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The Effect of Field Corn (Zea mays) Residues on the Germination and Growth of Field Corn

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THE EFFECT OF FIELD CORN (ZEA MAYS) RESIDUES

ON THE GERMINATION AND GROWTH OF FIELD CORN

by

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VITA

The author, Alice J. Stubblefield, was born on January 1, 1944, in Pittsview, Alabama.

Her elementary and secondary education were obtained in the public schools of McDonough, Georgia, from which she graduated in 1962.

In May, 1966, she received the Bachelor of Science degree with a major in Biology from the University of Dubuque, Dubuque, Iowa.

From September, 1967, to June, 1977, she was employed by the Chicago Board of Education as a Biology teacher.

In September, 1978, she entered the Graduate School of Loyola University of Chicago as a candidate for the degree of Master of Science. At Loyola her work centered around plant physiology.

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INTRODUCTION

It is now an established fact that some green plants contain substances capable of inhibiting germination and growth, and also acting in some cases as phytocides (Evernari, 1949).

When the same crop is grown in the same soil for long periods of time, subsequent plantings often grow poorly in comparison with similar plantings in virgin soil or soil never cropped to the species concerned. Thus, it was frequently observed in early experimental work that as a piece of soil was continuously cropped to field corn, the yields decreased; further, these decreases could not be made up by adding fertilizer.

The occurrence of substances of biologic origin in soils detrimental to plant growth has been demonstrated by many investigators (Bonner, 1950; Borner, 1960; Martin, 1957; Patrick and Koch, 1958). These substances appear to originate from many sources, including the decomposition products of plant residues. Plant residues from various sources constitute an important component of the soil. These materials, in the form of living, dying, and dead plant tissues, each with immense chemical diversity, are ultimately decomposed through the action of biotic and abiotic agencies. During the decomposition many complex interactions,

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transformations, and syntheses also occur. Thus, at any one time the soil and the environment of plant roots could contain a vast variety of chemical compounds, many of which no doubt have important effects on all·phases of plant development (Patrick, 1970). One of the important questions associated with plant residues is whether substances possessing phyto- toxic properties are formed during their decomposition or otherwise. Because of its possible impact on soil produc- tivity, this question has been investigated by agricultural scientists and others for over two hundred years. A consider- able amount of literature and much controversy have accumulated regarding the beneficial and detrimental effects of plant residues and their decomposition products.

Studies on the allelopathic properties of plant exudates and leachates have shown the occurrence of inhibitory compounds in a variety of plant extracts. Inhibitors are sometimes brought about by environmental conditions, but more often by conditions within the plant itself. The causes of these inhibitions are manifold. One of them lies in the production of substances inhibiting particular physiological processes which are indispensable for normal development (Evenari, 1949). The toxic compounds of many plants have been shown to inhibit growth in themselves under certain limited environmental conditions. Whether or not the same compounds are active in the soil under field conditions in inhibiting and limiting growth of plants is a separate

question which remains to be investigated. The majority
of the investigations directly concerned with the roles of
allelopathy in agriculture have involved effects of decom-
posing crop residues. There are many questions that must
yet be answered, especially those relating to conditions in
the field. How many inhibitors are produced, and in what
sequence are they produced? One of the major questions of
chemical ecology deals with what becomes of phytotoxins
after their release into the environment.

In the soil we are dealing with dynamic systems, where
all effects are transitory, where production, transformation,
and destruction of these substances go hand in hand (Patrick,
1970). During this active phase, phytotoxins could and
often do induce important effects on living plants that come
under their influence. Detection of and identification of
phytotoxins, and indeed other biologically active compounds
in soil, have for these reasons always been difficult.

It is highly probable that numerous phenolic compounds
are returned to the soil in plant residues, are present in
dead microbial cells, or are synthesized or released in the
soil through microbial metabolism. As stated before, they
are in a dynamic state as they are continually being decom-
posed and re-synthesized (Haider and Martin, 1975). Since
many factors come into play in soil, the chemistry of soil
phenolic acids is highly complex.

Within the last few years many investigators have succeeded in the identification of substances liberated from higher plants by means of chromatography (Borner, 1958). Some of the compounds present in corn plant materials and the related soils are suspected of being toxic to seed germination and growth of corn. Therefore an extensive study of these probable phytotoxins has been made. On the assumption that a toxic substance is partly responsible for reduced germination and growth of corn in soil previously cropped to corn for long periods, experiments were conducted to determine whether aqueous extracts of corn residues are inhibitory to the growth and/or germination of corn, and whether such an inhibitory effect, if present, is operative in nature. From this point of view, this researcher tried to determine the effect of phytotoxic substances of plant residues before, during, and after decomposition, as well as to evaluate the possible ecological significance of such substances as host-conditioning factors in the etiology of plant disease.

REVIEW OF RELATED LITERATURE

The presence of phytotoxic substances and the influence
of these naturally occurring soil organic substances on plant
growth have received attention dating back to the work of
DeCandolle in 1832. The best known and perhaps most widely
discussed work on toxic substances in soil is that of
Schreiner and co-workers in the early years of the twentieth
century (Schreiner and Shorey, 1907; Schreiner and Reed,
1908; Schreiner and Lathrop, 1911). Schreiner et al. found
that in certain soils where fertility had been reduced, for
example, by cropping to one plant for many years, water ex-
tracts which were toxic to the growth of wheat could be made.
After extracting the soil with 2% NaOH for 24 hours, they
isolated and identified picaline carboxylic acid, dihydroxy-
stearic acid, agroceric acid, and agrostearol. The
infertility of certain soils appeared to be correlated with
the presence of one or more of these compounds.

Proebsting and Gilmore (1940) investigated the diffi-
culty of re-establishing peach trees in old peach orchards.
They found that the addition of peach roots to healthy soil
inhibits the growth of the peach seedlings and they presented
evidence to show that a toxic factor was found in peach root
bark residue. They obtained stunting by the addition of the
water extract of peach root bark to sand cultures of growing

peach seedlings. Similar stunting was obtained when they
added Amygdalin and emulsin solutions to the growing peach
seedlings and no stunting when they used Amygdalin or emulsin
alone. Patrick (1955) was able to demonstrate that roots,
remaining in soil, release Amygdalin which is broken down by
soil microorganisms into glucose, hydrocyanic acid and
benzaldehyde. Amygdalin, a natural constituent of the peach
root bark, was detected in amounts of 50mg per gram root bark.
While Amygdalin is non-toxic to peach seedlings, the breakdown
product, benzaldehyde, produced inhibition of respiration
and browning of the root tips.

A second instance of toxicity owing to plant inhibi-
tors produced by roots was clearly demonstrated in the ex-
periments of H.M. Benedict (1941). Benedict worked with a
brome grass (Bromus inermus). After growing several years,
this grass attains a condition known as "sod-binding" in
which the stand begins to thin out and the plants die back.
Benedict suspected that this dying out of old brome grass
stands might be due to the accumulation of toxic substances
produced by bromegrass roots. He showed that dried roots
of bromegrass, even in small amounts, were inhibitory to the
growth of bromegrass seedlings. This was demonstrated in
nutrient solution leached through an old culture of brome-
grass and supplying this leached nutrient to seedlings.
It was also demonstrated by the incorporation of dried
bromegrass roots in sand in which further bromegrass

seedlings were grown. Benedict did not, however, show that any toxic substances from bromegrass roots actually accumulate in sod in old bromegrass stands to the extent necessary for inhibition. In short, while he established the presence of a toxic substance in bromegrass roots, he did not establish that root inhibition is active under field conditions.

In numerous experiments Bonner (1946) found that older guayale plants inhibited the growth of seedlings of the same species. The first of these experiments involved leaching of nutrient through sand cultures of one year old guayale plants. This leached nutrient was then supplied to younger plants also grown in sand culture. It was found that whereas guayule plants fed with fresh unleached nutrient grew rapidly, plants supplied with nutrient which had been leached through the sand containing an old guayule plant were greatly inhibited in growth. This effect was not due to the removal of nutrient by the older guayule plant. From these results they concluded that the toxin was liberated from older plants. The isolated toxin was identified as trans-cinnamic acid. Trans-cinnamic acid was isolated in crystalline form from the toxic liquor and was shown to cause 50% inhibition of growth at a concentration of 30 mg/l.

The assumption that root residues have an effect on apple replants is based on the results of Fastabend (1955). He revealed that apple roots and water leached through sick soil produced the soil sickness if added to healthy soil.

In experiments concerning the chemical nature of the inhibiting compounds it was possible to detect phlorizin, a natural constituent of apple root bark, in water leached through soils containing apple roots and in water containing apple root bark. Closely related to the apple and peach toxicity problem is the "slow decline of citrus" in California. In this case also there is a decrease in growth if citrus is replanted on soils which have previously produced citrus trees (Martin and Ervin, 1958).

Collison reported as early as 1925 on the detection of inhibitory substances in wheat straw. Collison demonstrated that extracts of wheat straw were toxic to young seedlings in water culture causing stunting and root discoloration. He concluded that specific chemicals present in the straw were responsible for plant injury; composting of the straw resulted in their disappearance. Many years later these investigations were resumed by Winter and Schonbeck (1956). They found that straw as well as roots of cereals contain cold water-soluble substances which inhibit the development of various plants. The inhibiting effect of wheat roots which grew in straw or root extracts of barley, rye, wheat and oats was still visible at a concentration of straw to water of 1 to 400. Thus it was demonstrated that phytotoxic substances were given off from cereal remains and were present in the soil under natural conditions.

McCalla and Duley (1948) reported that stubble mulch farming reduces the stand and growth of corn under some conditions, and they found that soaking corn seeds in an aqueous extract of sweet clover for 24 hours reduced the subsequent percentage germination and growth of tops and roots on agar in petri dishes. Alfalfa extracts had less inhibitive effects, and wheat straw either stimulated growth or caused no changes compared with water controls. They found that adverse effects of stubble mulching on corn were particularly striking during periods of wet, cool weather. Norstadt and McCalla (1963) followed up the earlier investigation of McCalla and Duley (1949) in which it was suggested that effects of crop residues might be due to a combination of toxins from the residues and from microorganisms that were caused to grow more profusely by substances in the residues.

Guenzi and McCalla (1962) collected crop residues and air dried and ground them to pass a 40-mesh screen. The materials, consisting of wheat and oat straw, soybean and sweet clover hay, corn and sorghum stalk, and bromegrass and sweet clover stems, were extracted with hot and cole water using 1 part residue to 15 parts water. One-half of each water extract was autoclaved for 1 hour at 20-lb. pressure. All residues were found to contain water-soluble substances that depressed plant growth of corn, wheat, and sorghum. The non-autoclaved extracts of the residues inhibited seed germination and shoot growth more than the autoclaved

extracts in most cases, but the autoclaved extracts were more depressive to root growth.

McCalla and associates (1964), in extensive studies of stubble-mulch farming, reported that while the practice of leaving crop residues on the soil surface was effective in combating erosion, plant growth was often depressed during wet cool springs. They also obtained plant injury with extracts. Such results led many investigators to conclude that formation of phytotoxic decomposition products is mainly associated with decay of readily decomposable organic matter under adverse aeration conditions. They believed that the occurrence of such substances under more normal soil conditions would be relatively rare.

Guenzi et al. (1967) investigated changes in the pathogenic activity of water extracts of residues of corn, wheat, oat and sorghum residues during decomposition in the field during a 41-week period. Wheat was used as the test plant in the assays. Toxicity of extracts of corn residues remained high during the first weeks of decomposition, but decreased rapidly thereafter. Patrick and Koch (1958) did a comprehensive investigation of the effects decomposing residues of timothy, corn, rye, and tobacco have on the respiration of tobacco seedlings. They found that during the decomposition of residues of all four species, substances were formed which were inhibitory to respiration in tobacco seedlings and that greater inhibition occurred if decomposi-

tion took place under saturated soil conditions. Patrick (1971) reported that toxins identified by gas chromatography from decomposing rye residue were acetic, butyric, benzoic, phenylacetic, hydrocinnamic, 4-phenylbutyric, and ferulic acids.

There appears to be little doubt that substances toxic to living plants can originate from excretions of roots and underground stems of certain living plants, or may be liberated from plant residues during their decomposition. Previous investigators have tested the toxicity of corn extracts on other plants such as wheat, tobacco or rye, but there has been little work on the effects of the extracts of corn residues on corn itself at various stages of growth. Also, if toxic substances are in fact given off from corn, it is not clear whether they originate from excretions of leaves, roots and stems of the living plants or are liberated from residues during their decomposition. Various types of phenolic acids have been determined in hydrolyzed extracts of mature corn. This study will attempt to determine, through chromatograms, the type(s) of phenolic acid(s) present during the intermediate stage of growth.

METHODS

Corn seeds of the variety Iochief obtained from a local
supply house were sown in flats in rich garden soil and
germinated in the greenhouse at 70-80 degrees Fahrenheit.
Plants were supplied three times weekly with California
Nutrient solution which was found to be near optimal for the
growth of corn. Plant material for use was collected at
three different stages of growth: young, intermediate, and
nearly mature, in order to determine the effect of maturity
on the degree of toxicity of products. The young stage
was collected 5-6 weeks after planting and was about 12-18
inches tall; the intermediate stage, 6-8 weeks after planting,
20-36 inches tall; and the nearly mature stage, 10-14 weeks
after planting, 35-46 inches tall. At the end of each growth
stage entire plants, including roots, were harvested. The
soil used in the various tests was obtained from the same
flats which grew each of the above crops. Three methods used
for extracting toxic material from the corn plants were
leaching, maceration and decomposition.

LEACHING

To test whether toxic material could be leached from
the leaves, samples of leaf washings were taken after each
growth period. Leaves were sprayed with distilled water and

resulting leachate collected in large sterile containers.
An effort was made to keep the mass of leaves constant
between batches, and the same amount of water used each time.
To 10kg of leafy stalks, 2500ml (equivalent to 2.5mm rain-
fall) of distilled water was added to simulate natural
rainfall or fog drip for 10 minutes. The leachate was
collected and the pH determined. It was filtered twice
(under suction) with Whatman No. 1 and No. 42 filter paper
successively. The leachate was used immediately for bioassay
analysis.

MACERATION

To test whether the living tissues of plants contained
substances having inhibitory effects on germination and
growth of seeds and seedlings, some of the plant material
was macerated in a Waring blender immediately after leaching.
In each test, 125gms of plant material plus 500ml distilled
water were used. The pH was determined, after which it was
filtered and centrifuged. The relative toxicity of the
extracts was determined immediately.

DECOMPOSITION

Experiments were carried out to determine whether the
decomposition of plant residues, in essentially identical
soil but under different moisture conditions, would give
rise to substances which exert different effects on the
germination and growth of corn seedlings. At the end of

each growth stage, entire corn plants were harvested, cleansed of soil and cut into pieces approximately 1 inch long. Duplicate samples of plant material were allowed to decompose in soils held at two moisture levels, saturation and field capacity. The soil used was obtained from the same flats which grew each of the stages of plants. To 500gms of soil, 125gms fresh weight plant material (roots, stems, and leaves) were mixed thoroughly and placed in 1000ml sterile beakers. Controls consisted of the same amount of fallow soil (no plant material). Distilled water was then added to the beakers. Two series of beakers were prepared simultaneously, saturated in a ratio of about 1:20 (solids completely submerged) and field capacity (amount of moisture which is retained in the soil after drainage of excess water-- about 50% of saturated). The beakers were covered tightly with aluminum foil and incubated at 60-70 degrees Fahrenheit for periods varying from 0 to 22 days. For each growth stage enough beakers were prepared to allow extraction from the contents of four beakers after decomposition periods 0, 7, 14, and 22 days. In another series of tests, the soil and plant material for each growth stage was autoclaved for 40 minutes before being allowed to incubate for 14 days. After each incubation period, four beakers of each crop (two field capacity and two saturated) were removed, 500ml distilled water added, stirred and allowed to settle. The liquid was then decanted and filtered three times. The

liquid was filtered first through several layers of sterile cheesecloth to remove large soil particles and undecomposed plant debris. Later, the aqueous extracts were filtered successively through Whatman No. 1 and No. 42 filter paper and the pH recorded. After preparation, the extracts were tested for phytotoxic properties. Bioassays were carried out in which the effects of extracts on germination and growth of corn seeds and seedlings were determined.

TESTS FOR TOXICITY

Germination

Filter paper was placed in the bottom of sterile petri dishes and moistened with 15ml of each filtrate from each extraction period. 0-day extractions consisted of leached extracts, macerated extracts, saturated and field capacity extracts. The remaining extraction periods--7, 14, and 22 day--consisted mainly of autoclaved and non-autoclaved series and the regular saturated and field capacity series. Ten corn seeds were sprinkled on filter paper impregnated with filtrates and incubated at room temperature. In the controls, pH7 phosphate buffer solution was used instead of the various filtrates. Each test was replicated four times. Results were obtained after 6 days of incubation. The test for toxicity was based on the percentage of inhibition of germination and radicle growth of seeds placed in toxin preparations as compared to the germination and growth of identical seeds in buffer solution.

Radicle observation and growth

Seeds were germinated on moist filter paper in sterile petri dishes. After 6 days at room temperature, when the radicles were approximately 5-7cm long, seedlings were selected at random and transferred to sterile dishes which contained 20ml of extract from the various test material. Four replicates, each with 20ml extract and four 6-day germinated seedlings were used. Again, controls used buffer solution plus seedlings. After 12 hours direct exposure of seedlings to aqueous extracts, visual effects, if any, were recorded.

In other tests carried out in flats in the greenhouse, sterile garden soil was used. 25ml of residue extract for each 100gms of soil were added to some flats, whereas buffer solution plus garden soil (same amounts) were added to others. Sturdy radicles were planted in both groups of flats. The seedlings were watered three times weekly with a balanced nutrient solution. After a nine day growth period, plants were removed from trays and their roots gently washed free of soil. With this method it was possible to obtain seedlings with clean roots virtually free of injury. Growth of seedlings was measured to the nearest centimeter and expressed as percentages of controls.

Older plant growth

Experiments were carried out in the greenhouse with older plants to determine whether certain of the toxic

effects could be observed under greenhouse conditions. In
this experiment, 2500gms sterile garden soil were placed
in deep flats. There were three replicates of the plant
extracts used. Flats of the same soil to which no plant
extracts were added served as the controls. Six corn plants
previously germinated in the greenhouse, each approximately
4 inches in height, were transplanted into each flat. The
plants were observed for 15 days for signs of severe stunting
or death. During this time period, all plants received the
same watering schedule (80ml/flat). After 15 days, results
were determined. Of the surviving plants, heights were taken
from the ground level to the tip. The toxicity index was
based on the percentage of dry weight and plant height
inhibition as compared to the controls.

ISOLATION AND IDENTIFICATION OF TOXIC SUBSTANCE

The techniques of Guenzi and McCalla (1965) were used
to isolate the toxic substance from corn plant. Plant
residues taken from the intermediate stage of growth were
dried at 70-80 degrees Fahrenheit for 24 hours and ground
in a Thomas Wiley mill to pass a 10"-mesh screen. Acid
and Alkaline hydrolysis were used to free the toxic substance
from the bound form, if any existed. Ten grams of air
dried, ground plant residue were used for each acid and
alkaline hydrolysis. First, plant residues were extracted
with 80% ethanol for 4 hours in a soxhlet extractor and

then evaporated to near dryness at 50 degrees Centigrade in vacuo. Three hydrolyzing treatments were carried out on the residues previously extracted with 80% ethanol and dried. Ten gram samples of residue were hydrolyzed with:

(1) 2n NaOH at room temperature for four hours. The Alkaline extract was acidified to pH 2 with HCl and extracted with diethyl ether. The ether solution was shaken with 5% $NaHCO_3$ and the ether portion discarded. The alkaline portion was acidified to pH 2 and re-extracted with ether. The ethereal solutions were dried over anhydrous $CaSO_4$ for 24 hours, concentrated and transferred to 2ml vials. (2) 2n NaOH and autoclaved for 45 minutes. After autoclaving, the same procedures as for the sample one were followed.

(3) 2n HCl for 1 hour under reflux. Extract with diethyl ether and concentrate ether extracts for chromatographic analysis.

Chromatography techniques as described by Smith (1960) were used. To separate the toxic substance, two directional ascending chromatograms on Whatman No. 1 paper were used. Two solvents were used: first, benzene-acetic acid-water (165:34:1, v/v/v/) and second, Sodium formate-water-formic acid (10:200:1, w/v/v/). Separate chromatograms were sprayed with diazotized sulfanilic acid and diazotized p-nitroaniline to obtain characteristic color reactions with acids. Identifications were made by comparing residue samples with color reactions of authentic samples of phenolic acids,

ferulic acid, p-coumaric, syringic, vanillic, and p-hydroxy-
benzoic acids. No attempts were made to determine the
toxicity of the various samples.

RESULTS

The experiments indicate that <u>substances unfavorable</u> <u>to the growth of corn plants emanate from plant extracts</u> <u>of corn.</u> The toxicity of extracts from corn plants of different ages was variable, but did show some consistent effects due to the age of the plants. Corn bioassays revealed considerable differences in the activity of extracts. Some had stimulatory properties, others had no effect, and others delayed or inhibited the germination of corn seeds and reduced the growth of seedlings.

The data show that in many cases neither young, intermediate, nor nearly mature corn plants yielded very significant toxic effects on germination and growth. Although results presented show conclusively that there is a powerful inhibitor of growth of corn seeds and seedlings contained in corn residue, the inhibition was not pronounced in certain stages of growth. The results reveal that extracts obtained from the soil and residues in the relative proportions found in the fields showed more effect on seeds and seedlings than the saturated conditions. A delay in germination and reduction of root growth and root injury were obtained with several extracts of the decomposed residue. In some cases similar phytotoxicity was also exhibited with leaf leachate and macerated extracts. No

phytotoxicity was obtained with extracts of soil from which
no leachate, macerate, or decomposing residues had been
added. The amount of toxicity also varied with the decom-
position period. Substances which had significant toxic
properties were frequently observed when the plant material
had been decomposing in the soil for approximately 7-15
days. Toxic extracts showed inhibition up to 70% in some
cases. Following this period, it was shown that the quantity
of extractable toxins greatly decreased with time and
stimulatory effects were sometimes observed. Highest ac-
tivity was exhibited by extracts obtained during the 1-2
weeks of decomposition. Corn residues allowed to decompose
at field capacity became more toxic during the early stages
of decomposition than the saturated condition. Saturated
extracts lost their toxicity more rapidly than extracts
from the field capacity series. Autoclaving appeared to
increase the production of toxic substances in many cases.
Color differences were also noted between extracts obtained
from the autoclaved and non-autoclaved series. After the
14-day decomposition period , extracts of samples from the
autoclaved series were light brown in color, while those
from non-autoclaved samples were usually dark green to
black. It was also noted that the toxicity of the corn
extracts was independent of their pH values. There was
no degree of consistency between the relative toxicity of
the extracts and their pH values. As they matured

the extracts became slightly more alkaline, but not significant. Therefore the pH did not have an appreciable effect on the germination and growth of corn.

Each experimental treatment and control was set up at least in triplicate. The bioassay results were analyzed by means of the Students -T- test standard deviation. A difference in inhibition of 50% or more between two means may be taken as significant at the 1% level, while difference of 30% between two groups of ten may be taken as significant at the 5% level.

With the variation seen between growth stages, the results from each stage will be presented individually.

STAGE I--YOUNG PLANT

Leachate and macerated extracts

Extracts of both leaf leachate and macerated plants showed greatest toxicity during this stage of growth. There were no major inhibitory effects on germination and radicle length in the aqueous extracts. Radicle growth was slightly more inhibited than germination (figures 5A, 5B). When in direct contact with the soil in which seedlings grew, the toxicity level increased (figures 5C, 5D). The young stage was significantly toxic to soil seedlings and older plants. Both leaf leachate and macerate showed significances of $p < .0005$ for radicle growth in soil. Germinated seedlings, after being placed in soil and given added extracts, enhanced inhibition. Seedlings in leachate

did not grow. Fungus developed and surrounded the root
system. Even though the macerate extract showed plants
with green healthy shoots, root growth was slightly
stunted. After 15 days of growth, older plants which
received leached and macerated extracts were significantly
smaller than the controls. Leaf leachate showed significance
of .001 $< p <$ .0025 for dry weight and macerate showed
significance of $p <$ .0005. Their height and dry weight
accumulation in comparison with controls were greatly
retarded, and there was even death of some plants (figures
5E, 5F).

Decomposed residue extracts

Saturated extracts showed slight stimulatory effects
on germination 0-day, at a time when very little decomposi-
tion could have taken place. Inhibition was not significant
with increased phases of decomposition (figures 7A, 7B).
After being exposed to the aqueous extracts in dishes,
radicles of germinated seeds continued to grow normally,
but with slight darkening on root tips. Small enlargements
on root tips were seen with the 22-day extract. Inhibition
was greatest in the later phases of decomposition, 2-3
weeks. As germination inhibition increased, radicle growth
showed a decrease in inhibition with stimulatory activity
in later phases of decomposition. Inhibition was very
significant in weight reduction of older plants. Field
capacity and saturated series showed weight reduction sig-

nificance of p$\angle$.0005. Again, the greatest toxicity was
seen in the later stages of decomposition, 7-14 days, with
decreasing inhibition as decomposition progressed (figures
1A, 1B, 1C, 7C, 7D, 7E). Soil seedlings were greatly in-
hibited during 0-day phase from the field capacity series,
with inhibition slightly less in saturated series (figure
7C). In most cases, where seedlings were in contact with
soil, the saturated series showed greater levels of toxicity
than the field series. The greatest experimental inhibition
in this entire study was noted during the 7-14 day decompo-
sition of the saturated series in this young stage of growth
(figure 7C). 0-day decomposition for both the field capa-
city and saturated series showed a significance of p$\angle$.0005
for soil radicle length inhibition. 14-day, field capacity
and saturated series showed a significance of
.005$\angle$p$\angle$.01 for soil radicle length inhibition, whereas
the saturated 22-day decomposition series showed a signi-
ficance of .01 $\angle$p$\angle$ .025. Autoclaved extracts from the
young stage of growth did not show any pronounced inhibi-
tion except for the soil seedlings grown for nine days in
this extract (figures 6A, 6B, 6C, 6D). Great inhibition
was exhibited with the saturated autoclave extracts, showing
a significance of p$\angle$.005. Field capacity autoclave
extracts showed slightly less, but significant toxicity
levels of .0005$\angle$p$\angle$.001.

 Since inhibition was noted during the early phase,
0-day, of decomposition for the young stage of growth,

observations were made on germination and growth with
extracts from original soil to see whether this could
partially be attributed to toxic products already in the
soil. There were no significant effects of this extract
on germination, but again, when placed in contact with soil,
seedling inhibition was enhanced (figure 4A).

STAGE II--INTERMEDIATE PLANT

Leachate and macerated extracts

During this stage of growth, germination inhibition
was more from the macerated extracts than the leaf leachate,
even though not very significant (figures 5A, 5B).
Macerated extracts showed no effect on radicle length in
aqueous extracts whereas germination was greatly delayed.
In most instances, seeds germinated in leachate showed
good growth and were similar to controls. With the macerated
extracts from the intermediate stage of growth, toxicity
had more effect on the germination of seeds and soil seedl-
ings than with height/weight reduction of older plants
(figures 5B, 5D, 5F). Young seedlings placed in soil
were inhibited greater with both leachate and macerate
extracts of stage II growth (figures 5C, 5D). Leaf leachate
had a significance of $.0025 < p < .005$ for soil radicle
inhibition and the macerate had a significance of
$.025 < p < .05$. Again, with soil contact, young seedlings
and older plants toxicity levels increased in the leachate
extracts. This was slightly less than the young stage of

growth, but still fairly toxic in comparison to the controls.

Decomposed residue extracts

The inhibitory effect upon radicle growth in the
aqueous extracts during the intermediate stage of growth
was much greater than that on germination, especially during
later phases of decomposition, 14-22 day (figures 8A, 8B).
The 22-day saturated series showed a significance of
$.0005 < p < .001$ for the radicle growth in the aqueous
extracts. The relatively high germination in this growth
stage was not accompanied by good seedling growth. After
a few days of growth, the controls of older plants were as
high as 28cm, whereas those treated with extracts varied
from 17 to 19 cm. The effects of inhibition first appeared
after 7-10 days (figures 8A, 8B, 8D, 8E). The seedlings
grown for nine days in soil containing extracts showed root
growth inhibition which steadily increased as the decompo-
sition phase progressed (figures 2, 8C). Highest levels
of toxicity were shown in both young soil seedlings and
older plants during later phases, 7-14 day of decomposition
(figures 8C, 8D, 8E). For radicle length inhibition of soil
seedlings, significant values were seen more with the
saturated series than with the field capacity series:
0-day saturated = $.01 < p < .025$; 2-day saturated =
$.001 < p < .0025$; 14-day saturated = $.05 < p < .10$; and
22-day saturated = $.001 < p < .0025$. The field capacity
series showed significant values at: 7-day field =

.025 $\angle$ p $\angle$ .05, and 22-day field = .05 $\angle$ p $\angle$ .10. For the
older plants, both the 14-day field capacity and saturated
series showed significant values of p $\angle$ .0005 for mean
weight reduction. The primary root was shorter and secon-
dary roots were few and stunted. Seedlings or older plants,
when planted in soil containing residue extracts, either
died or were greatly retarded in growth in comparison with
similar seedlings not so planted. Plants growing in toxic
extracts were stunted and their roots showed considerable
root damage. Little growth of plant, either in top or
root, was observed after transplanting. Some plants just
withered away gradually. Roots at harvest time indicated
that the size of the plant could be roughly correlated with
the extent of root damage. The smaller plants showed much
more root decay than the larger, more vigorous plants. In
the intermediate stage of growth, the extracts were fre-
quently so toxic that it was possible to use as a criterion
of activity the death or survival of older plants rather
than inhibition of growth. After the 16-22 day period, the
toxicity of the extracts containing decomposition products
of corn had decreased and almost disappeared and in some
cases showed stimulatory effects (figures 8A, 8D, 8E).

Autoclaved samples did not radically affect the
germination of seeds. Again, radicle growth was very
similar to the healthy controls (figures 6A, 6B). Saturated
autoclaved samples greatly affected soil seedlings, de-
creasing slightly in older plants, but still significant

(figures 3, 6C, 6D). The 14-day autoclaved field capacity
series had a significant value of .005 $<$ p $<$ .01 for soil
radicle reduction whereas the saturated autoclaved series
showed a significance of p $<$ .0005. Both the autoclaved
field capacity and saturated series showed significant
values of p $<$ .0005 for weight reduction in older plants.
When seedlings were in direct contact with aqueous extracts
of decomposed material, greying appeared on radicle. Small
enlargements or nodules also appeared. Although all parts
of the seedlings were in contact with the toxic extracts,
the visible injurious effects appeared to be restricted
mainly to the region of the root, and in most cases root
development was not inhibited but discoloration appeared.

STAGE III--NEARLY MATURE PLANT

Leachate and macerated extracts

No major inhibitory effects on germination and
radicle growth were seen with the leaf leachate and mace-
rated extracts from any phase of the nearly mature stage of
growth (figures 5A - 5F). Some signs of stimulation were
seen in young soil seedlings and radicle growth of germin-
ated seeds using the leachate extracts from this stage
(figure 5A). Frequently, germinated seeds of both leachate
and macerated extracts showed good growth and were similar
to controls. Again, when in soil, the toxicity levels
increased slightly particularly with macerated extracts
when applied to soil seedlings and older plants (figures

5D, 5F). Soil seedlings when exposed to leachate extracts showed great stimulation of growth, but seedlings placed in direct contact with aqueous extracts of macerated plants showed enlargements or nodules on root tips.

Decomposed residue extracts

There was a decrease in inhibition in almost all areas of testing with extracts from the decomposed plant products of the nearly mature corn plants, in comparison to stages I and II. This stage showed slight decreasing effects on germination with slight increasing effects on radicle length as the decomposition progressed, although neither were very significant (figures 9A, 9B). No marked adverse effects on corn root growth were obtained with extracts from this stage. In most instances, some stimulation was obtained with such extracts. Most of the substances exerted relatively mild effects and were inactivated quite rapidly during subsequent stages of the decomposition (figures 9B, 9C). When residues of nearly mature plants were added to soil, toxic products did not arise until during the relatively late stages of decomposition (figure 9C). The greatest levels of toxicity were noted during the 9-15 day decomposition. In most cases, the field capacity series showed more inhibition than the saturated series. Significant values were noted for the 0, 14 and 22-day decomposition periods for the field capacity series: 0-day field capacity = $.01 < p < .025$; 14-day field capacity =

.01 $\angle$ p $\angle$ .025; and 22-day field capacity = .025 $\angle$ p $\angle$ .05.
There were many circumstances with both field and saturated
series that consequently no inhibition was obtained and
often root tips were slightly stimulated, but more so with
saturated series. Greatest inhibition was recorded with
soil seedlings exposed to 14-day decomposition products
(figure 9C). Some seedlings grown for nine days in soil
containing extracts had stunted roots, while others showed
regions of discoloration and apparently killed, but numerous
secondary roots were put out which remained uninjured.
The seedlings grown for nine days in soil containing no ·
extracts developed long, healthy roots which represented
quite normal growth. In older plants the color of the leaves
of the treated plants became yellow-green, as compared with
the dark green leaves of the controls.

Autoclaved samples showed greatest inhibition during
young soil seedlings and older plant growth (figures 6C,
6D). Significant values of p $\angle$ .0005 were noted in both
autoclaved field capacity and saturated series for soil
radicle length reduction. Germination and aqueous radicle
growth were not significantly affected. The field capacity
autoclaved series showed stimulatory radicle growth and
germination during this stage of growth (figures 6A, 6B).

ISOLATION AND IDENTIFICATION OF TOXIC SUBSTANCE

Since results presented show that there is an
inhibitor of growth of corn in corn residue, the isolation

of the inhibitor was therefore undertaken. Many phenolic

acids have been shown to be toxic to germination and growth

of many plants. The toxic principle was found to be soluble

in ether and heat stable. Larger amounts of phenolic acids

were liberated by alkaling hydrolysis of 80% ethanol ex-

tracts than by acid hydrolysis. Autoclaving with 2n NaOH

produced increased amounts of toxic substance. From the

chromatographic results, two phenolic acids were detected--

ferulic acid and syringic acid. The bronze-yellow color

of ferulic and syringic acids were detected by their reac-

tion with the spray reactant--diazotized sulfanilic acid

and diazotized p-nitroaniline. Syringic acid occurred less

frequently in samples than ferulic acid.

The following photographs reveal the effects observed when corn seedlings were exposed to toxic and non-toxic extracts obtained from various decomposition periods in the soil. Initially, the seedlings used in each decomposition test were identical in appearance, all having come from the same sample on the 6th day of germination.

Figure 1-A: Control--young stage of growth, 14-day decomposition period.
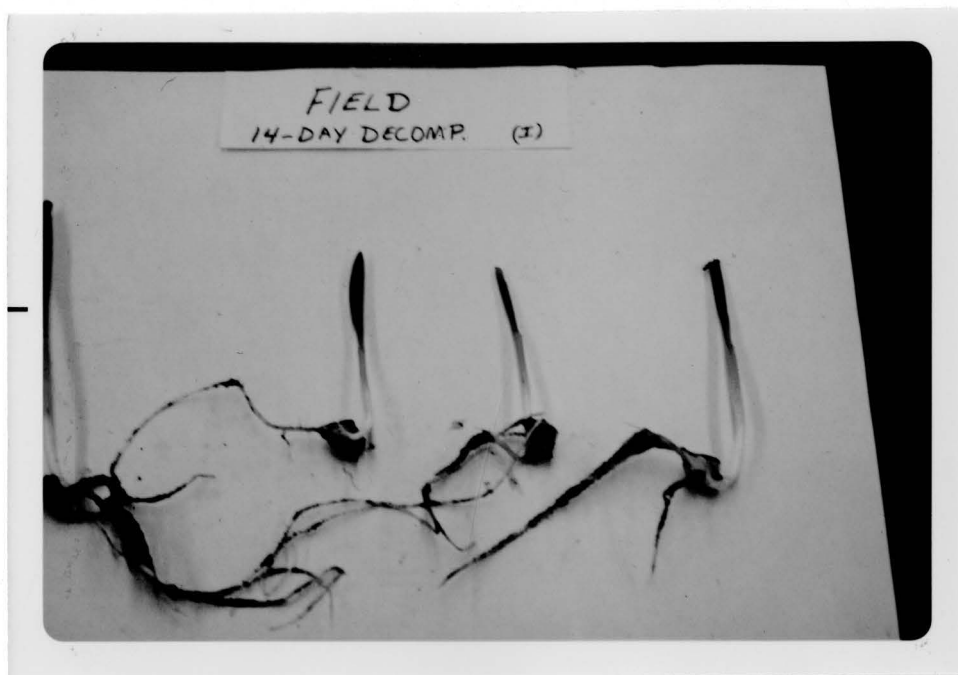
Figure 1-B: Field capacity--young stage of growth, 14-day decomposition period.
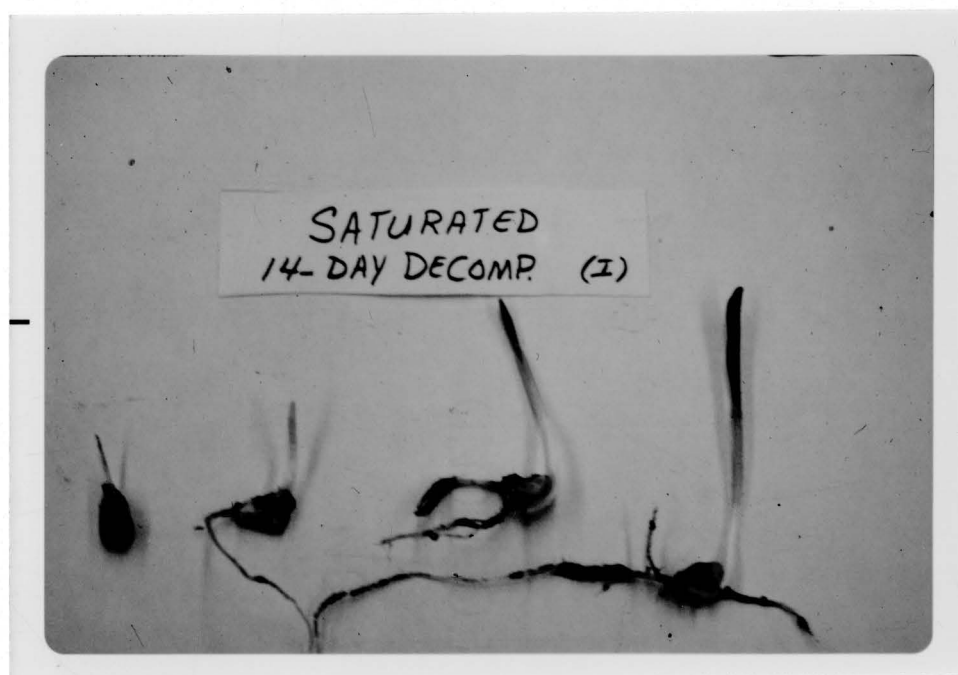
Figure 1-C: Saturated series--young stage of growth, 14-day decomposition period.

Figure 2: Comparison of saturated, field capacity, and con-
trol--intermediate stage of growth, 7-day decomposition period.
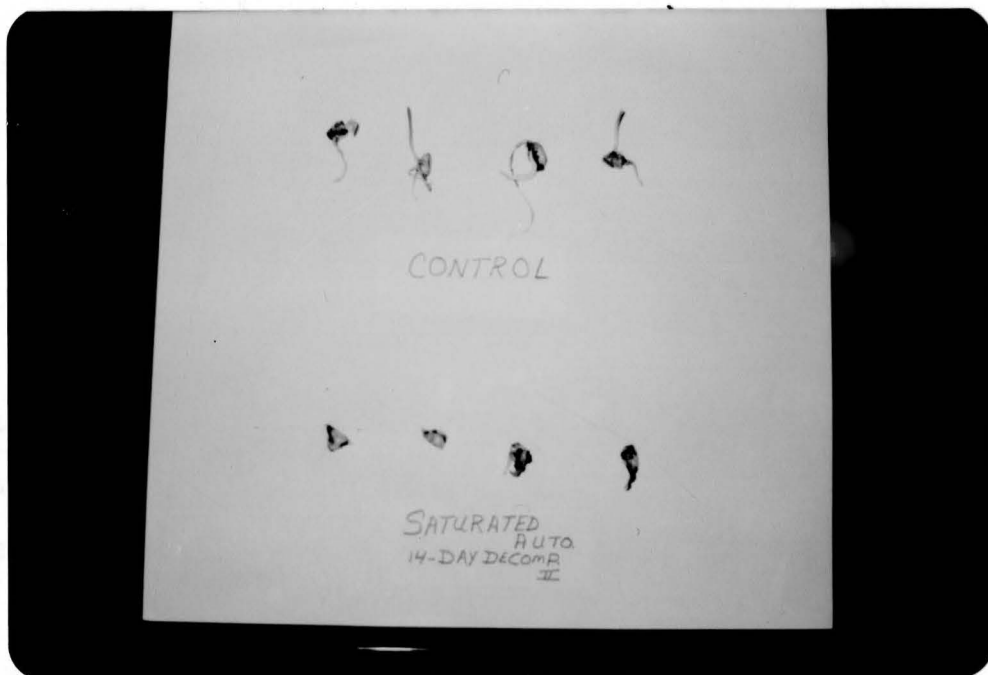
Figure 3: Autoclaved control and saturated--intermediate
stage of growth, 14-day decomposition period.

Figure 4-A: Relative toxicity of original soil at various
growth stages, based on percentage inhibition or stimulation
of radicle length in soil.

F = Field capacity

S = Saturated

Figure 4-B: Mean pH values of decomposed plant residue

at various stages of growth.

F = Field capacity

S = Saturated

C = Control
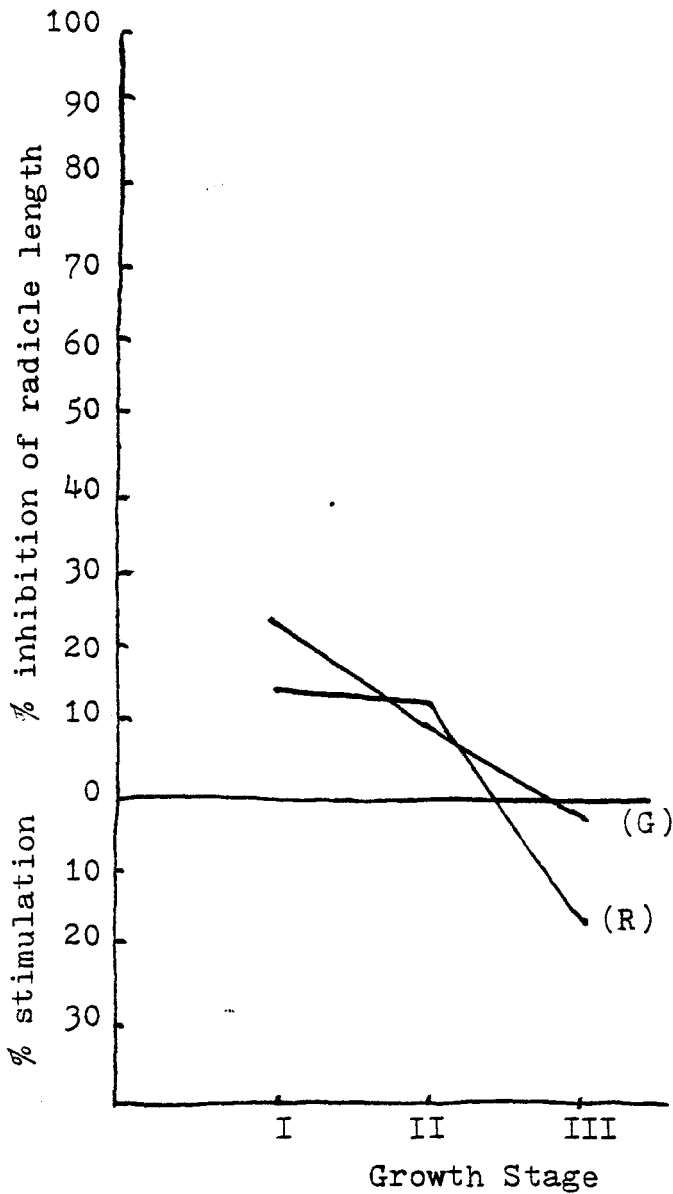
Figure 5-A: Relative toxicity of leaf leachate at various growth stages, based on percentage inhibition or stimulation of germination and radicle length in aqueous extracts.

G = Germination

R = Radicle length
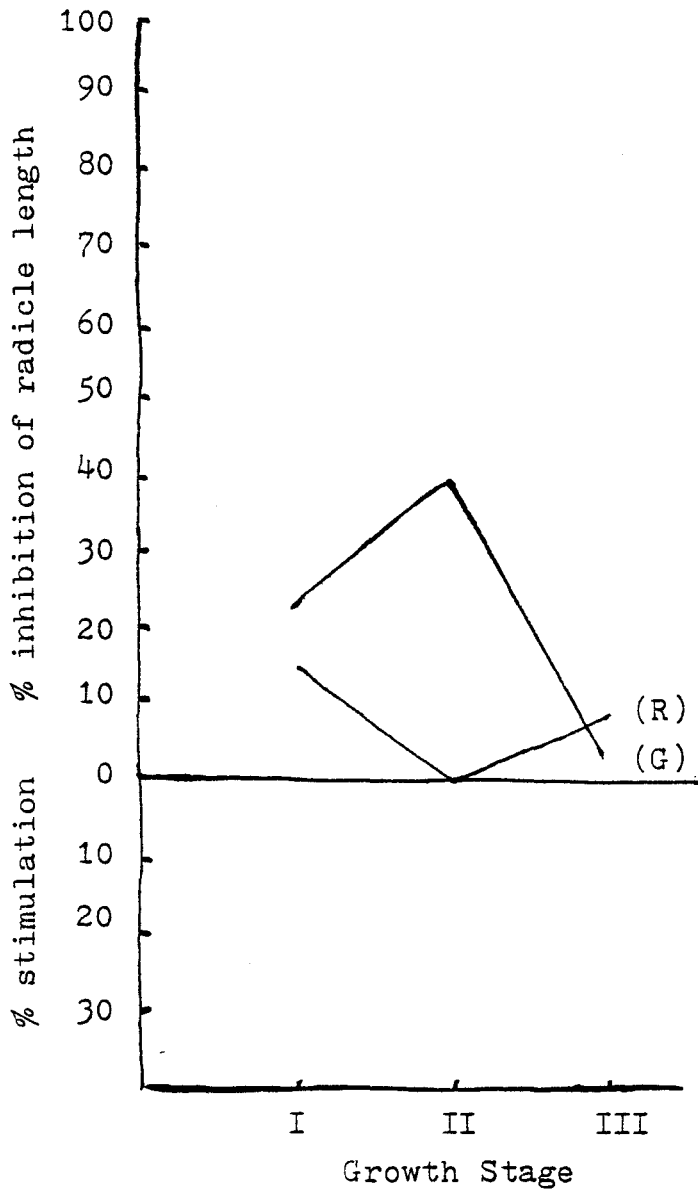
Figure 5-B: Relative toxicity of macerated plant at various growth stages, based on percentage inhibition or stimulation of germination and radicle length in aqueous extracts.

G = Germination

R = Radicla length
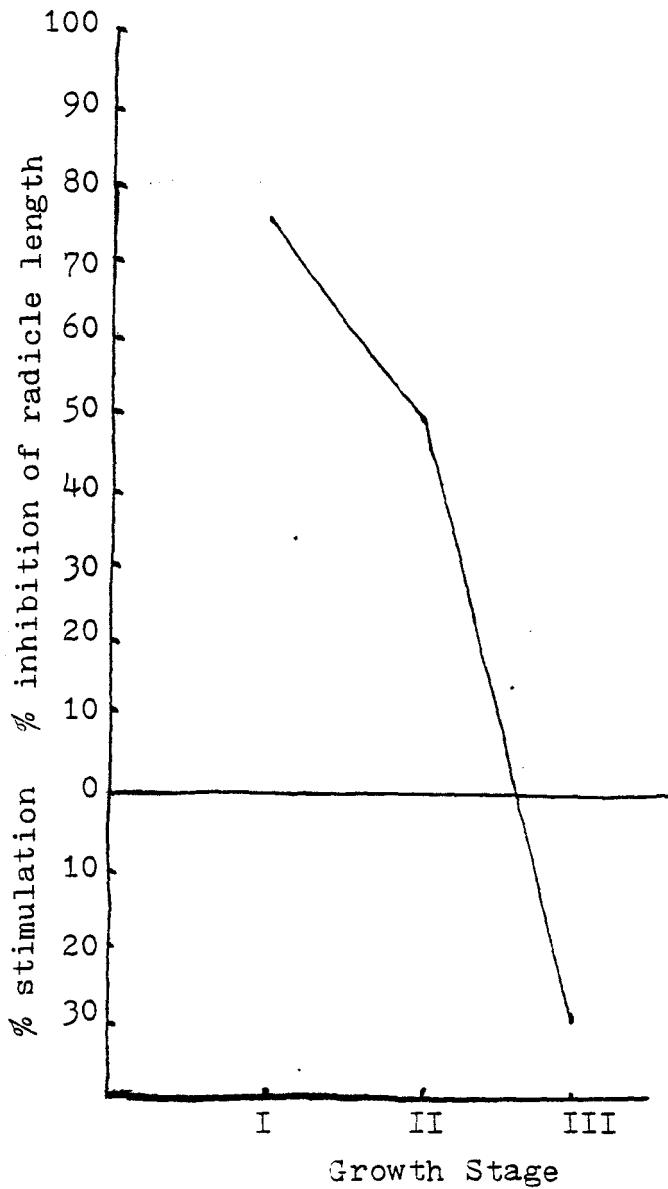
Figure 5-C: Relative toxicity of leaf leachate at various growth stages, based on percentage inhibition or stimulation of radicle length in soil.
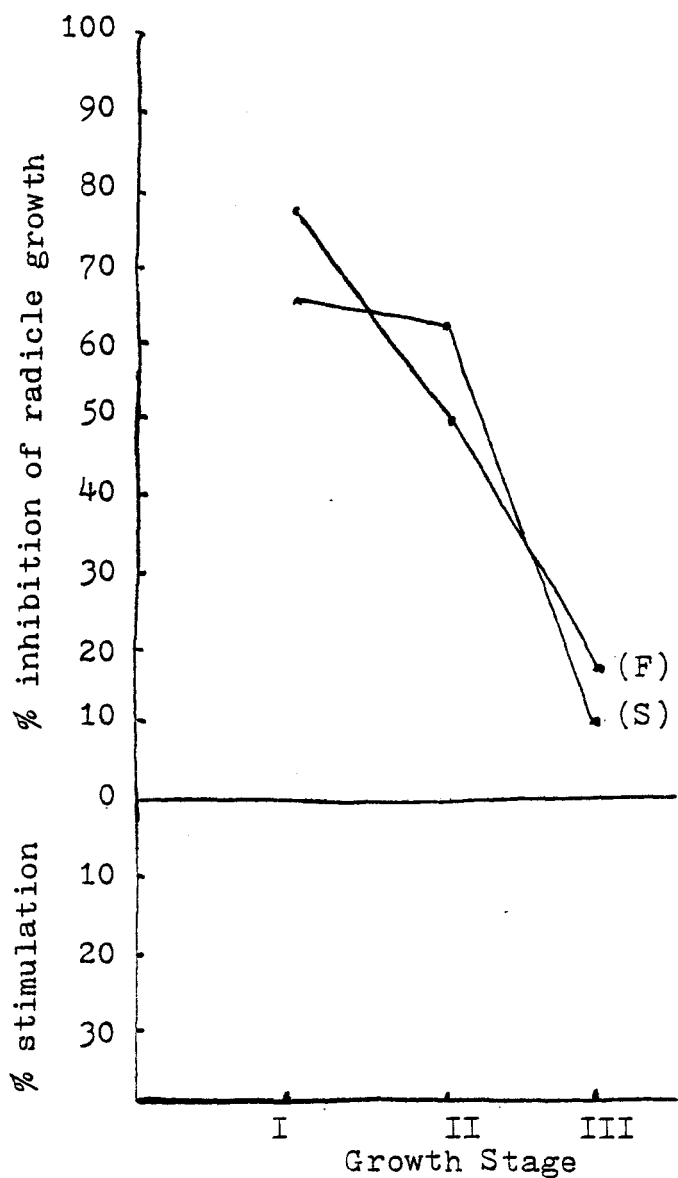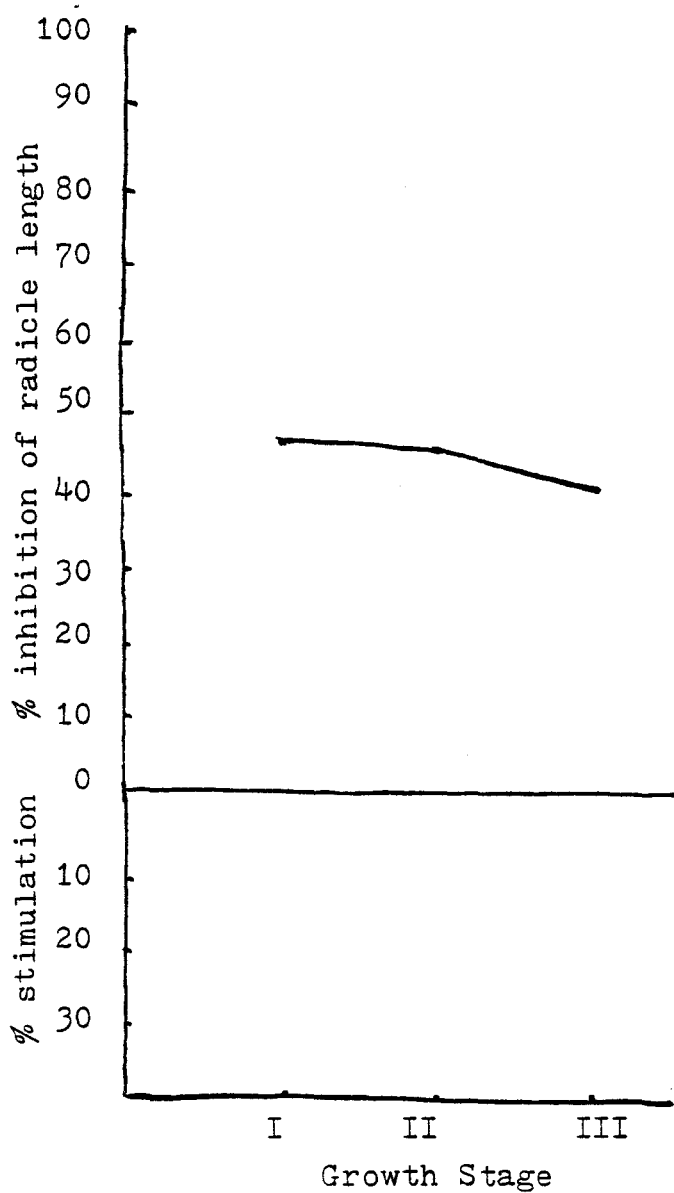
Figure 5-D: Relative toxicity of macerated plant at various
growth stages, based on percentage inhibition or stimulation
of radicle length in soil.
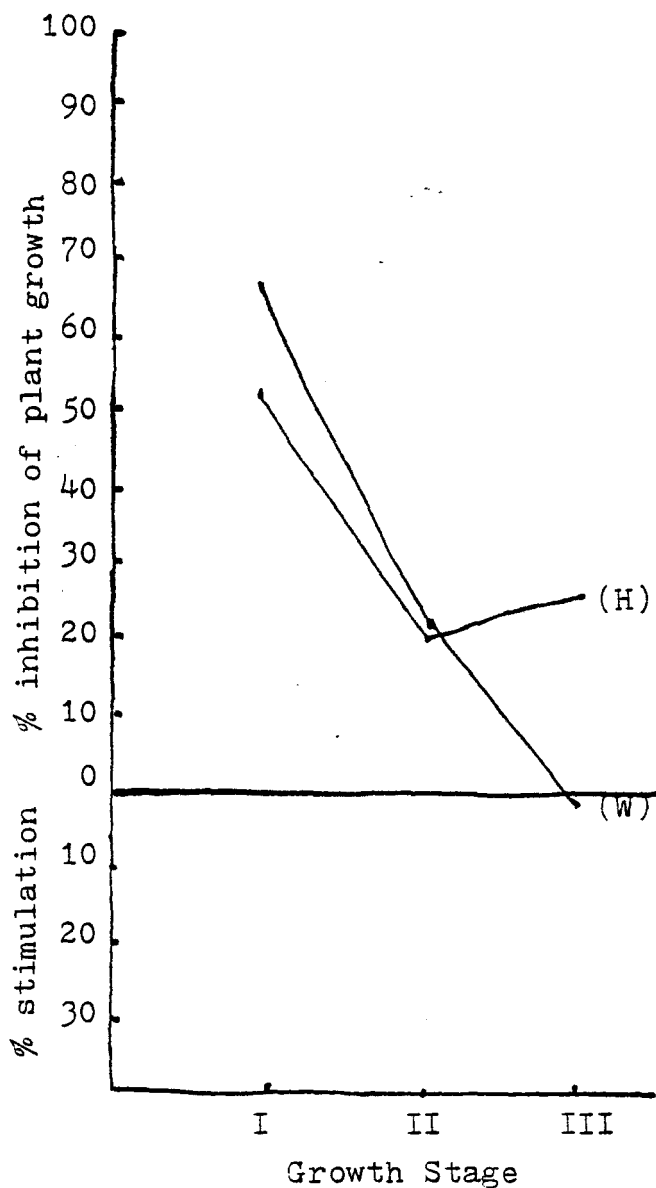
Figure 5-E: Relative toxicity of leaf leachate at various growth stages, based on percentage inhibition or stimulation of plant growth.
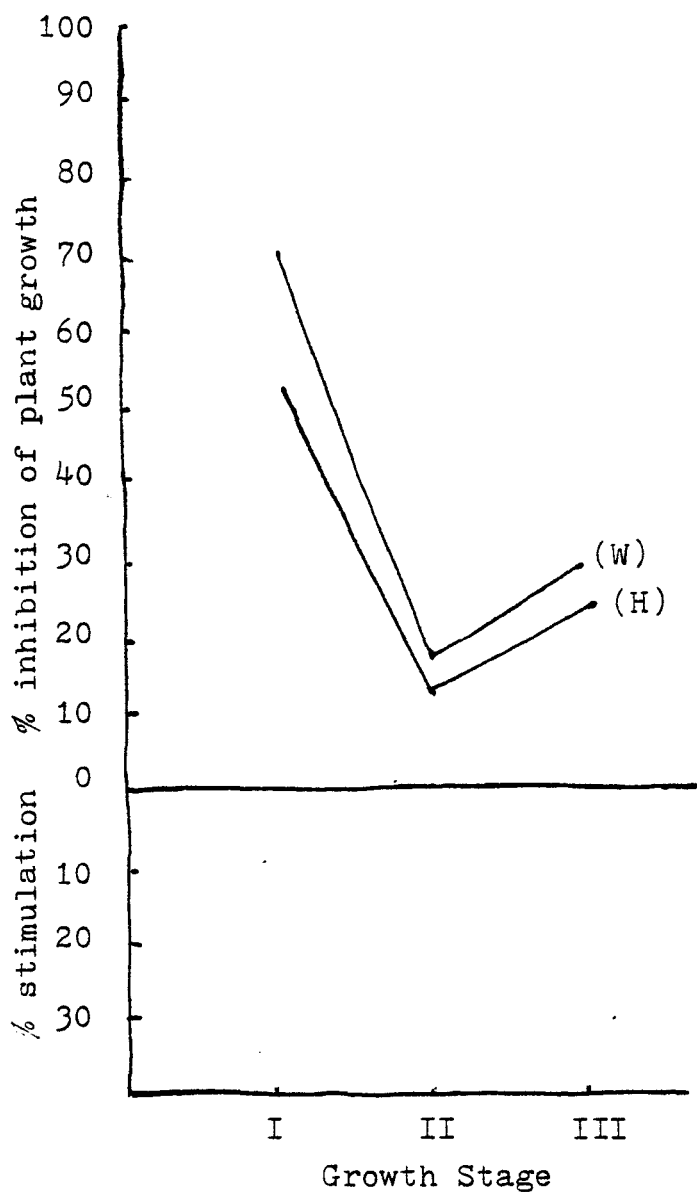
W = Weight

H = Height

Figure 5-F: Relative toxicity of macerated plant at various growth stages, based on percentage inhibition or stimulation of plant growth.

W = Weight

H = Height
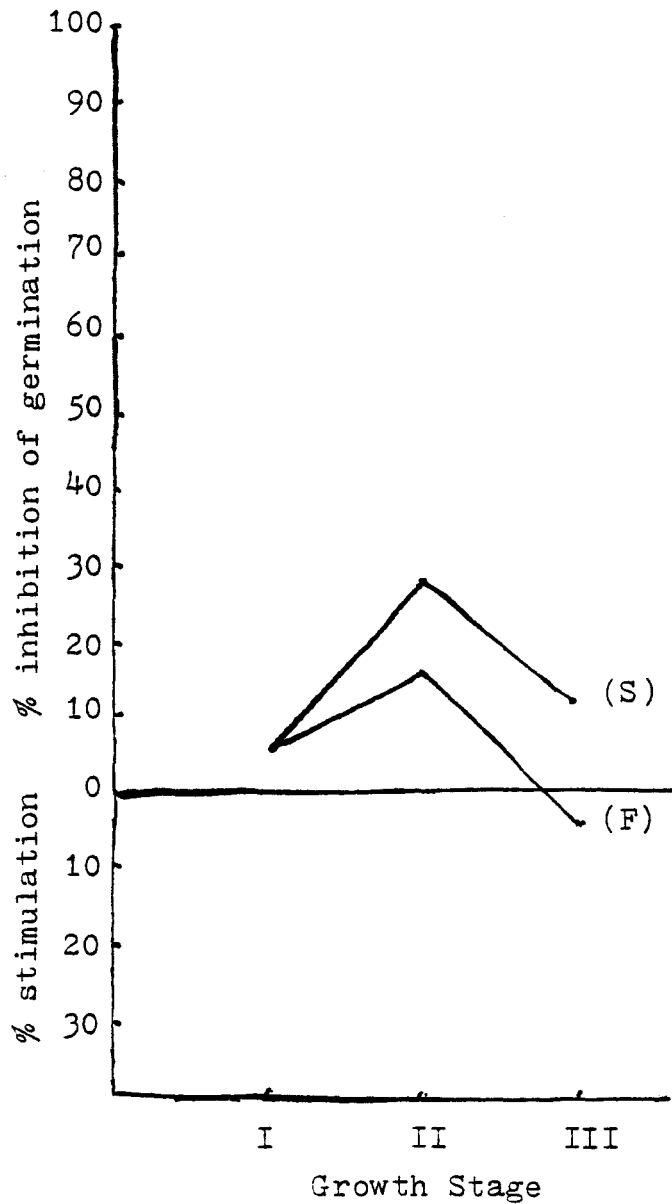
Figure 6-A: Relative toxicity of autoclaved decomposed plant residue at various growth stages, based on percentage inhibition or stimulation of germination.

F = Field capacity

S = Saturated

Figure 6-B: Relative toxicity of autoclaved decomposed plant residue at various growth stages, based on percentage inhibition or stimulation of radicle length in aqueous extracts.

F = Field capacity

S = Saturated
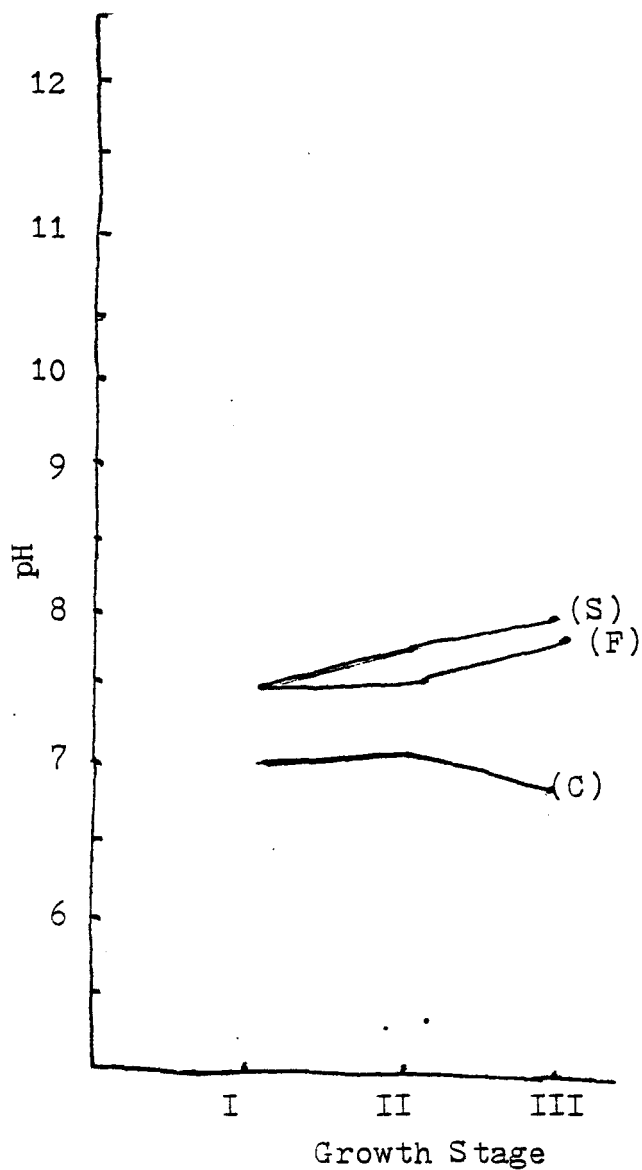
Figure 6-C: Relative toxicity of autoclaved decomposed plant residue at various growth stages, based on percentage inhibition or stimulation of plant growth.

FW = Field capacity weight   FH = Field capacity height

SW = Saturated weight   SH = Saturated height
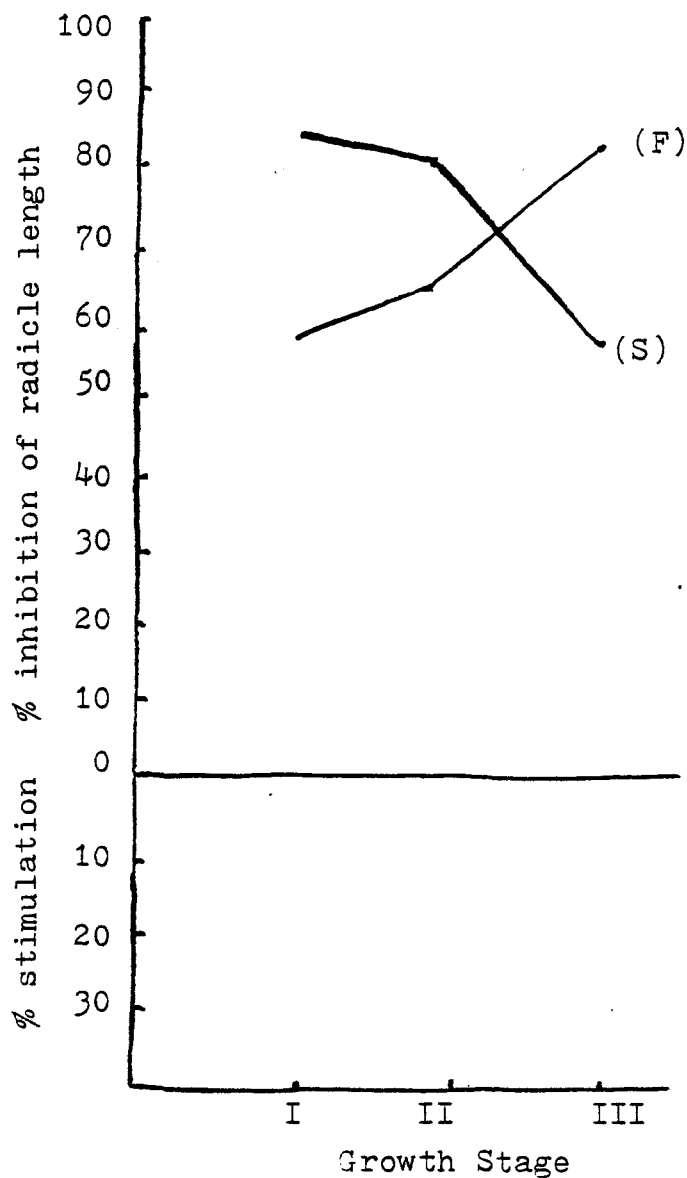
Figure 6-D: Relative toxicity of autoclaved decomposed plant residue at various growth stages, based on percentage inhibition or stimulation of radicle length in soil.

F = Field capacity

S = Saturated

Figure 7-A: Growth stage I--relative toxicity of decomposed plant residue based on percentage inhibition or stimulation of germination.

F = Field capacity

S = Saturated

Figure 7-B: Growth stage I--relative toxicity of decomposed plant residue based on percentage inhibition or stimulation of radicle length in aqueous extracts.

F = Field capacity

S = Saturated

Figure 7-C: Growth stage I--relative toxicity of decomposed plant residue based on percentage inhibition or stimulation of radicle length in soil.

F = Field capacity
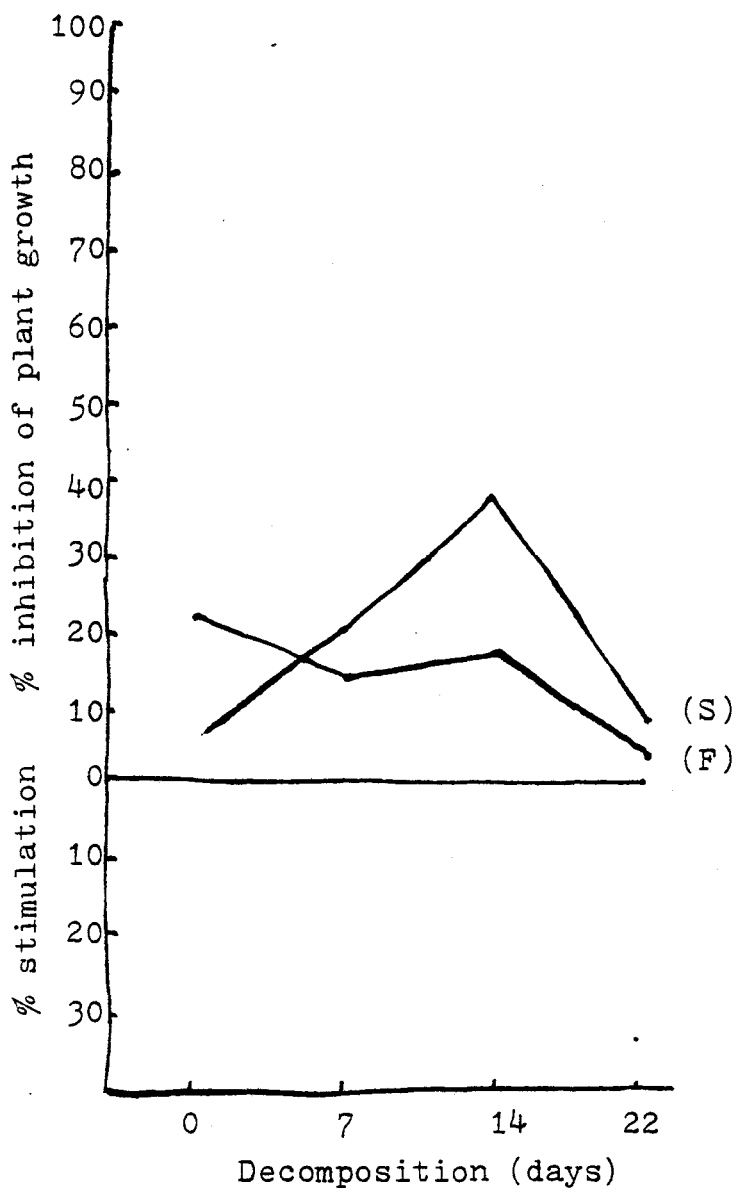
S = Saturated

Figure 7-D: Growth stage I--relative toxicity of decomposed
plant residue based on inhibition or stimulation of plant
growth in height.

F = Field capacity
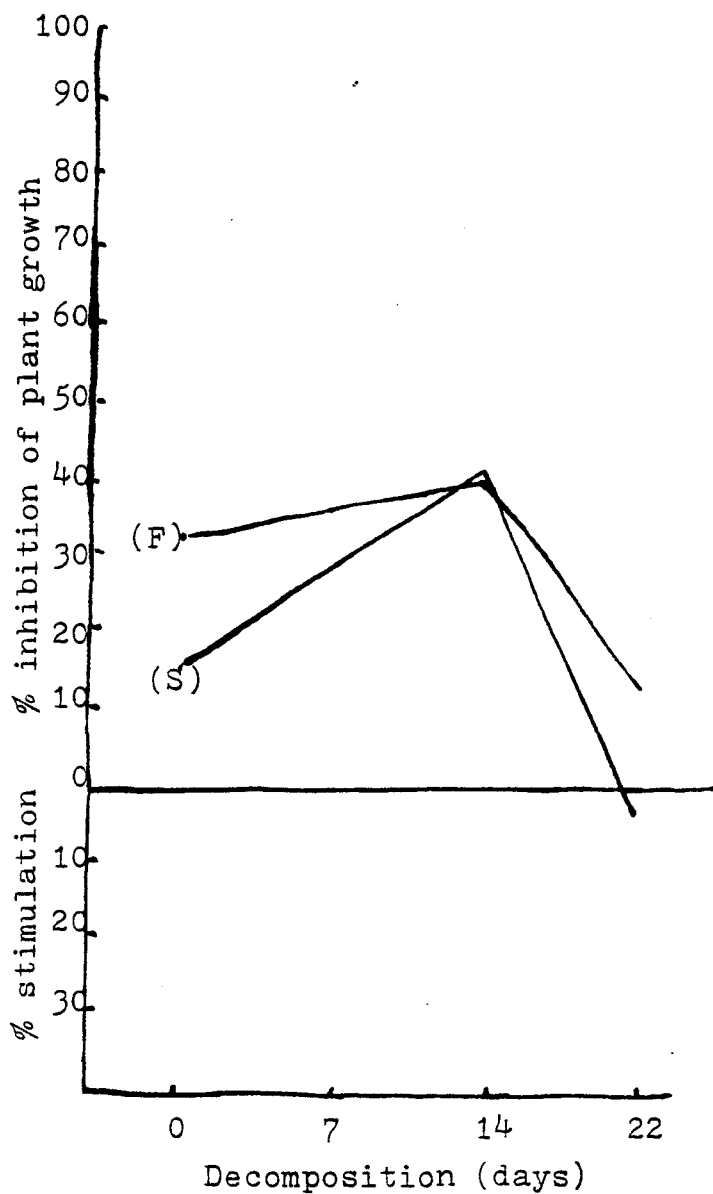
S = Saturated

Figure 7-E: Growth stage I--relative toxicity of decomposed
plant residue based on percentage inhibition or stimulation
of plant growth in weight.

F = Field capacity
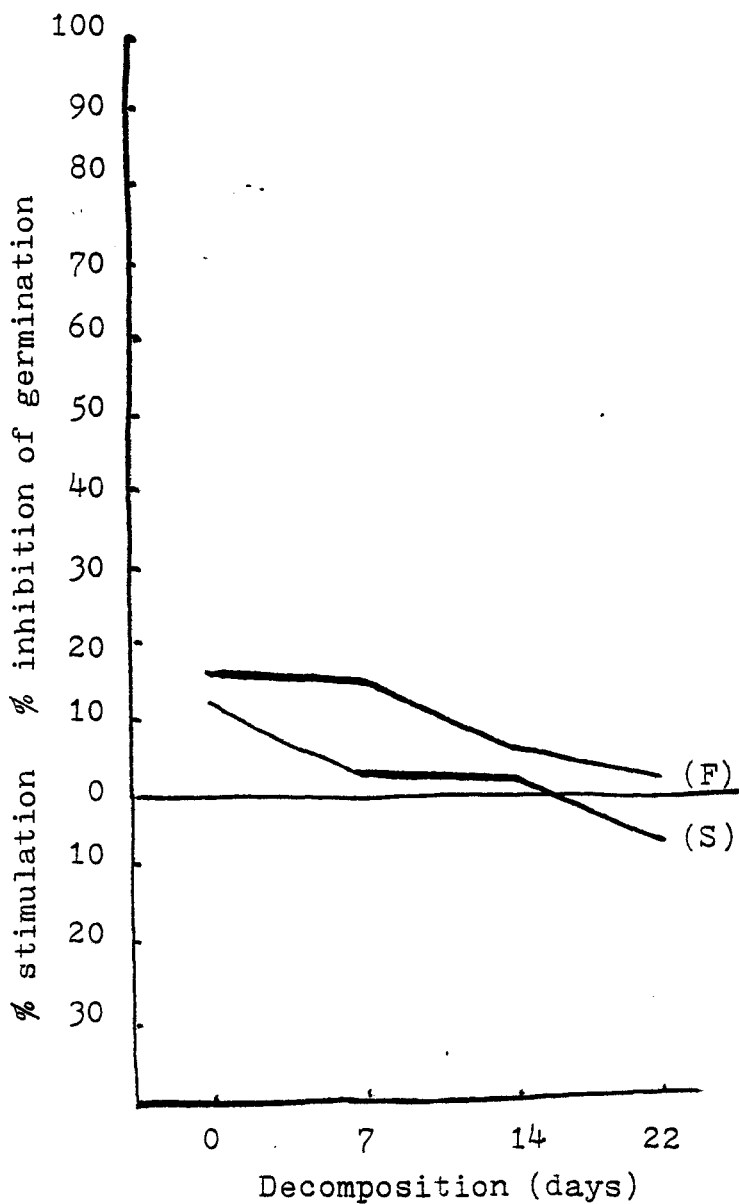
S = Saturated

Figure 8-A: Growth stage II--relative toxicity of decomposed
plant residue based on percentage inhibition or stimulation
of germination.

F = Field capacity
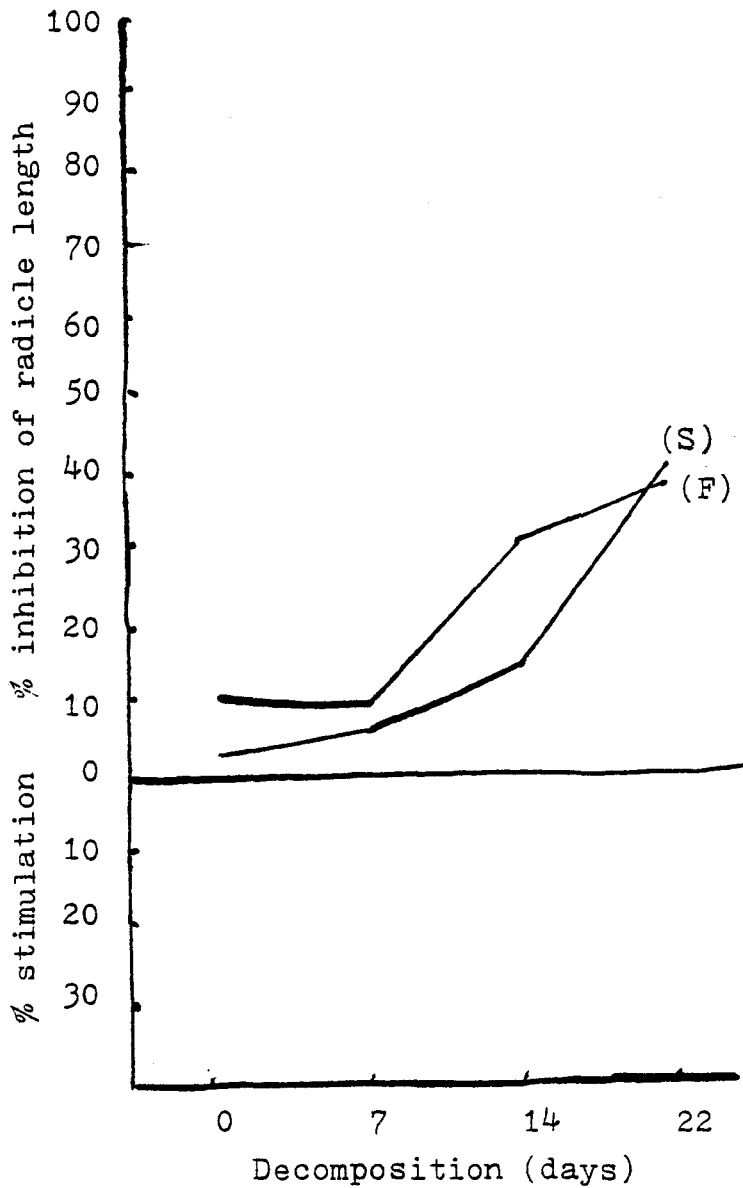
S = Saturated

Figure 8-B: Growth stage II--relative toxicity of decomposed plant residue based on percentage inhibition or stimulation of radicle length in aqueous extracts.

F = Field capacity
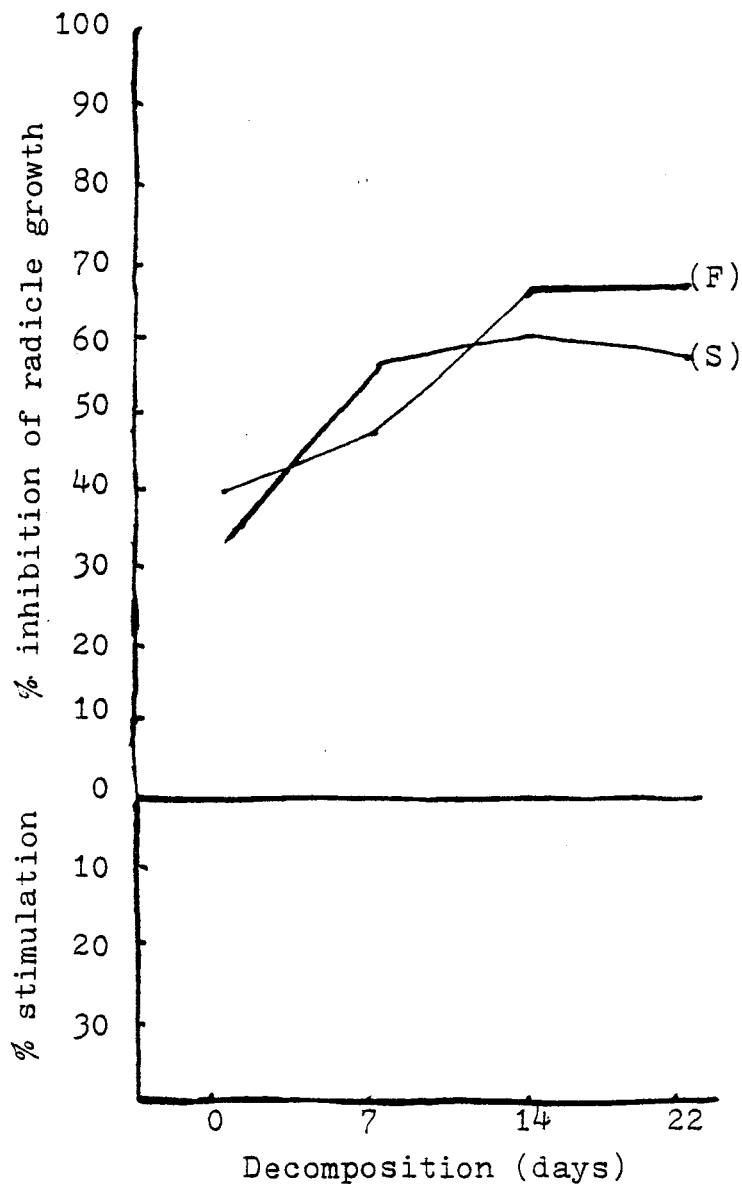
S = Saturated

Figure 8-C: Growth stage II--relative toxicity of decomposed plant residue based on percentage inhibition or stimulation of radicle length in soil.

F = Field capacity
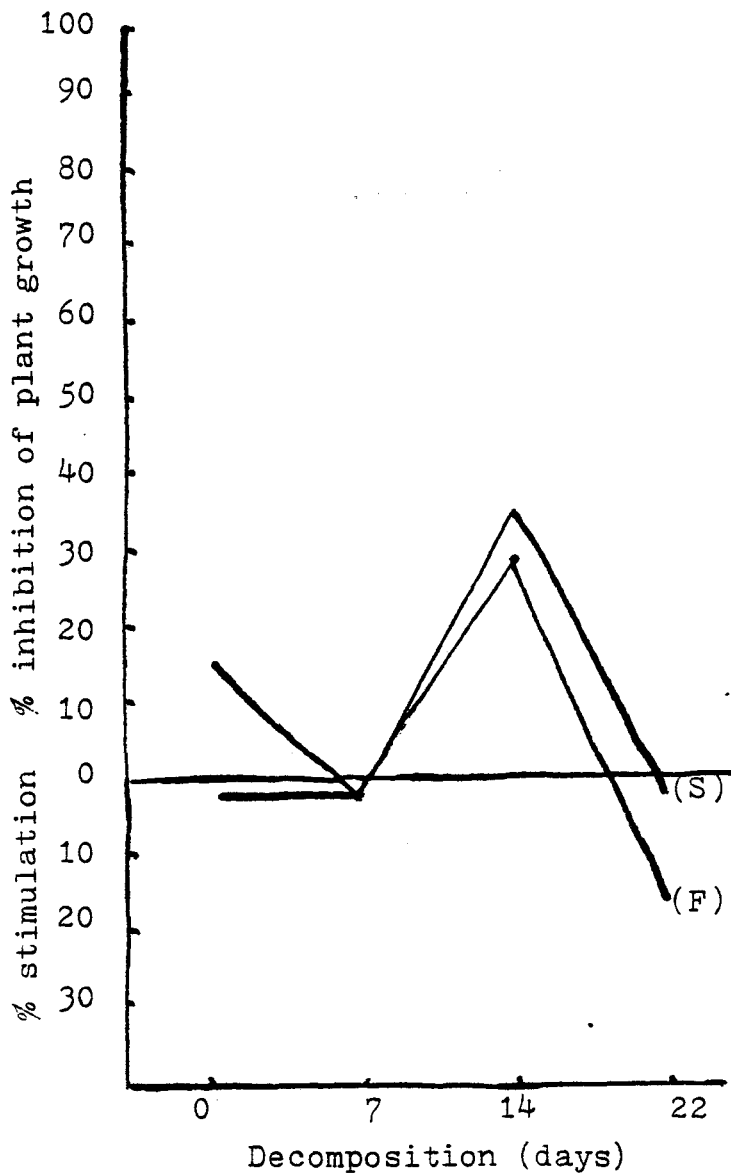
S = Saturated

Figure 8-D: Growth stage II--relative toxicity of decomposed plant residue based on percentage inhibition or stimulation of plant growth in height.

F = Field capacity
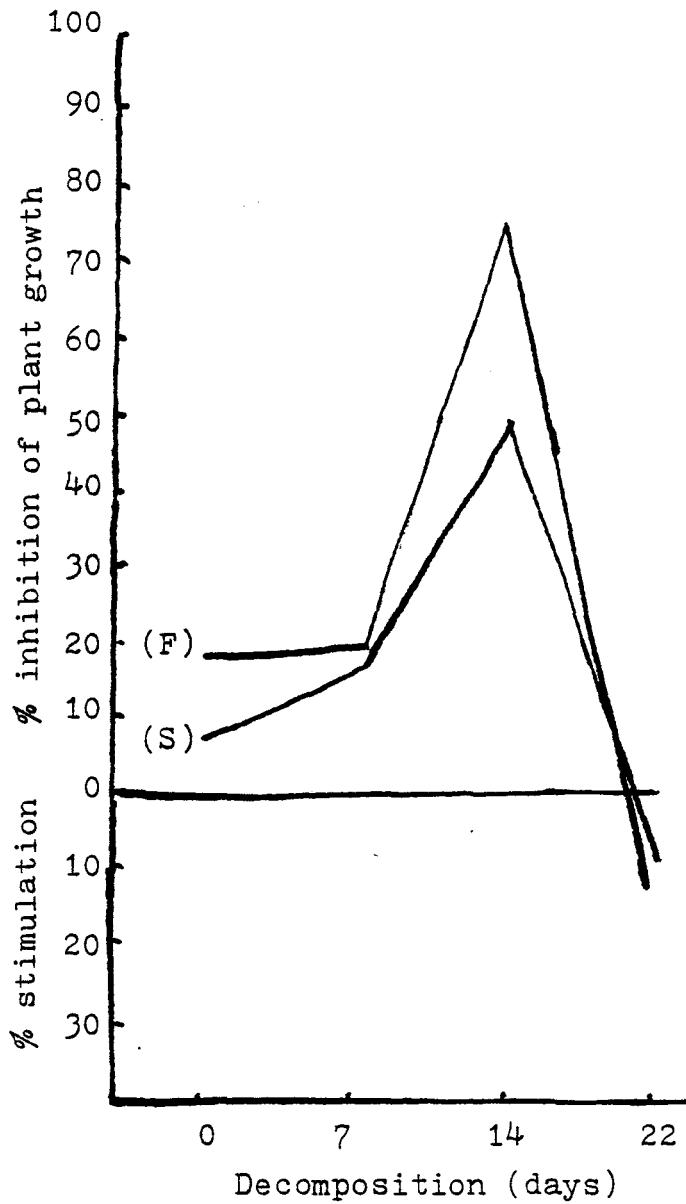
S = Saturated

Figure 8-E: Growth stage II--relative toxicity of decomposed plant residue based on percentage inhibition or stimulation of plant growth in weight.

F = Field capacity
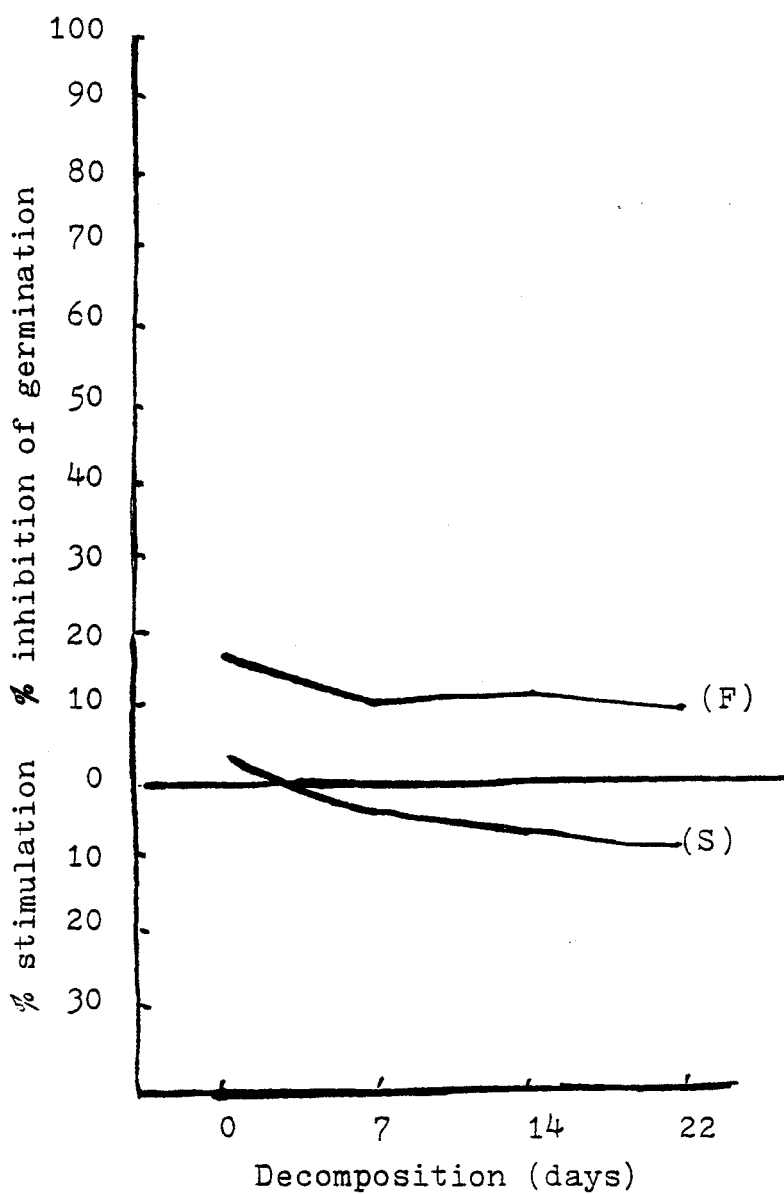
S = Saturated

Figure 9-A: Growth stage III--relative toxicity of decomposed plant residue based on percentage inhibition or stimulation of germination.

F = Field capacity
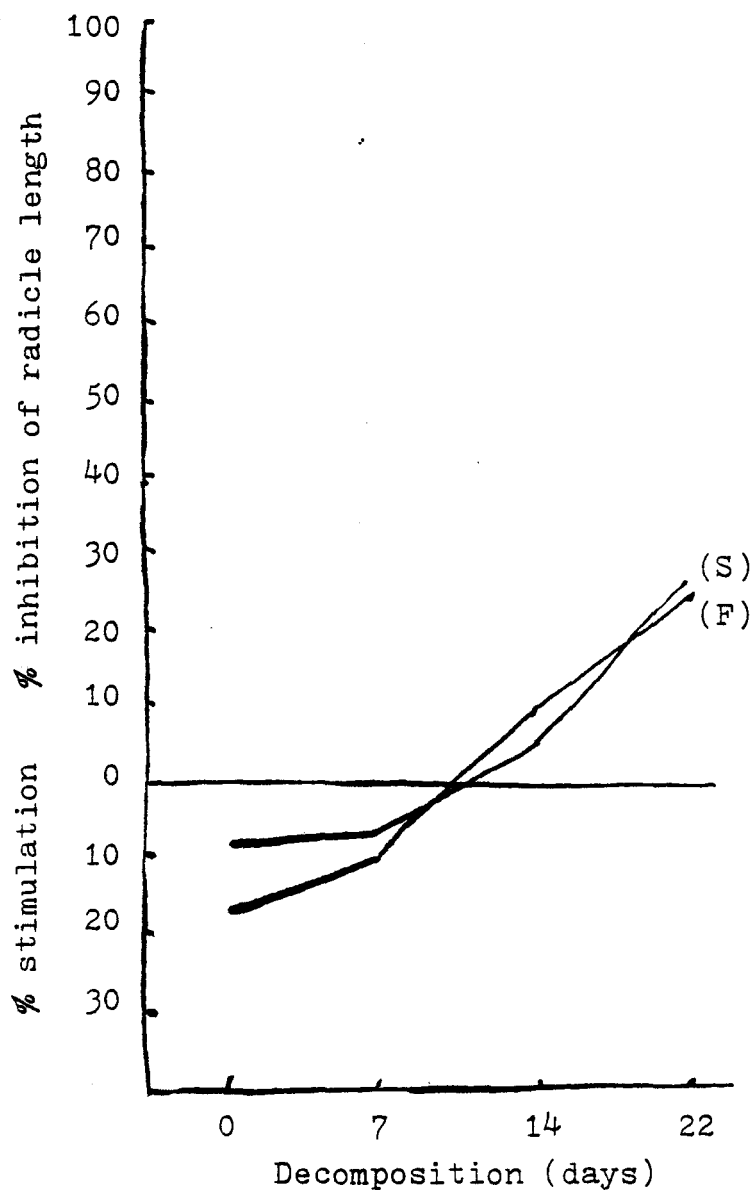
S = Saturated

Figure 9-B: Growth stage III--relative toxicity of
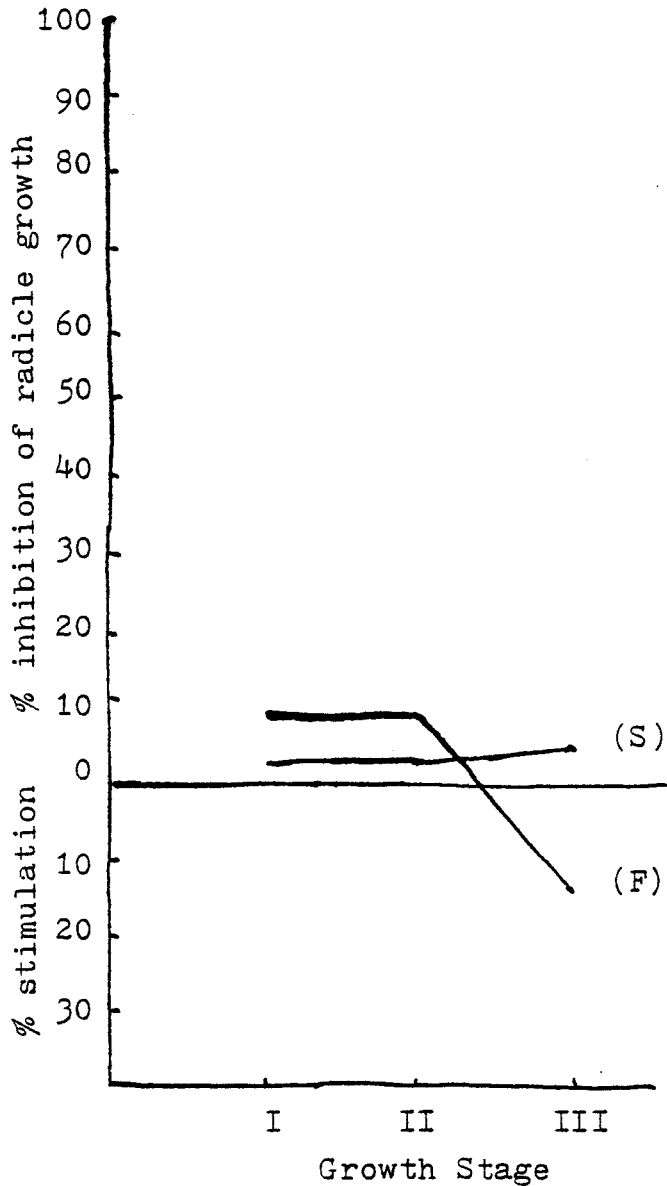decomposed plant residue based on percentage inhibition
or stimulation of radicle length in aqueous extracts.
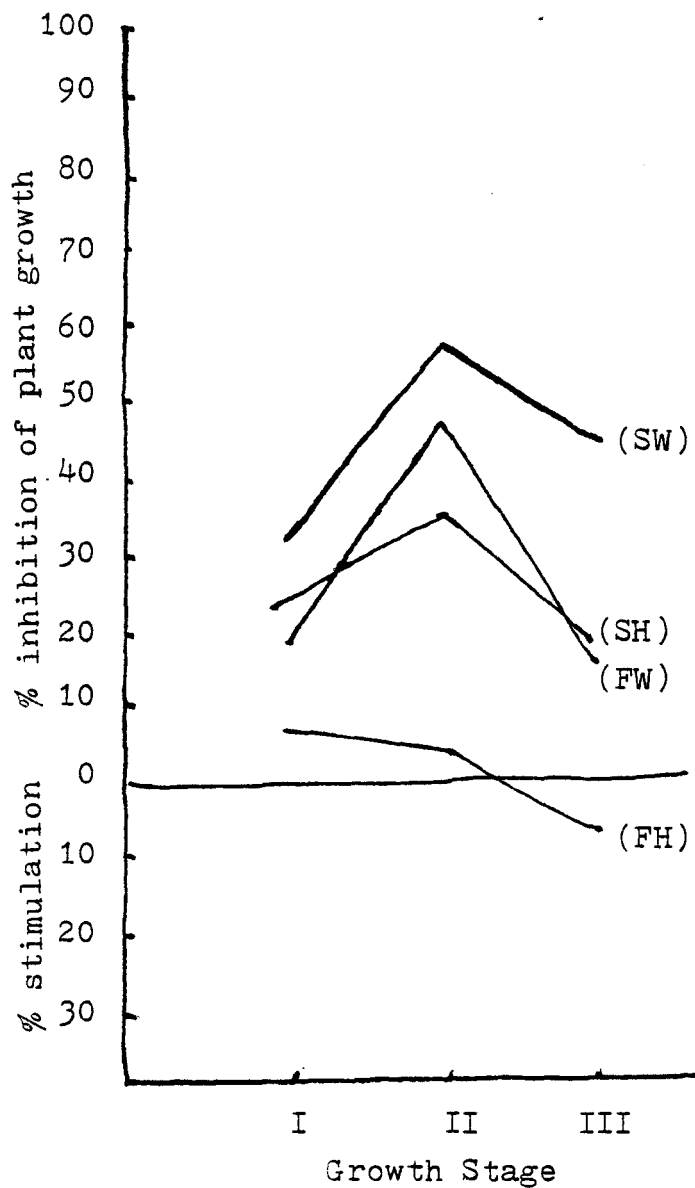
F = Field capacity
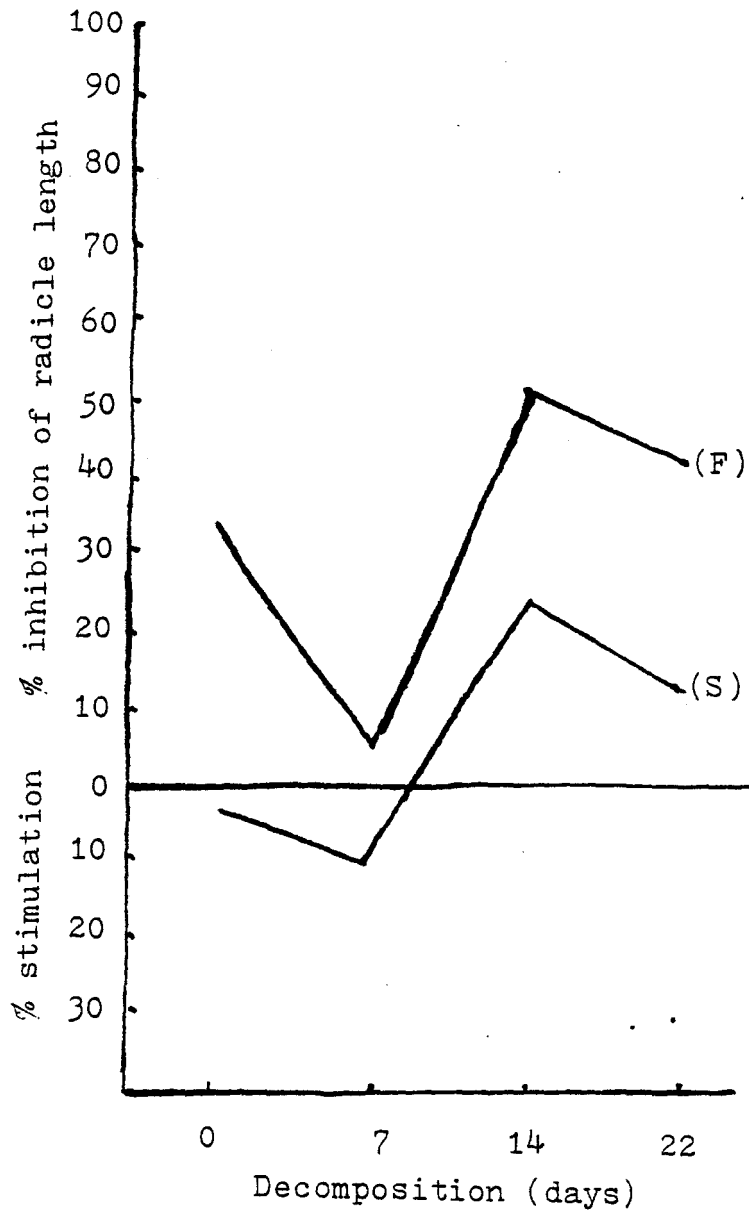
S = Saturated

Figure 9-C: Growth stage III--relative toxicity of decomposed plant residue based on percentage inhibition or stimulation of radicle length in soil .
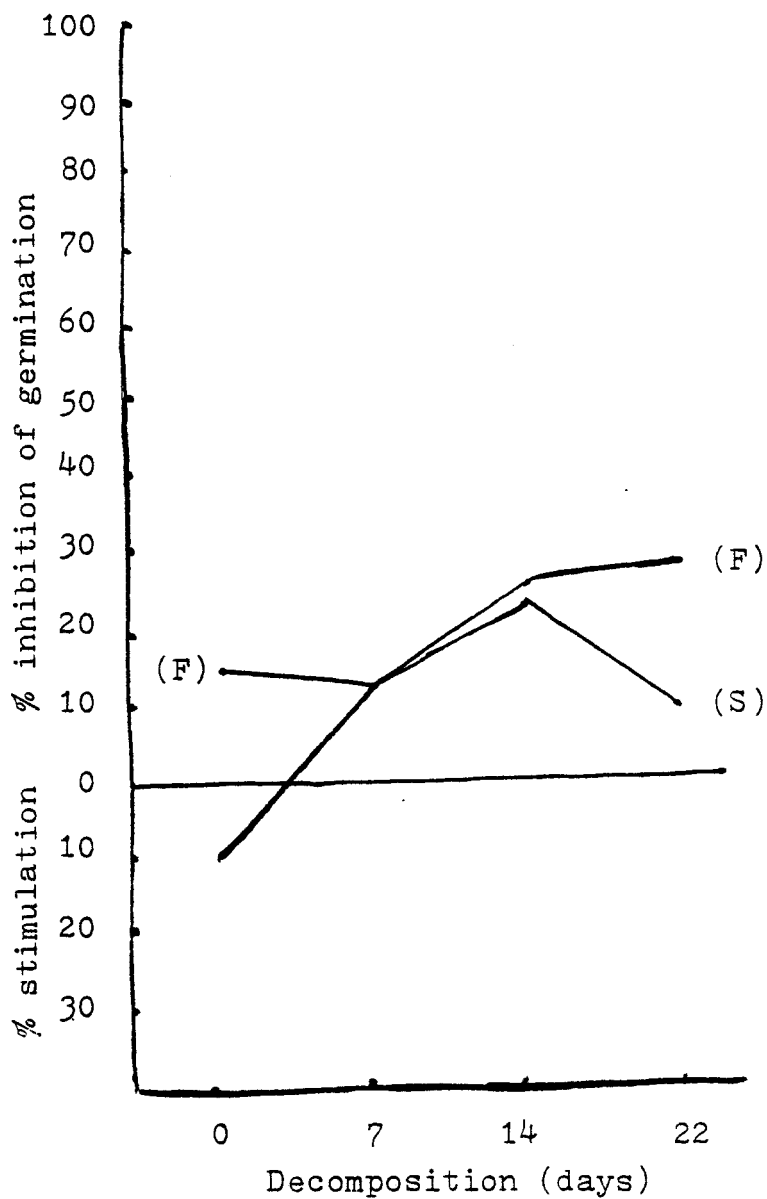
F = Field capacity
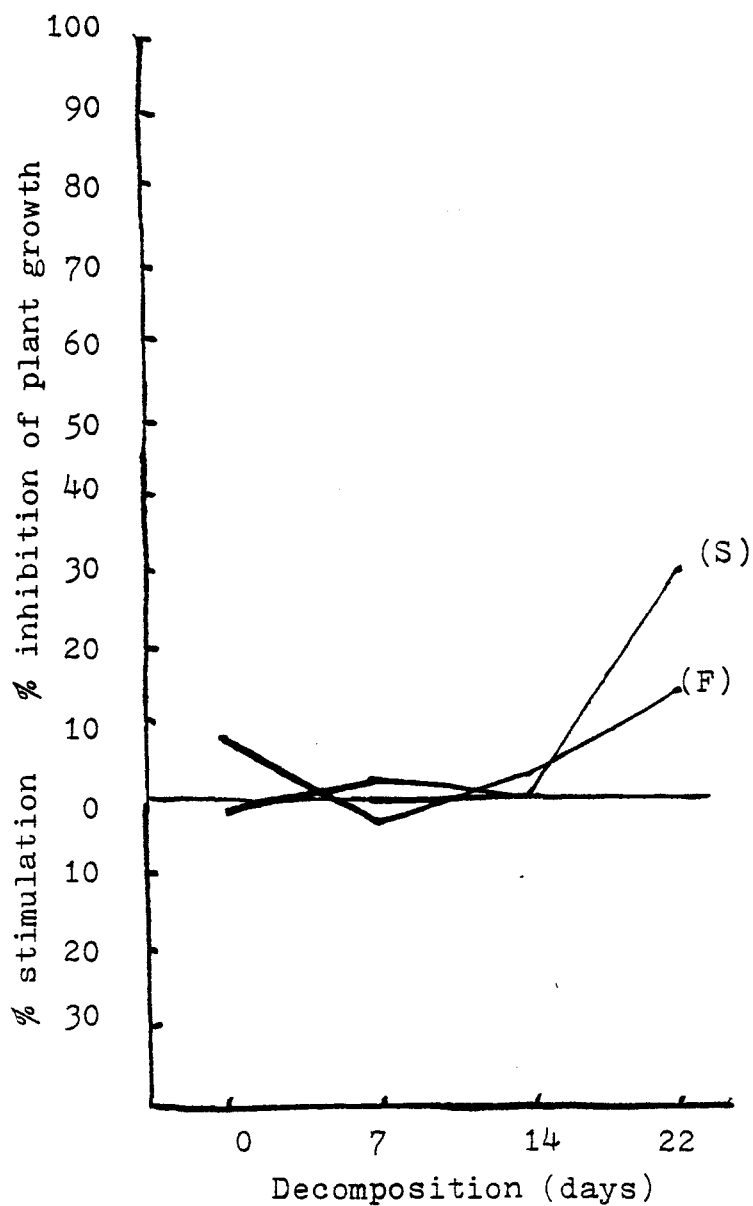
S = Saturated
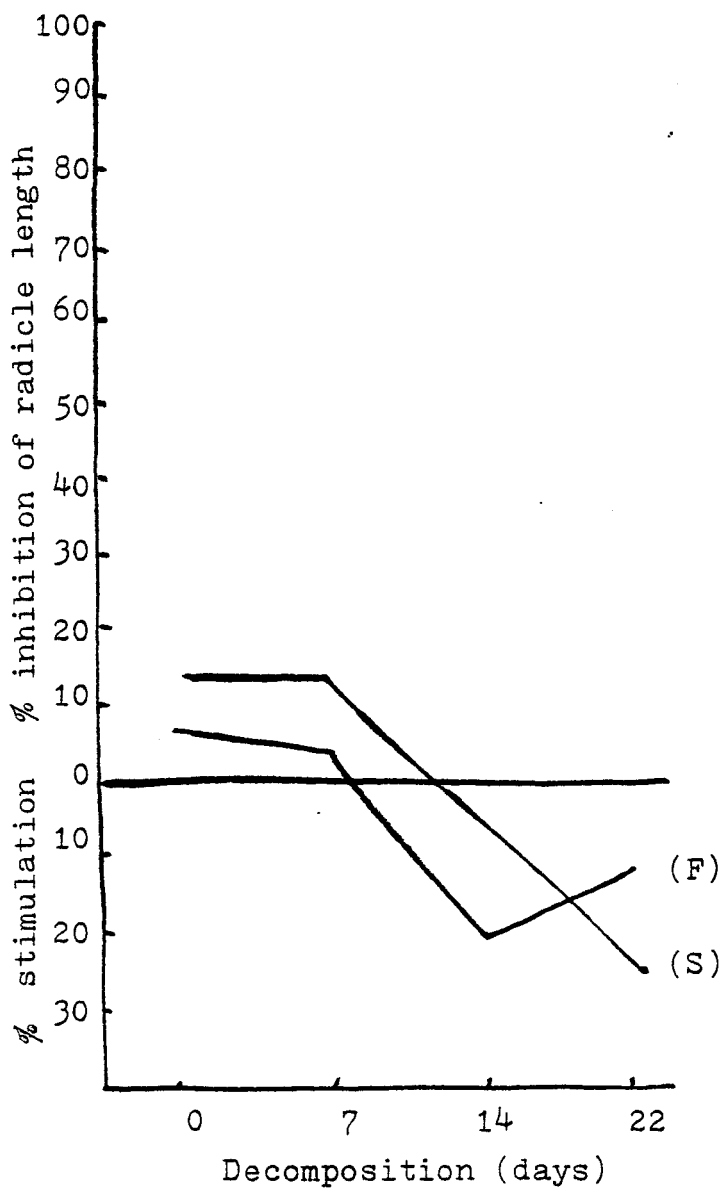
Figure 9-D: Growth stage III--relative toxicity of
decomposed plant residue based on percentage inhibition
or stimulation of plant growth in height.

F = Field capacity
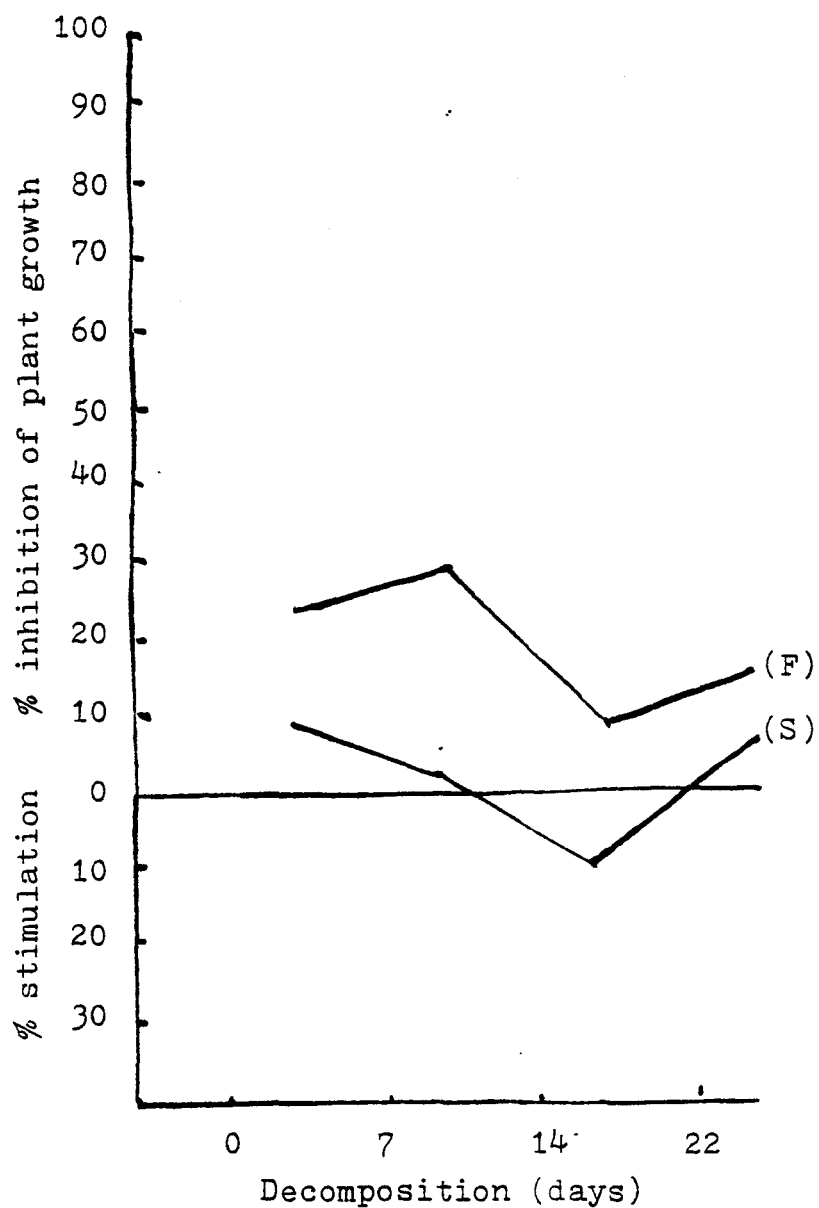
S = Saturated
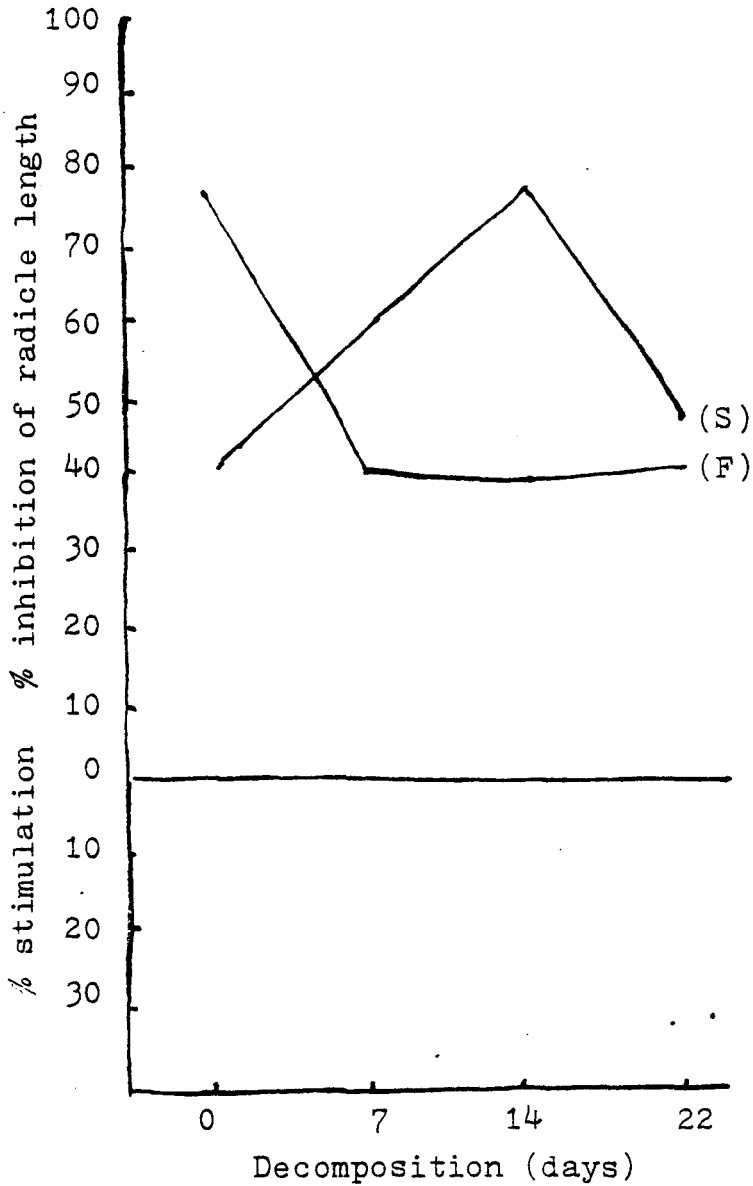
Figure 9-E: Growth stage III--relative toxicity of decomposed plant residue based on percentage inhibition or stimulation of plant growth in weight.

F = Field capacity

S = Saturated

YOUNG GROWTH STAGE (I)				
Moisture Level	Decomp. period (day)	Control radicle length cm.	Test radicle length cm.	Germina-tion percent
Field Capacity	0	3.56	3.40	70
	7	8.64	8.38	70
	14	6.22	7.76	63
(autoclaved)	14	6.22	5.72	80
	22	4.45	4.98	59
Saturated	0	3.60	3.10	90
	7	8.80	7.60	70
	14	7.30	7.80	65
(autoclaved	14	6.45	6.35	80
	22	4.00	5.40	75

Table 1: Mean values of decomposed plant residue on radicle length and germination in aqueous extracts; germination is expressed as percent of control.

INTERMEDIATE GROWTH STAGE (II)				
Moisture Level	Decomp. period (day)	Control radicle length cm.	Test radicle length cm.	Germina- tion percent
Field Capacity	0	4.47	4.00	70
	7	8.38	7.70	68
	14	7.00	5.00	75
(autoclaved)	14	7.70	7.11	68
	22	5.00	3.10	83
Saturated	0	4.75	4.85	73
	7	10.30	9.65	78
	14	8.80	7.62	78
(autoclaved)	14	6.70	6.60	58
	22	9.20	5.59* ($\pm$ .658)	90

Table 2: Mean values of decomposed plant residue on radicle length and germination in aqueous extracts; germination expressed as percent of control.

*Significantly different from control at .05 level or better.

($\pm$) Standard deviation

NEARLY MATURE GROWTH STAGE (III)				
Moisture Level	Decomp. period (days)	Control radicle length cm.	Test radicle length cm.	Germination percent
Field Capacity	0	6.10	7.37	65
	7	6.86	7.62	65
	14	6.43	5.84	70
(autoclaved)	14	6.43	7.37	85
	22	6.60	5.08	70
Saturated	0	7.20	7.73	75
	7	6.90	7.50	75
	14	5.80	5.43	85
(autoclaved)	14	6.30	6.10	70
	22	6.70	4.78* ($\pm$ 1.04)	85

Table 3: Mean values of decomposed plant residue on radicle length and germination in aqueous extracts; germination expressed as percent of control.

*Significantly different from control at .05 level or better.

($\pm$) Standard deviation

YOUNG GROWTH STAGE (I)			
Moisture Level	Decomp. period (days)	Control radicle length cm.	Test radicle length cm.
Field Capacity	0	14.48	3.30* ($\pm$ .494)
	7	12.70	7.62
	14	16.26	9.91* ($\pm$ 2.71)
(autoclaved)	14	13.00	5.50* ($\pm$ 1.65)
	22	13.21	7.87
Saturated	0	11.50	6.84* ($\pm$ .247)
	7	15.00	6.00* ($\pm$ .824)
	14	18.00	4.00* ($\pm$ 1.02)
(autoclaved)	14	16.75	3.00* ($\pm$ .259)
	22	13.50	7.00* ($\pm$ 1.29)

Table 4: Mean values of decomposed plant residue on radicle length in soil.

*Significantly different from control at .05 level or better.

($\pm$) Standard deviation

INTERMEDIATE GROWTH STAGE (II)			
Moisture Level	Decomp. period (days)	Control radicle length cm.	Test radicle length cm.
Field Capacity	0	12.45	7.62
	7	14.73	7.87* ($\pm$ 2.02)
	14	11.94	4.32
(autoclaved)	14	11.00	4.00* ($\pm$ .092)
	22	12.95	4.57* ($\pm$ 1.31)
Saturated	0	14.00	9.64* ($\pm$ 1.82)
	7	13.07	6.05* ($\pm$ 2.02)
	14	12.20	5.13* ($\pm$ 2.53)
(autoclaved)	14	16.10	3.06* ($\pm$ 1.39)
	22	14.07	6.20* ($\pm$ 1.98)

Table 5: Mean values of decomposed plant residue on radicle length in soil.

*Significantly different from control at .05 level or better.

($\pm$) Standard deviation

NEARLY MATURE GROWTH STAGE (III)			
Moisture Level	Decomp. period (days)	Control radicle length cm.	Test radicle length cm.
Field Capacity	0	11.65	7.75* (± 2.19)
	7	15.75	15.24
	14	15.49	7.62* (± 1.94)
(autoclaved)	14	14.83	2.70* (± 1.06)
	22	14.22	8.38* (± .880)
Saturated	0	12.20	12.80
	7	16.70	18.53
	14	14.00	10.82
(autoclaved)	14	15.80	7.00* (± 1.27)
	22	13.01	11.40

Table 6: Mean values of decomposed plant residue on radicle length in soil.

*Significantly different from control at .05 level or better.

(±) Standard deviation

INTERMEDIATE GROWTH STAGE (II)					
Moisture Level	Decomp. period (days)	Control weight gms.	Test weight gms.	Control height cm.	Test height cm.
Field Capacity	0	4.57	3.80	30.53	25.78
	7	4.71	3.81	25.25	25.86
	14	3.90	1.00* ($\pm$.282)	27.28	19.18
(autoclaved)	14	5.00	2.77* ($\pm$.174)	28.20	27.20
	22	2.46	2.80	18.09	22.07
Saturated	0	4.90	4.52	28.70	29.30
	7	3.50	2.88	26.75	27.20
	14	4.90	2.51* ($\pm$.174)	24.00	15.83
(autoclaved)	14	3.50	1.55* ($\pm$.198)	30.00	19.81
	22	2.60	2.82	19.00	19.45

Table 8:   Mean values of decomposed plant residue on plant growth.

*Significantly different from control at .05 level or better.

($\pm$) Standard deviation

NEARLY MATURE GROWTH STAGE (III)					
Moisture Level	Decomp. period (days)	Control weight gms.	Test weight gms.	Control height cm.	Test height cm.
Field Capacity	0	3.06	2.28	21.74	20.09
	7	2.90	2.03	21.08	22.05
	14	2.42	2.17	22.61	22.35
(autoclaved)	14	2.51	2.12	24.00	26.03
	22	1.97	1.62	23.37	20.57
Saturated	0	3.25	2.92	22.75	22.80
	7	2.80	2.74	19.40	19.30
	14	2.67	2.93	23.18	23.18
(autoclaved)	14	3.40	1.82* ($\pm$1.51)	20.50	16.86
	22	2.00	1.86	25.00	17.58

Table 9: Mean values of decomposed plant residue on plant growth.

*Significantly different from control at .05 level or better.

($\pm$) Standard deviation

AREAS OF TESTING	CONTROL			LEAF LEACHATE			PLANT MACERATE		
	TEST MATERIALS								
	STAGES OF GROWTH								
	I	II	III	I	II	III	I	II	III
Germination (%)	81	83	78	60	75	80	60	50	75
Radicle Length (aqueous) cm.	3.6	4.5	6.1	3.1	4.0	7.4	3.1	4.6	5.6
Radicle Length (soil) cm.	14.5	12.5	11.7	3.6* ($\pm$.494)	6.2* ($\pm$1.90)	16.0	7.6* ($\pm$1.06)	7.1* ($\pm$1.24)	7.0
Height (cm.)	29.2	30.5	21.8	14.0	24.5	16.4	14.0	26.7	16.7
Weight (gms.)	1.43	4.57	3.06	0.48* ($\pm$.233)	3.60	3.07	0.43* ($\pm$.061)	3.75	2/18

Table 10: Mean values of leachate and macerate on germination, radicle length, and plant growth; germination is expressed as percent of control.

*Significantly different from control at .05 level or better.

($\pm$) Standard deviation

DISCUSSION

It has been shown that certain organic compounds which arise from corn plants and accumulate in the soil surrounding the roots of corn are definitely toxic to the growing corn seedling.

It was shown experimentally that by taking essentially similar healthy corn plants and exposing one group to corn extracts, the other not to extracts, and then subjecting all the plants to the same environmental conditions, defects were considerably more severe in the plants which had been exposed to extracts of leaf leachate, macerated plants, and decomposed plant residue. From the investigations, it was found that toxic substances were present in the corn plant, were released by decay as well as by leaching and maceration, and were inhibitory to corn seedlings. Even if questionable samples were eliminated, there was definite evidence that at least some inhibitors got out of corn plants by some method. These substances exhibited a wide range of activity including inhibition of seed germination and growth, discoloration and death of plant roots.

Nielsen et al. (1960) definitely demonstrated that certain crop plants contain water-soluble materials that inhibit seed germination and seedling growth of several crop plants. Taking the effects of toxicity on the corn

plant as a whole, it can be noted that corn plants are doing different things simultaneously and this affects no two groups of plants in exactly the same way.

The germination, growth inhibiting, and phytocidal effects of extracts of corn which were surveyed in this paper may have been brought about by one or more chemical substances which could come from the plants. There was no relation between the inhibiting action of the extracts and their acidity. This experiment shows that pH alone was not enough to explain the inhibition, for then the neutralization would have been much more pronounced. All this proves is that pH might contribute to inhibition, but is not its sole cause. Even though toxicity has been found to be more severe under conditions which were unfavorable for the growth of the plant, such as wet heavy soils, the opposite was found to be true in this study. This evidence was dissimilar to that of Patrick (1955) who stated that substances arising during decomposition of plant residues under conditions of high soil moisture were more toxic to plants than those formed during decomposition under normal moisture conditions. However, Patrick and Koch (1958) found that for temporary periods following rain or irrigation, favorable conditions may stay for varying periods of time. Conditions that lead to the formation of high concentrations of toxic products may therefore be more common than is generally realized, and not necessarily confined to saturated soils. Apparently,

the toxins do not move far from the original place of pro-
duction, and the extent of root injury and the total effect
on the plant would depend on how often the growing root system
comes into contact with the plant residues when decomposition
products are toxic and also affected by the types of substance
being produced at that particular time. This may explain
some of the reasons for the inconsistent results and dis-
crepancies obtained concerning the production and existence
of substances under field and saturated conditions. The
research reported here indicates that the toxicity of ex-
tracts was variable for different groups of plants. The
results of the leaf leachates and macerations indicate
that the tissues of corn plants do contain substances having
inhibitory effects on the germination and growth of corn.
Most of the toxic substances which were obtained in the
leachates and macerations appeared during the young stage of
growth with decreasing effects following. According to
Patrick and Koch (1958), this decline or lack of toxicity
might mean that some of the specific toxic components in
the tissues of the plant may not be released at times by
leaching with water or maceration of the tissues. It is
also possible that these toxic components might occur at
stages of the plant's maturity other than those tested.
This may also hold true for the declines which appeared in
the toxicity of certain phases of the decomposition periods.

Waksman (1929), restated by Alexander (1977), found that young plants were higher in water-soluble constituents than were mature plants. The possibility therefore exists that the differences observed between plant materials are attributable to their age rather than to specific characters, since the test for toxicity depended on a water extraction. Since the compounds obtained from leaf leachates and macerations are normal constituents of the plant, it seems unlikely that they arise secondarily from the bacterial decomposition of dead plants, but they represent an actual excretion from the tissues of the living plant. Although the leaves are not a principal contributor to the toxicity, their significance must nevertheless be considered in leachates. The leachate and macerated extracts were not as toxic during the intermediate stage of growth as those from the decomposed material, indicating that leaves of corn are not the only or main toxic source.

In view of the marked changes in chemical composition of plants during various stages of growth, experiments were next carried out to determine the effect of maturity on the degree of toxicity of decomposition products. A variety of factors may have an effect on the amount of inhibition recorded. For instance, the overall results seemed to indicate that the inhibitive effects of the decomposed material result from a combination of toxins present in the plant material plus toxins produced by microorganisms that

are stimulated to grow more luxuriantly by material in the decomposed residue. Whether the toxic substances were synthesized by soil microorganisms using plant material or the breakdown products were inherent in the plant tissues was not definitely determined. The results of the work of Patrick, Toussoun and Snyder (1963) on barley and rye suggested that in many instances the inhibitive effects were probably a combination of both the synthesizing by microorganisms using plant material and the breakdown products in the tissues of plants.

Under saturated and field conditions, toxicity due to products of decomposition appeared early and was quite high for a short time. Even though germination and radicle growth inhibition existed, it did not show any tremendous inhibition with extracts from any particular growth stage of decomposed residue. Greatest inhibition was noted when young seedlings and older plants were in direct contact with soil rather than in aqueous extracts as during the germination tests. According to Wang, Yang and Chuang (1966), many substances, at least in part, are present in absorbed and bonded forms in soils, and the effect of these substances upon plant growth would not be the same in the soils as it would be in aqueous forms. The level of toxicity of these substances in soil can increase tremendously under certain conditions where most plants would be seriously retarded.

Young seedlings germinated for six days were affected with extracts from all growth stages. In some cases, root

growth was retarded and in other cases rotted and completely killed. This showed the great sensitivity of the roots to the toxic substances. Bioassay of corn seedlings consistently gave 30-70% inhibition of root growth. Patrick and Koch (1958), in their study on wheat, felt that localized zones probably occur in most soils where ideal conditions exist at least briefly, thereby exhibiting a wide range of activity, including growth inhibition, discoloration and sometimes death of the plant roots. In this study, the roots of corn seedlings were found to be most sensitive to the toxins produced by plant residues. The roots had a good chance of contacting localized zones of toxin production in the soil. Those seedlings which were not entirely killed not only showed reduction in growth, but their root tips were dark in color, indicating damage. This agrees well with the findings of Patrick and Toussoun (1965); Patrick (1971), Chou and Muller (1972). It thus seems that decomposed plant material is the primary source of toxic production in corn. The reason for the variability in accumulation of toxicity in several samples is still not very clear. It would seem, however, that toxicity may occasionally accumulate in soil under conditions where the permeation of soil by roots is more thorough than under other conditions.

In all stages of growth, extracts appeared to be greatest during the 7-14 day decomposition period. This effect exhibits a certain specificity. After 7-14 days,

decomposition of plant material by microorganisms probably occurred, which may explain the delayed toxicity of extracts during various parts of the experiment. Corn seedlings planted during this period were greatly stunted or even killed. After this great period of toxicity, 7-14 day decomposition, in most cases the toxicity would gradually decrease. Patrick (1955) stated that the length of the toxic period and rate of decline would depend on the amount of plant material present, relative number of microorganisms in the area, and length of time that favorable conditions for microbial decomposition were maintained. After all the plant residue had been completely decomposed, most of the soil toxicity would also disappear. The disappearance would partly explain the observation frequently made from previous investigations that the toxicity of agricultural soils is often greatly diminished two or three years after cropping. It would seem obvious that the toxic principles released from the corn plant must be destroyed or inactivated in the soil under favorable field conditions.

Autoclaved decomposed samples produced a very toxic, brown substance which induced darkening and necrosis of root cells and eventually caused wilting or death of many plants. This data agrees with that of Guenzi and McCalla (1962), who found that the non-autoclaved extracts of the residues inhibited seed germination and shoot growth more than autoclaved extracts in most cases, but the autoclaved extracts were

more depressive to root growth. Even though there was a slight decrease in toxicity with the nearly matured extracts, the toxicity remained relatively high throughout the experiment with most autoclaved samples, field and saturated series. It appears that the toxic substance is very stable in soil which has been sterilized by autoclaving and does not disappear as rapidly from the soil. This again may substantiate the fact that the toxic principle is destroyed in soil, probably by the activity of microorganisms since this disappearance does not take place as rapidly in sterilized soil.

Chou and Muller (1972) stated that although the chemical structure and function of humic acids in the soil are still unclear, experiments strongly suggest that humic acid may interact with natural growth inhibitors, such as phenolic compounds in the soil, bringing about a decreased activity of the toxins. This may also be another possible reason for the gradual decline in toxicity as the decomposition periods were carried out. According to McNaughton (1968), phenolics are the principal active agents in preventing seed germination, but it is either other substances or phenolics at quite low concentrations that are responsible for the inhibition of seedling growth. In view of this fact about phenolic acids, samples were taken of plant material during the intermediate stage of growth since high levels of inhibition seem to have appeared more frequently at this stage; these samples were then prepared for isolation

and identification of the toxic principle. At this point
in the research, the toxic substance was presumed to be a
type of phenolic acid. Both acid and alkaline hydrolysis
showed signs of a toxic substance. More was found in the
alkaline hydrolysis with a small amount in the acid hydroly-
sis. Most of the toxins were detected in the autoclaved
alkaline hydrolysis. This result agrees well with Guenzi
and McCalla (1966), whose results indicated that 2n NaOH
at room temperature hydrolyses the ester linkage, which
suggests that the majority of the toxic substances exist
in a combined form in the plant. Only small amounts of phe-
nolics, if any, probably exist as free acids or as glycosides
(hydrolyzable by 2n HCl). If the alkaline portion hydrolyses
the ester linkage, then it could be assumed that most of
the toxic material in corn residue was linked in this manner.
However, since autoclaving increased toxic concentration,
another type or types of linkage must be involved. The
mechanisms involved in the cleavage of the ester linkage was
not investigated. Guenzi and McCalla (1966) identified
p-coumaric, p-hydroxybenzoic, syringic, and ferulic acids
in toxic extracts of stubble mulched fields. Two similar
phenolic acids were detected in this study: those of ferulic
and syringic acids. There can be, however, no certainty
that the compounds isolated are in fact identical with those
which arise from normally growing corn plants. It should
be borne in mind, however, that at least one of the toxic

agents, ferulic acid, is believed to be a normal constituent of the corn plant.

Bonner (1960) found certain phenolic acids unstable in soils incubated at room temperature and with a water content approximating field capacity, but stable in soil which had been sterilized by autoclaving. This agrees somewhat with the data presented here and further explains the slow decline in toxicity levels of decomposed extracts.

Knoesel (1959) found that phenolic acids in soil caused a substantial shift of microbiological balance. Thus it is possible that phenolic acids affect plant growth through their influence upon soil microorganisms. When soil humic substances are degraded by means of chemicals, they yield a large number of phenolics. Such degradation by microbial activity could be expected to take place also in soil. Therefore, it would appear likely that simple phenolic acids would be rapidly utilized by the soil population or would be protected by or linked into humic complexes.

There is little doubt that substances toxic to living corn plants can come from excretions of organs of plants or may be given off from plant residues during their decomposition. The length of time these substances remain in the soil and their relative importance in inhibiting the growth of corn plants are at present very controversial. The answers are difficult to determine accurately and would vary with the many factors which may be different for each field.

Although the results from these tests are not entirely con-
clusive, it is evident that decomposition of corn residues
under natural field conditions is at times accompanied by the
formation of phytotoxic substances. It is believed that
these toxins may perform a very important role in the field
as the primary cause of some root rots and in making plants
more susceptible to attack by organisms not normally
regarded as pathogenic. Plant toxins' overall ecological
significance needs further investigation to further determine
various morphological and physiological changes that toxic
substances induce in the plant.

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The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

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