acid during a 24-hour period.

The growth rate of Rhodotorula rubra on ferulic acid was measured by following increases in optical density and decreases in ferulic acid content of the medium. The decrease in ferulic acid was also followed with Cephalosporium curtipes and biomass measurements were taken. In these studies, the inoculum was prepared by growing cells for 24 hours on a reciprocating shaker in M-9FA medium. Then 1 ml of inoculum was transferred to a 250 ml Erlemeyer flask containing 50 ml of the same medium. The O.D. of R. rubra cultures was determined by growing the organism in 250 ml Erlenmeyer flasks equipped with side-arms which fit into a Spectronic 20. Measurements were taken at 600nm. Two flasks were harvested at each time interval to determine the concentration of the remaining ferulic acid. Rhodotorula cells were removed from the medium by centrifugation. Because Cephalosporium curtipes did not pellet well upon centrifugation, it was removed from the medium by filtra-

tion through a 0.45 micron millipore filter and then dried for 24 hours at 60 C after which the hyphal weight was determined.

The loss of ferulic acid during the growth cycle was followed by extracting ferulic acid from the cell-free spent medium. The first step of the extraction procedure was the adjustment of the medium to pH 2.0 with concentrated HCl. The ferulic acid was extracted with anhydrous ethyl ether, the ether was evaporated, and the residue taken up in a known volume of 95% ethanol. Optical density was determined at 309nm and amounts of ferulic acid were determined from a standard curve. This wave length is slightly different from that cited in the decomposition section because the maximum absorption of ferulic acid varies, depending upon the concentration of water present.

It was of interest at this point to determine if other species of Cephalosporium and Rhodotorula could utilize ferulic acid as a sole source of carbon. Four species of Rhodotorula and three species of Cephalosporium were selected from the American Type Culture Collection on the basis of the original source of isolation. I specifically wanted soil isolates. All seven selected species were grown for 72 hours in shaker flasks using M-9 plus ferulic acid. Any increase in optical density was taken as a sign of growth. As a control the seven organisms were also grown in M-9 plus glucose.

Identification of Intermediates

The growth rates of Cephalosporium and Rhodotorula were also determined on vanillic, p-hydroxybenzoic, cinnamic and synapic acids. These compounds were chosen because of their structural similarity to ferulic acid. Vanillic and p-hydroxybenzoic acids were also chosen because they have been reported to be intermediates in ferulic acid breakdown.

Several authors (Henderson 1963, Tom and Wood 1970) reported that the sequence of ferulic acid breakdown is from ferulic to vanillic to protocatechuic acid followed by ring cleavage to beta-keto-adipic acid. In order to determine whether intermediates were produced, a portion of the ether fraction was chromatographed on Whatman 3 MM paper. The chromatograms were developed in BAW followed by 6% AA as previously described. Cephalosporium and Rhodotorula were also grown on M-9 with glucose as the carbon source and the spent medium was extracted as previously described. Paper chromatograms were prepared of this spent medium and of that attained when the organisms were grown on specific phenolic acids. Resulting phenolic compounds were identified only if they were produced in the phenolic acid medium and not in the glucose medium. The identification procedures used closely followed those described by Rice (1965b).

CHAPTER III

RESULTS

Disappearance of Ferulic Acid from Decomposing Leaves

Analysis of the decaying leaves placed around the hackberry tree showed that over 65% of the extractable ferulic acid was lost in the first 100 days, and by day 300 less than 0.1% remained in the leaves (Fig. 1). The rate was much faster during the early part of the experiment (January-April) than during the last part (April-October).

A statistical comparison between the results from individual months was made with Student's t test, and there was a significant difference at the 0.05 level between samples for adjacent months from day 1 to day 150. Furthermore, there was a significant difference at the 0.05 level between day 150 and day 300, but differences in amounts for adjacent months during this time were not significant. The experiment was terminated on day 300 because of the low amount of ferulic acid remaining in the leaves.

Concentrations of ferulic acid in the soil under the bags of decaying leaves remained relatively constant except

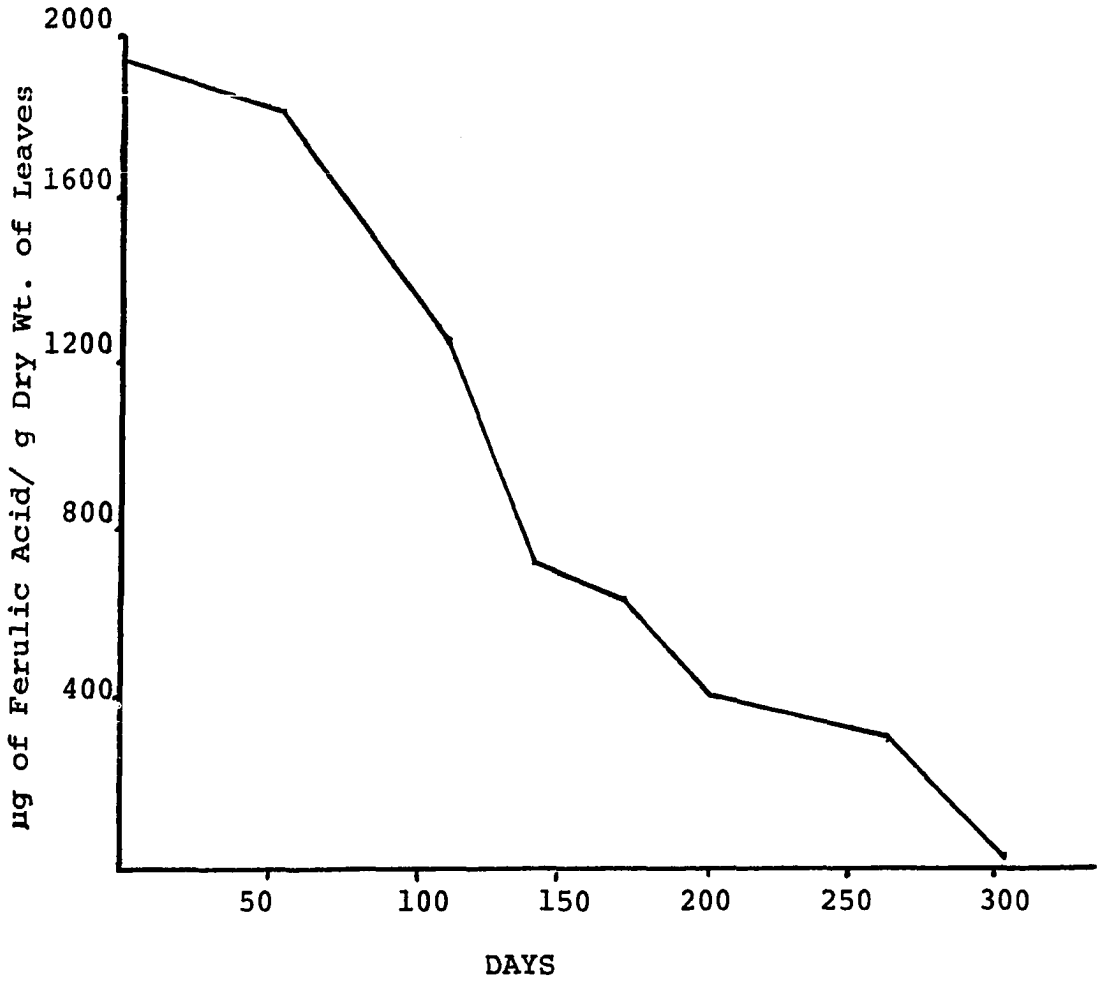


Figure 1. Change in concentration of ferulic acid during decomposition of leaves of Celtis laevigata. Concentration is plotted against time in days starting from day 0 which was December 10, 1971.

for minor month to month fluctuations which were not statistically significant (Table 1). The only exception to this was the concentration at day 100 which was significantly higher than the concentration at the previous period and from that at the following sampling period. There was no corelation between the ferulic acid in the soil and the amount lost from the leaves through decomposition.

Other phenolic acids identified from the soil were p-hydroxybenzoic, p-coumaric, and vanillic acids (Table 2). These were not found in the leaves with the Guenzi-McCalla technique.

Analysis of the roots of hackberry trees showed that they contained 2000 ppm of ferulic acid.

Effect of Ferulic Acid on Microbial Activity

Similar curves of CO_2 evolution indicated that addition of ferulic and vanillic acid to soil elicited similar responses from the soil population (Fig. 2). Addition of cinnamic acid resulted in a similar curve except that the lag phase was 14 days long.

The concentration of microorganisms in the soil increased greatly on addition of ferulic acid (Fig. 3), and the increase was about twice as great as in unamended soil. A pseudomonad and an actinomycete-like organism (Skerman 1967) accounted for virtually the total number of microorganisms that responded to the amended soil. These two

Table 1. Concentration of ferulic acid in soil under bags of decomposing hackberry leaves.

Days[a]	0	30	100	130	161	191	222	253	300	365
PPM[b]	25	26	47[c]	22	22	30	34	27	32	23

[a] Number of days from start of decomposition.

[b] Each figure is average of five analyses.

[c] Difference from concentrations at day 30 and day 130 significant at 0.05 level.

Table 2. Chromatography of phenolic acids extracted from leaves and soil.

Compound	Rf's on Whatman #1[a]		Fluorescense		Reagent color[b, c]		
	BAW	6%AA	long uv	short uv	p-Nit	Sulfanilic	$FeCl_3$-$K_3Fe(CN)_6$
Ferulic acid	.83	.46	bl	bl	br	org	bl
Suspected ferulic	.80	.44	bl	bl	br	org	bl
Vanillic acid	.88	.56	none	viol	viol	org	bl
Suspected vanillic	.84	.55	none	viol	viol	org	bl
p-Coumaric	.85	.52	none	abs	viol	org	bl
Suspected p-coumaric	.85	.48	none	abs	viol	org	bl
p-Hydroxy benzoic acid	.88	.66	none	abs	red	yel	dk bl
Suspected p-hyroxybenzoic	.89	.67	none	abs	red	yel	dk bl

[a] See text for solvent systems.
[b] Diazotized p-nitroaniline (Bray et al. 1950), diazotized sulfanilic acid (Bray et al. 1950), ferric chloride-potassium ferricyanide (Smith, 1960, p. 329).
[c] bl, blue; viol, violet; br, brown; dk, dark; abs, absorption.

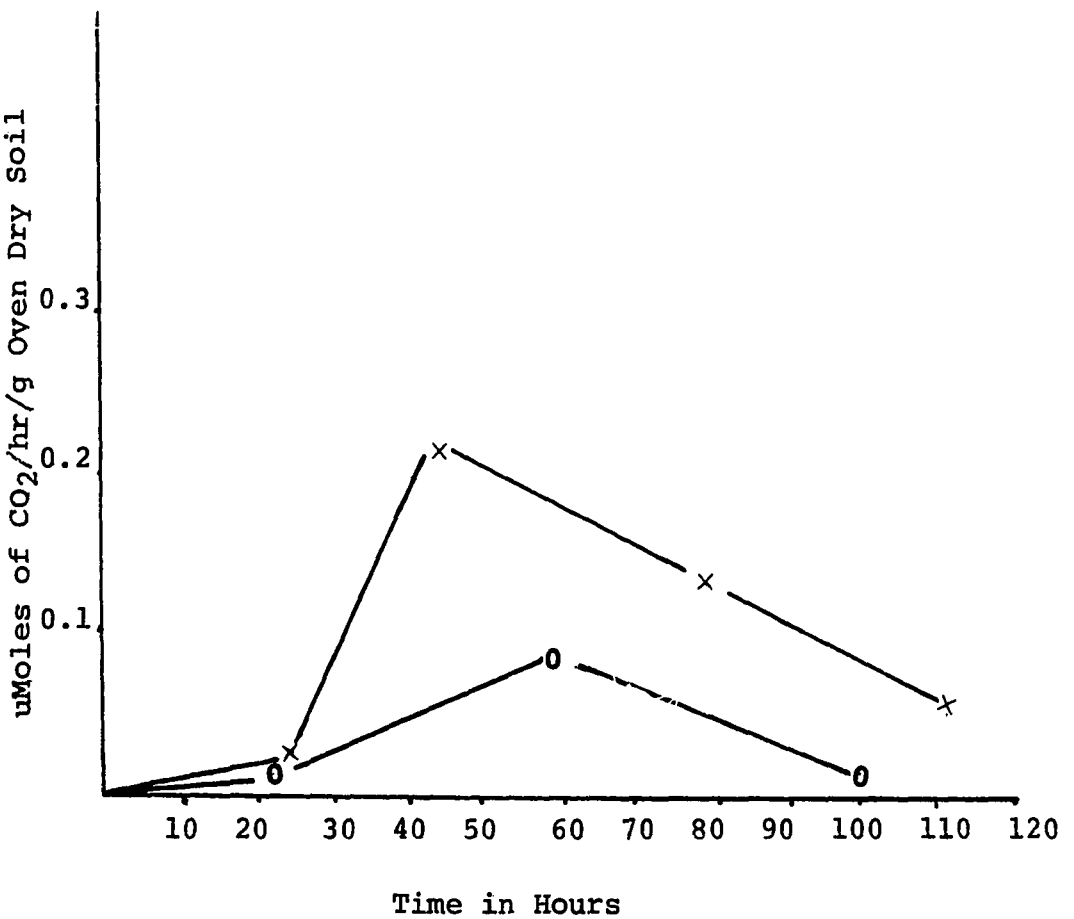


Figure 2. Effect of ferulic and vanillic acids on CO_2 evolution from the soil: x, effect of ferulic acid; 0, effect of vanillic acid.

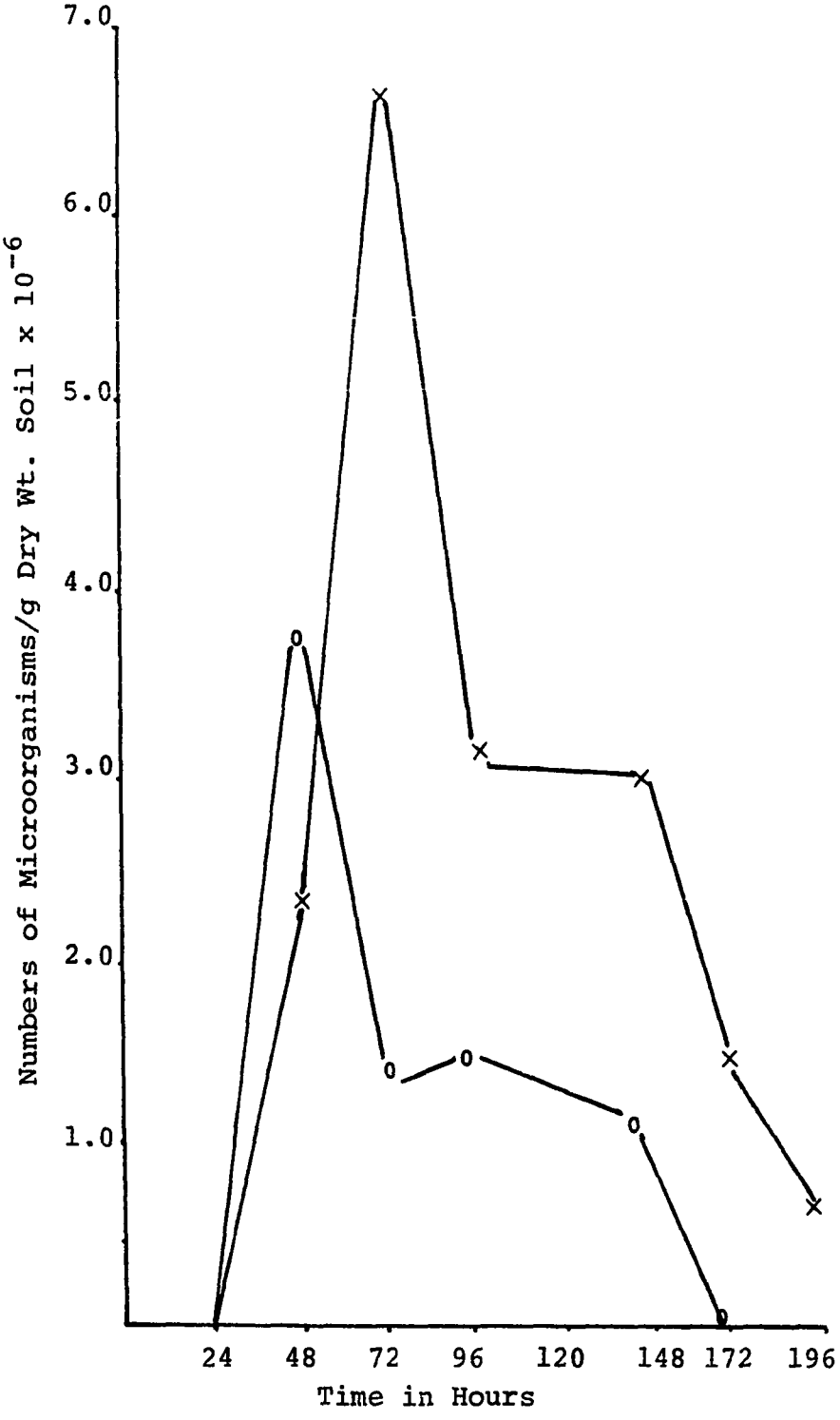


Figure 3. Change in microbial numbers in soil amended with 500 ppm of ferulic acid, x, 0, unamended soil.

organisms were discarded from later metabolic studies because of their slow growth on ferulic acid.

Identification of Organisms

The preliminary isolation was done with unamended soil from the climax tall grass prairie located near the hackberry tree which was used in the decomposition experiment. It yielded eight different colony types. Three of the colonies were hard and crusty and gave a gram negative reaction. One species of Penicillium (Barnett and Hunter 1972) and Cephalosporium curtipes (Gilman 1957) were also isolated. Three of the original isolates did not grow after the initial isolation. The Cephalosporium was used in later studies because of its rapid growth on ferulic acid. The second isolation technique employed soil enriched with 1000 ppm of ferulic acid. This procedure yielded nine morphologically different organisms of which eight were gram negative and one was gram positive. With physiological tests, several organisms were keyed to the genus Pseudomonas (Skerman 1967). The last isolations were obtained by percolating ferulic acid through a column of soil for 14 days. This procedure yielded only one major organism, which was identified as Rhodotorula rubra (Lodder and Kreger-van Rij 1952).

Further growth tests in a liquid medium with ferulic acid as the sole carbon source resulted in the elimination

of all of the original isolates except Cephalosporium curtipes and Rhodotorula rubra which were used in subsequent studies.

Metabolic Studies

The optimum pH for the utilization of ferulic acid by Rhodotorula rubra was 7.3, whereas the optimum for Cephalosporium curtipes was 5.8 (Table 3). In subsequent studies both organisms were grown at pH 5.0 because both organisms grew well at this pH with ferulic acid as the sole carbon source.

The efficiency of Rhodotorula in utilization of ferulic acid is indicated by almost total removal of the acid from the medium (Fig. 4). Rhodotorula also appears to grow almost as well on vanillic, cinnamic, and p-hydroxybenzoic acids (Fig. 5). Cephalosporium was not as efficient as Rhodotorula in removal of ferulic acid from the medium (Fig. 6A). When both organisms were grown for 24 hours on ferulic acid, Rhodotorula removed over 95% of the carbon source, whereas Cephalosporium removed only about 80%. Cephalosporium grew much better on ferulic acid than it did on vanillic, cinnamic, or p-hydroxybenzoic acids (Fig. 7).

One of four species of Cephalosporium obtained from the American Type Culture Collection was able to utilize ferulic acid as the sole carbon source, but all could utilize glucose (Table 4). Two of the three species of

Table 3. Effect of pH on utilization of ferulic acid during a 24 hour period by Rhodotorula and Cephalosporium on M-9FA with an original concentration of 500 PPM of Ferulic acid.

Rhodotorula rubra		Cephalosporium	
pH	ppm of ferulic acid utilized	pH	ppm ferulic acid utilized
7.3	436	6.2	226
6.0	300	5.8	441
5.3	300	5.2	413
3.0	24	3.5	100

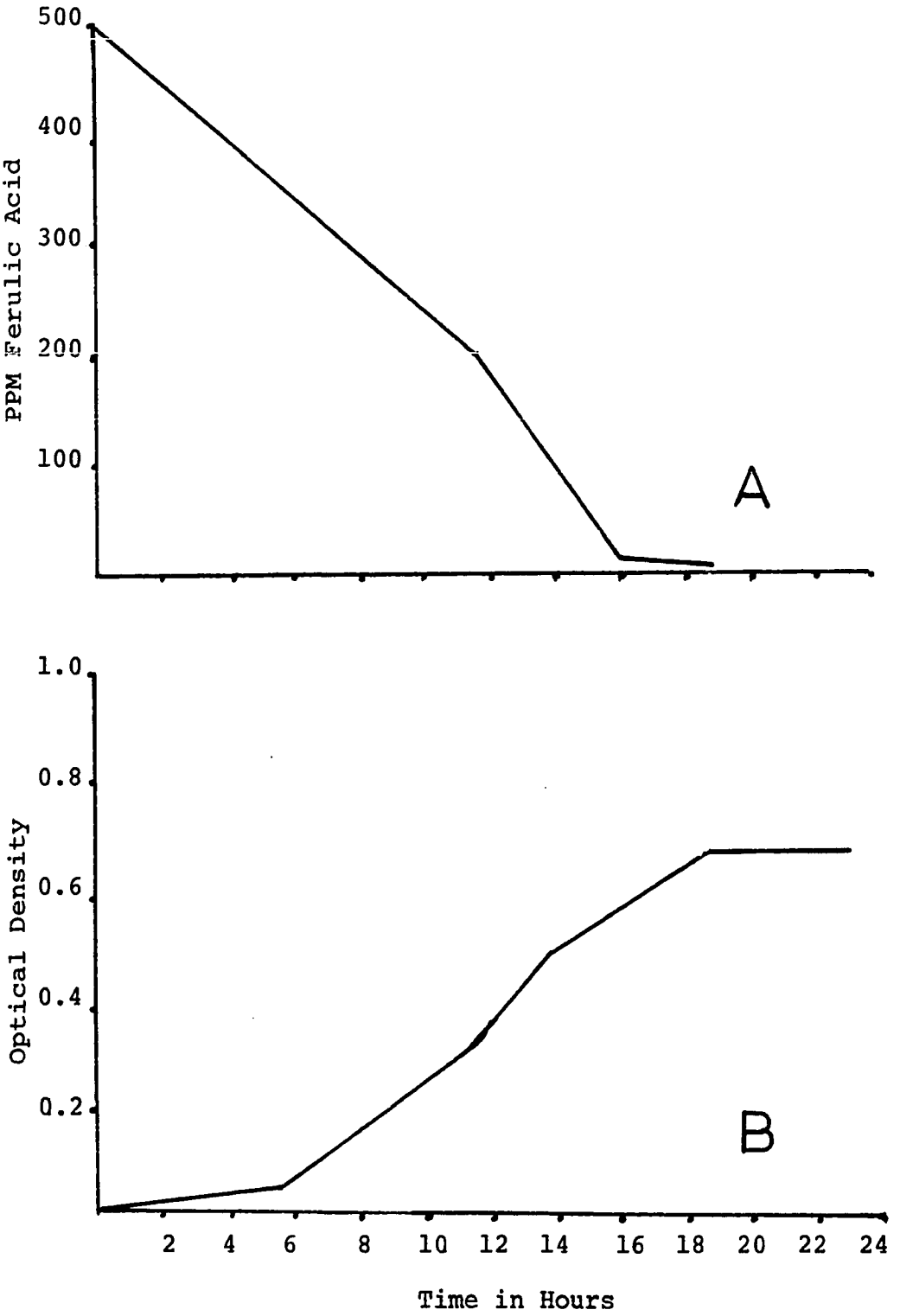


Figure 4. Growth of Rhodotorula rubra in M-9 with ferulic acid as sole source of carbon: A, change in concentration of ferulic acid during growth cycle; B, change in optical density during growth cycle.

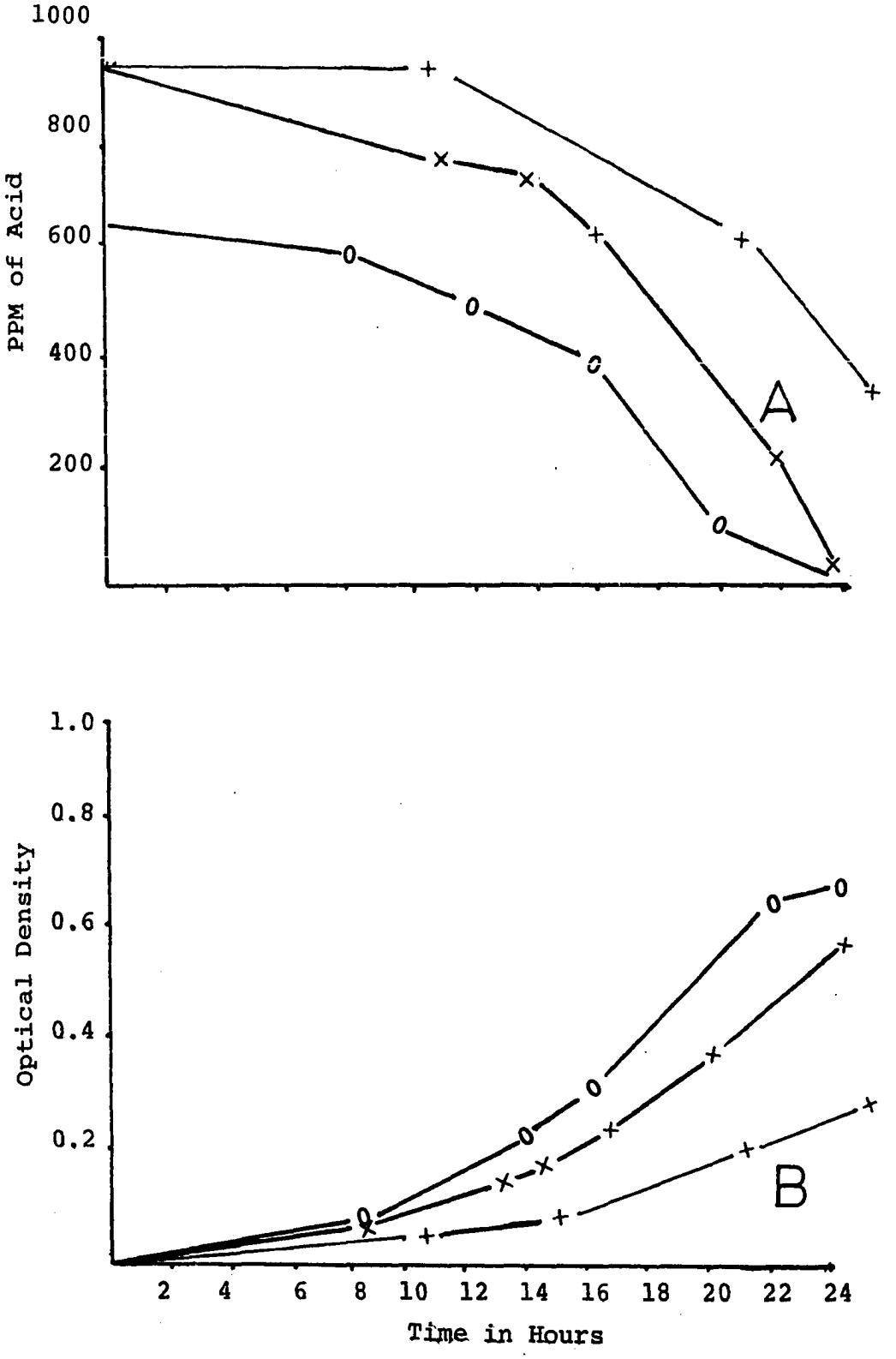


Figure 5. Growth of Rhodotorula rubra in M-9 with sole source of carbon: being vanillic acid, x, cinnamic acid, 0, or p-hydroxybenzoic acid, +, A, ppm of the carbon source plotted against time: B, change in optical density during growth on different carbon sources.

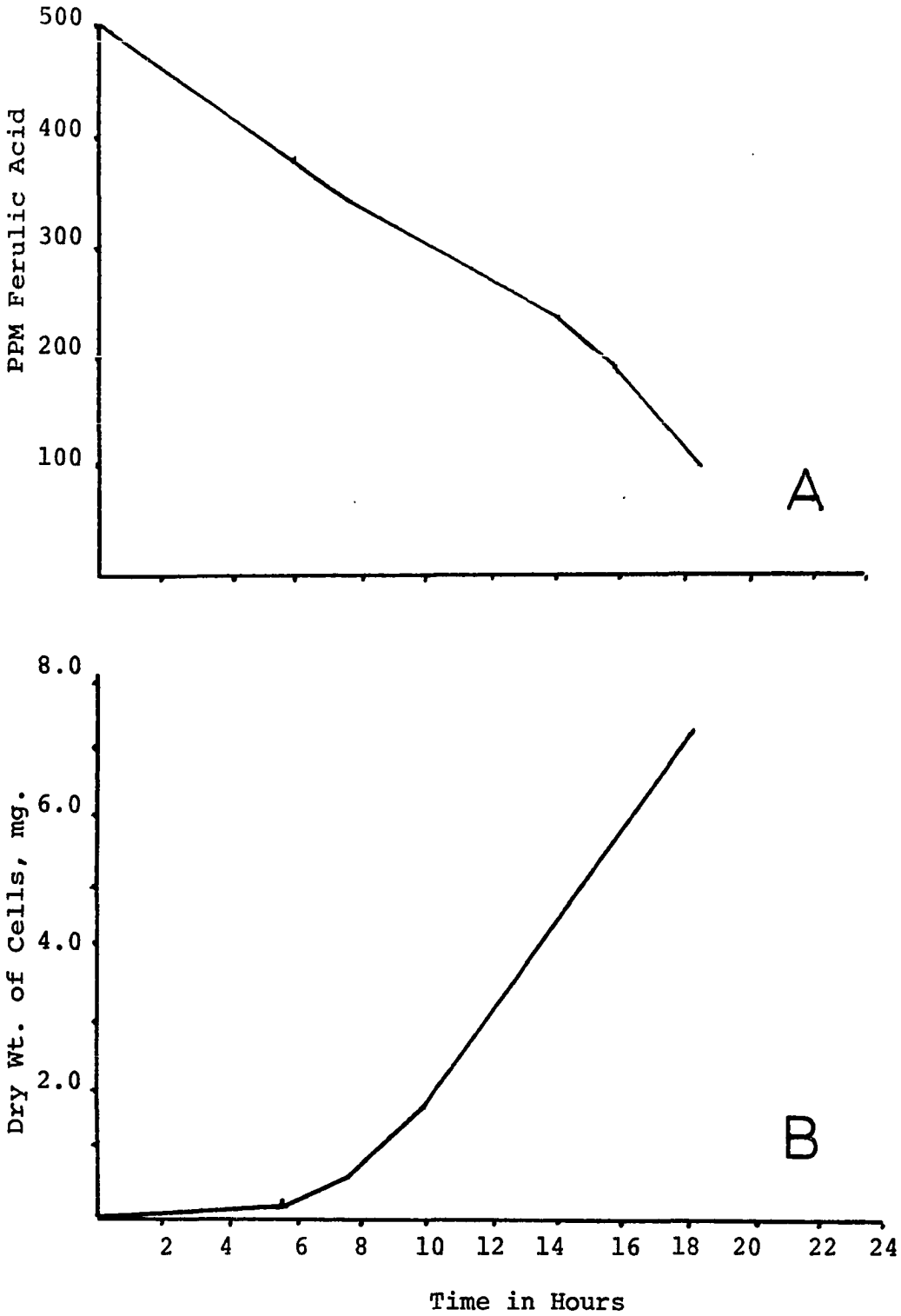


Figure 6. Growth of Cephalosporium curtipes on ferulic acid as sole source of carbon: A, change in concentration of ferulic acid; B, change in biomass of culture.

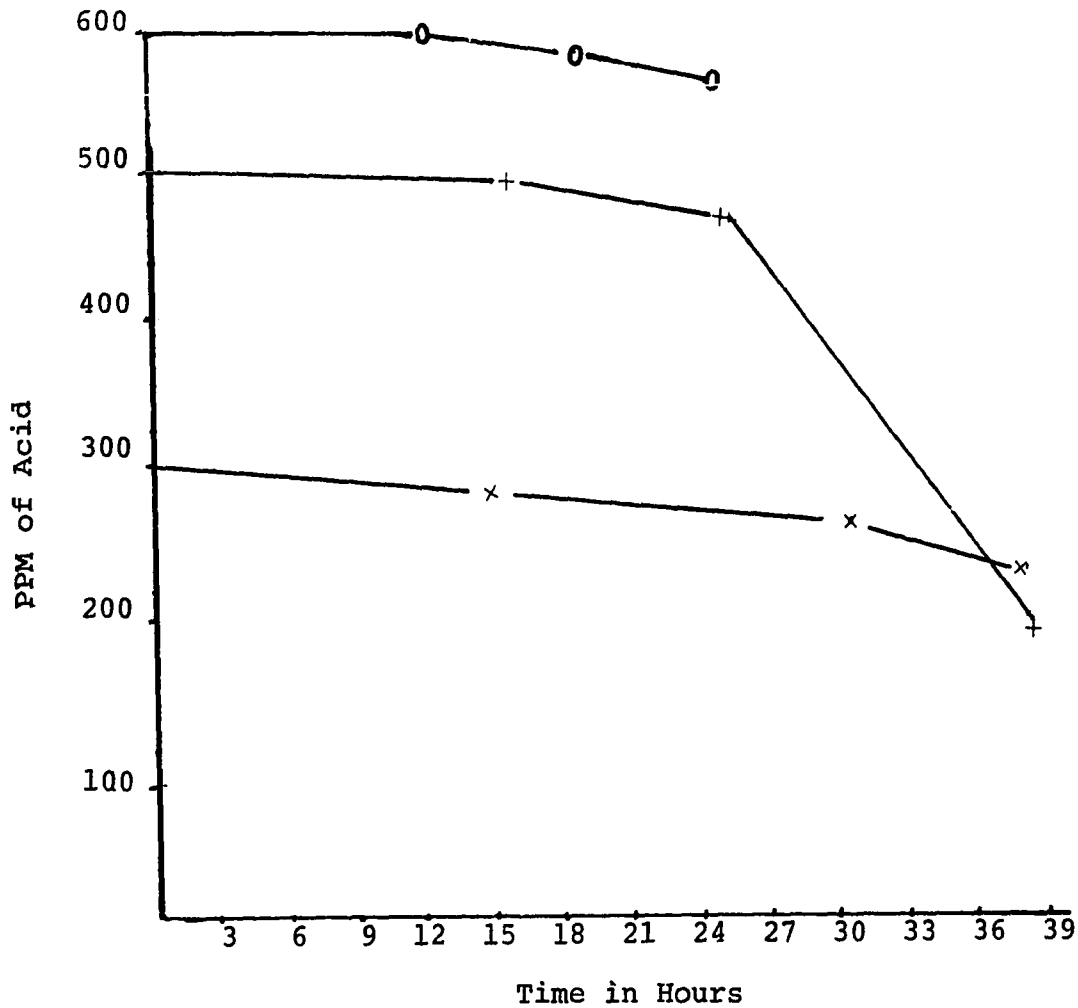


Figure 7. Change in concentration with time of vanillic acid, x; p-hydroxybenzoic acid, +; and cinnamic acid, 0; when used by Cephalosporium curtipes as sole source of carbon.

Rhodotorula from the same source were able to utilize ferulic acid, and two were able to use glucose (Table 4). No additional experiments were performed using these organisms.

Identification of Intermediates

It was assumed that any intermediate produced from the action of the organisms on the carbon source would accumulate in the medium. Rhodotorula metabolizes ferulic acid in a manner similar to other organisms that have been studied. When Rhodotorula grew on ferulic acid, vanillic acid appeared in the medium immediately with a peak concentration appearing after 18 hours (Fig. 8). No other phenolic acids were detected during this time period. When Rhodotorula was grown on vanillic, cinnamic, and p-hydroxybenzoic acid, no phenolic intermediates were detected.

There were no intermediates detected when Cephalosporium was grown on ferulic, vanillic, or p-hydroxybenzoic acid.

Table 4. Ability of several species of Cephalosporium and Rhodotorula to utilize ferulic acid or glucose as the sole carbon source.

Organism	ATCC No.	Glucose	Ferulic
Cephalosporium furcatum	16218	+	+
C. khandalense	16091	+	-
C. nordinii	16236	+	-
C. roseum	16227	+	-
Rhodotorula glutinis	10788	+	+
R. rubra (mucilaginosa)	16639	+	+
R. lactosa	18177	-	-

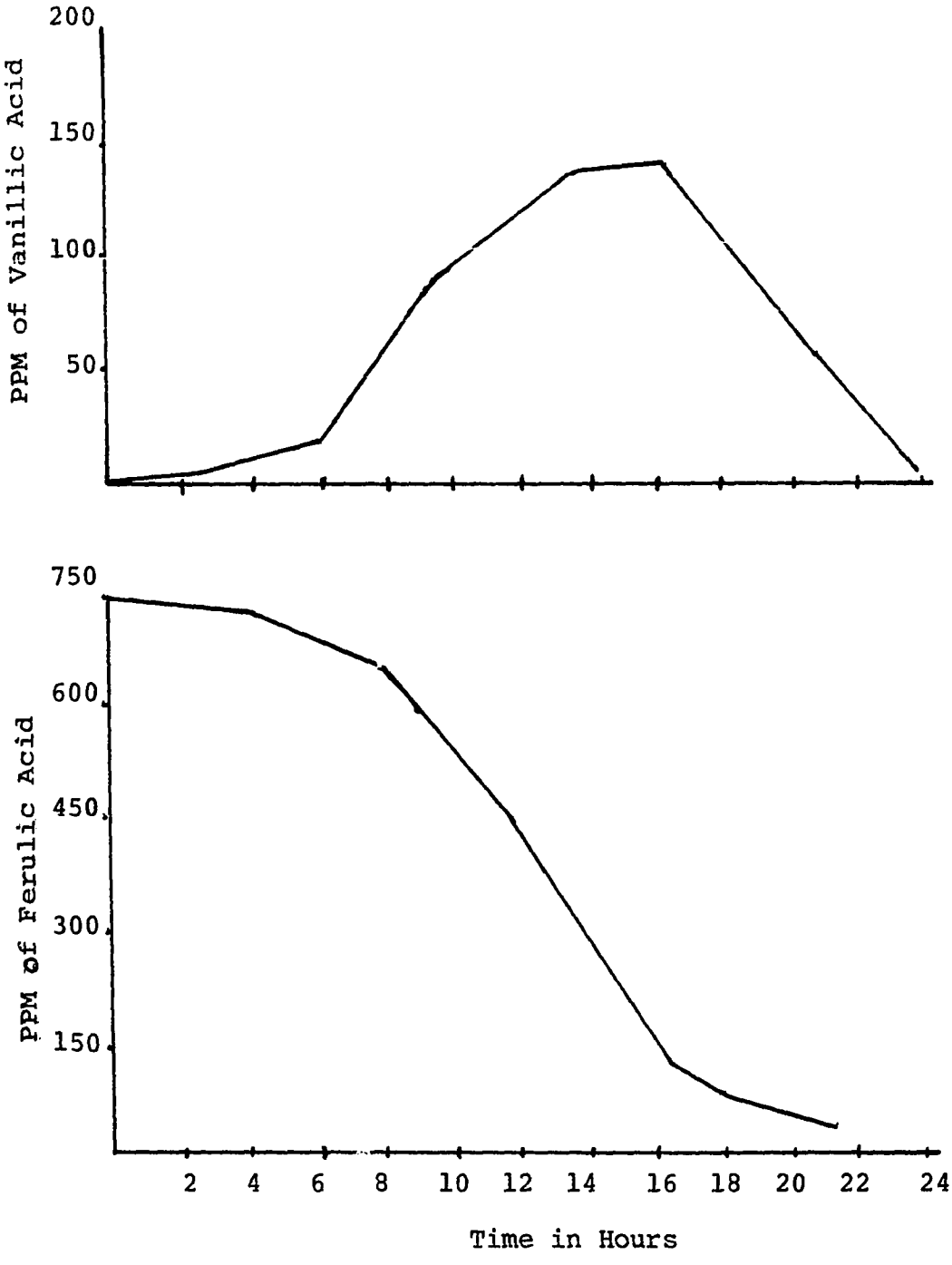


Figure 8. A shows utilization of ferulic acid by Rhodotorula rubra when grown in M-9; B, appearance of vanillic acid in medium during growth of Rhodotorula rubra on ferulic acid.

CHAPTER IV

DISCUSSION

At the outset of this research I suspected that ferulic acid in leaves would be lost at a rate corelated with that of the breakdown of lignin. The rate of lignin breakdown has been reported by several different authors (Phillips, Weihe, and Smith 1930, Martin and Wang 1944) to vary according to the type of plant material being decomposed, but in all cases, it has been shown to be very slow. On the other hand, the rate of disappearance of ferulic acid from the leaves as indicated by the Guenzi-McCalla technique, seems to be much faster than any reported rates of lignin breakdown. There is some evidence for the presence of other ferulic acid containing compounds in plants, and these have been shown to be rapidly decomposed by microorganisms (Sundman 1964a, 1964b, Tom and Wood 1970a). Among these are the lignans which have been reported to be widespread in the plant world (Robinson 1968), but I could find no data on the amounts present in plants. Thus their possible role in explaining the rapid loss of ferulic acid from hackberry leaves is only speculative. Ferulic acid occurs as the free

acid in some plants and also in numerous glycosides which are relatively easily decomposed (Harborne 1964).

It was assumed that ferulic acid would leach into the soil after being released from the bound form in the leaf and increase the concentration of this acid in the soil. However, the relatively constant amount of ferulic acid in the soil throughout most of the year indicated that this was not true. The March soil sample taken 100 days from the start of the experiment had an average concentration of ferulic acid that was significantly higher than that of soil samples taken at other times. This high concentration of ferulic acid did not corelate with the amount lost from the decaying leaves during the preceding month nor with increased rainfall, change in soil organic matter, or an increase in temperature during or before this sampling time. The lack of any significant corelation between the concentration of ferulic acid in the soil and its disappearance from organic matter on the surface suggests that loss of ferulic acid from surface material has little influence on soil concentration of this acid. The roots of hackberry were found to have concentrations of ferulic acid equal to those found in the leaves at the start of the decomposition experiment. This suggests that sources of ferulic acid in the soil may be exudation from living roots or leaching from dead or decomposing roots.

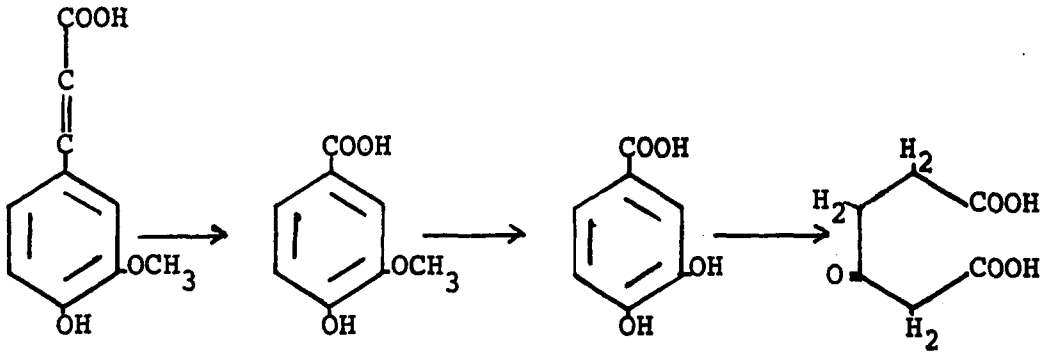
The concentration of ferulic acid in the soil under hackberry was slightly higher than the amounts reported by other workers. Guenzi and McCalla (1966b) and Wang et al. (1968) found less than 10 ppm in soil under various crop plants.

When ferulic acid was added to the soil, there was an increase in the evolution of CO_2 accompanied by an increase in total numbers of microorganisms in the soil. This increase in numbers was due almost entirely to two different organisms. One organism was identified as a pseudomonad, and the other appeared to be an actinomycete, but no positive identifications were made. The failure of the usual soil microorganisms to appear on sample plates indicated that they were inhibited by the ferulic acid.

Eighteen microorganisms were isolated which were able to grow in soil amended with ferulic acid. Many of these isolates were identified as belonging to the genus Pseudomonas; three were fungi, Cephalosporium curtipes, Penicillium sp., and Rhodotorula rubra; and the other organisms appeared to be actinomycetes. The isolation of bacteria, actinomycetes, and fungi which were apparently able to utilize ferulic acid as a carbon source agrees with reports of other workers (Henderson and Farmer 1955, Henderson 1956, diMenna 1959). Among the isolated organisms, Cephalosporium curtipes and Rhodotorula rubra grew best with ferulic acid as the sole carbon source. Several species

of Cephalosporium and Rhodotorula were subsequently selected from the American Type Culture Collection and tested for their ability to use ferulic acid as the sole carbon source. Only one species of Cephalosporium and two of Rhodotorula were found to be able to do this, and one of the species of Rhodotorula was a strain of R. rubra.

A proposed scheme for the metabolism of ferulic acid by certain microorganisms was developed by Henderson (1963).



Ferulic Acid Vanillic Acid Protocatechuic Acid β-Keto-adipic Acid

The strain of R. rubra used in this study appeared to employ part of this scheme because a definite conversion of ferulic acid to vanillic acid was observed. When Rhodotorula was grown on vanillic acid, however, no detectable amount of protocatecuic acid or any other phenolic compound was found. No attempt was made to look for nonphenolic compounds. C. curtipes appeared either to utilize ferulic acid directly or to use another pathway that does not involve phenolic compounds. Henderson and Farmer (1955) isolated many organisms

that metabolized ferulic acid and found many that did not utilize the scheme as outlined above. This indicates involvement of other pathways in ferulic acid metabolism.

In conclusion, it was observed that ferulic acid is rapidly lost from decomposing leaves. On the other hand, the amount of extractable ferulic acid in the soil remained relatively constant throughout the year except for the month of March. This indicates that loss of ferulic acid from decomposing surface material does not cause a significant increase in the ferulic acid content of the soil. The isolation of two soil microorganisms that actively metabolize ferulic and other phenolic acids provides a possible explanation for the lack of accumulation of these compounds in the soil. This decomposition of ferulic and other phenolic acids in the soil is of ecological importance because accumulation of such compounds to high concentrations could significantly reduce plant growth.

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