ALLELOPATHIC POTENTIAL
OF
CYPERUS ROTUNDUS L.

BY

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Dedicated
In
Loving Memory
To
my grandmother, Mae Bolt Charles
from whom I learned my
love for plants.

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throughout his college years made his educational dreams a reality. The author also wishes to express his appreciation, love and devotion to his wife, Phyllis Anne Robinson Gilreath, for her unselfish assistance and constant devotion through their graduate years.

Last, but not least, the author wishes to acknowledge all the little people who make life worth living . . . thanks Horatio, Charlie and Susie.

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Allelopathic Potential of
Cyperus rotundus L.

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The allelopathic effects of leachates from Cyperus ro-
tundus L. (purple nutsedge) plants and tissue residues were
studied in greenhouse sand culture experiments at Gainesville,
Florida. Cucumber (Cucumis sativus L.), lettuce (Lactuca
sativa L.) and tomato (Lycopersicon esculentum Mill.) were
used as bioassay plants. Growth of bioassay species was re-
duced by leachates from growing nutsedge plants, decaying
leaves and tubers with the greatest reduction from growing
nutsedge plants. A seasonal variation in results was noted
with maximum growth reduction occurring in fall and winter
studies.

Density of C. rotundus in a field was correlated nega-
tively with density of Richardia scabra L. and positively

correlated with densities of Euphorbia hirta L. and Geranium carolinianum L.

A study was conducted to evaluate tubers and CH_3OH extracts of tubers as phytotoxins under field conditions. Bioassay crops were cucumber, tomato, radish (Raphanus sativus L.) and lettuce. Plant density and weight were also obtained for 7 weed species. Soil incorporation of nutsedge tuber extract reduced the no. of cucumber, Eleusine indica (L.) Gaertn. and Digitaria sanguinalis (L.) Scop. plants as compared to the no. in the control treatment. Nutsedge tubers incorporated into the soil (Aeric Haplaquod) reduced the weight of Raphanus raphanistrum L. plants relative to the control.

Laboratory studies were conducted to determine if purple nutsedge tubers contained an allelopath and to chemically separate and characterize the compound(s) in tubers. Cucumber seed germination bioassays indicated the presence of a growth inhibitor in CH_3OH extracts of purple nutsedge tubers and the presence of an inhibitor in CH_3OH extracts of soil in which purple nutsedge had previously grown. The allelopath was shown to be heat stable and water soluble with an approximate molecular weight of 250 amu ($\pm$ 100 amu) as determined by gel chromatography.

Mass spectrometric analysis of a combined sample of the 3 most active separates obtained by gel chromatography suggested constituents included protocatechuic and linoleic acids.

Further separation of the allelopathic compound(s) from interfering compounds was attempted by high pressure liquid chromatography preceded by a separation procedure based on elution by a solvent sequence of increasing polarity. The allelopath consisted of 2 or more compounds, one of which was low in polarity, while the other was fairly polar. Attempts to further separate these components by high pressure liquid chromatography were not successful. It is suggested that the allelopath may actually be a complex of compounds that are water soluble, heat stable and have molecular weights of approximately 250 $\pm$ 100 amu.

INTRODUCTION

Cyperus rotundus L. (purple nutsedge) is considered to
be the world's worst weed. It is often found as sizeable in-
festations in vegetable and agronomic fields. Changes in
cultural practices and the increased use of herbicides which
reduce the number of controlled weeds and thus decrease their
competitive ability have resulted in purple nutsedge and oth-
er "resistant" species infesting larger acreages (60).

C. rotundus may be controlled to varying degrees by cul-
tural and chemical means. Both methods have limitations.
Cultural control results in incorporation of large quantities
of plant tissues into the soil and can lead to larger infes-
tations as tuber chains are broken, releasing tubers from
dormancy imposed by apical dominance, and tubers are spread
about the field (46). Chemical control is limited by the
availability of herbicides which are both effective against
nutsedge and registered for use on a given crop. Herbicides
which are effective against nutsedge are often phytotoxic to
the crop or have residual periods which determine what crops
can be grown after application of the compound. This is es-
pecially true with vegetable crops as the choice of compounds
is often not as great as with agronomic crops. Chemical and
cultural control both eventually result in addition of large
quantities of plant debris to the soil environment. These
tissue residues might result in a significant yield reduc-
tion for the grower if they release allelopathic compounds.

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Interference with plant growth by competition and production of allelopathic compounds has been ascribed to many plants including annual crops, trees and weeds (1, 2, 6, 12, 18, 22, 23, 24, 34, 37, 40, 48). Competitive effects of C. rotundus are well known (58, 59, 60), but less is known about its allelopathic potential. Most information which is available concerns growth reductions due to decaying tissues (10, 16) and water extracts of tubers (45) which may involve anaerobic decay by-products. None of this information proves that the allelopathic compound is produced directly by C. rotundus plants.

The experiments reported here were designed to evaluate the allelopathic potential of growing and decaying C. rotundus, determine whether its cultural and chemical control poses a significant problem with regard to possible allelopaths released by decaying tissues, and to chemically characterize the compound(s) involved.

REVIEW OF LITERATURE

Many plants have been shown to produce chemicals that
will reduce the growth and development of other plants.
These compounds are called allelopaths and the chemical in-
teraction between plants is allelopathy. The term "allelo-
pathy," originally coined in 1937 by Molisch (31), referred
to both beneficial and adverse chemical interactions between
plants. It has since been modified by Rice (43) to pertain
only to adverse chemical effects. Allelopathic interactions
are often thought to be restricted to higher plants, but
they may also involve lower plants and microorganisms, both
alone and in various combinations. An allelopathic effect
is dependent upon the addition of a chemical compound to the
environment by a plant; whereas, competition involves the re-
moval of something from the habitat. Thus, one should think
of allelopathy and competition as being 2 distinct phenomena
under the general heading of interference.

The earliest known written reference to allelopathy is
contained in a 300 year old Japanese document (44). It states
that water washings of leaves of <u>Pinus</u> <u>densiflora</u> Sieb. and
Zucc. were injurious to crops growing under this tree. The
classic example of allelopathy is the wilting of tomato plants
grown in close proximity to black walnut trees (<u>Juglans</u> <u>nigra</u>
L.) (6). Most of the scientific evidence for allelopathy was
not obtained until the 20th century with the vast majority of

the information appearing after 1960 (44). Thus, the study of allelopathy is a relatively young discipline.

Allelopaths have been found in all plant parts, excluding the hypocotyl. Roots have been shown to contain allelopaths which in some cases, such as with black walnut trees (26), require root contact between 2 species, while in other instances allelopathic root exudates have been produced (5).

Bode (4) demonstrated inhibitory activity of leaf leachates from wormwood (Artemisia absinthium L.). Allelopathic activity of leaf and stem residues has been observed for many plants, including wheat straw (Triticum spp. L.) (27), wormwood (11), timothy (Phleum pratense L.), corn (Zea mays L.), rye (Secale cereale L.), and tobacco (Nicotiana tabacum L.) (41). Still et al. (49) found that extracts of silver maple (Acer saccharinum L.) bark contained a phenolic inhibitor.

Flowers have also been shown to release allelopaths (43). Annual sunflowers (Helianthus annuus L.) produce floral compounds which are toxic to their own and other seedlings. As a result, in old-field succession, needlegrass (Aristida oligantha Michx.) was established as the dominant species (61).

Harborne and Simmonds (13) found catechol in the fruit of Citrus spp. L. A number of researchers have reported germination inhibitors released by various seeds (12, 18, 51).

Allelopaths are released to the environment by 5 main means: 1) root exudation (5), 2) direct tissue contact (26), 3) leachates from various tissues (4, 11, 27), 4) volatilization (33, 34, 35, 36, 37, 38), and 5) through decay (41, 48).

Rice (43) classified allelopaths in 15 categories as follows: 1) water soluble organic acids, straight-chain alchohol, aliphatic aldehydes, and ketones, 2) simple unsaturated lactones, 3) long-chain fatty acids, 4) naphthoquinones, anthraquinones, and complex quinones, 5) terpenoids and steroids, 6) simple phenols, benzoic acid, and various derivatives, 7) cinnamic acid and derivatives, 8) coumarins, 9) flavonoids, 10) tannins, 11) amino acids and polypeptides, 12) alkaloids and cyanohydrins, 13) sulfides and mustard oil glycosides, 14) purines and nucleosides, and 15) miscellaneous compounds, including abscisic acid. The most frequently identified allelopaths produced by higher plants have been phenols and benzoic and cinnamic acids and their derivatives (43).

Allelopathy is important not only with regard to direct effects on higher plants, but also through indirect effects due to changes in the soil microbial population, resulting in alterations in the nutritional status of the soil and unfavorable shifts in the microflora. Important examples are changes in the population of nitrifying bacteria, Nitrosomonas spp. and Nitrobacter spp. A decrease in their population would result in a decrease in nitrate (NO_3^-) nitrogen available for plant growth, and an increase in ammonium (NH_4^+) nitrogen. Plants are generally able to utilize either of these ionic forms of N; however, some plant species achieve better growth with one form than with the other (56). Thus, the negative influence of an allelopathic agent on nitrifying

bacteria might be manifested as a nutritional disorder of the affected plants or reduced growth. The inhibition of nitrification by allelopathy has been reviewed by Rice (43). This inhibition is widespread, and occurs as a result of forests (7, 17, 21), crop plants (8, 28, 30, 32, 54) and weed species, both broadleaved and grasses (29, 42).

C. rotundus, a sedge, belongs to the family Cyperaceae. It can be distinguished in the field from C. esculentus L. by the production of tuber chains along stolons, whereas, C. esculentus produces stolons which terminate in tubers (9). In addition, these species have different geographical limitations. C. rotundus is restricted to tropical, subtropical and temperate climates where the soil seldom freezes, and C. esculentus can thrive in cooler regions (15, 50).

The principal method of propagation of C. rotundus is by basal bulbs and tubers (53). The importance of reproduction by seed is questionable as conflicting results in seed germination have been reported (55). The reproductive capacity of C. rotundus tubers is quite high, i.e. up to 1,200 new shoots and tubers from 1 tuber in 20 weeks. The underground structures generally weigh 4 to 5 times more than the foliage (14). Betria and Montaldi (3) observed that maximum tuber formation in Argentina occurred in fall and winter and dropped to a minimum in spring and summer.

Allelopathic information on C. rotundus is limited to decaying residues and tuber extracts (10, 16, 45). Friedman and Horowitz (10) demonstrated a reduction in radicle length

of barley (<u>Hordeum</u> <u>distichum</u> L. cv. 'Esperanza'), black mustard (<u>Brassica</u> spp. L. cv. 'Alsace') and wheat (<u>Triticum</u> spp. L. cv. 'Dwarf 68') when seeds were germinated in contact with water extracts of tissues of bermuda grass (<u>Cynodon</u> <u>dactylon</u> L.), <u>C</u>. <u>rotundus</u> and johnsongrass (<u>Sorghum</u> <u>halepense</u> L.), which had been allowed to decay in soil for 2 and 4 months. In a subsequent experiment, they employed the same technique but varied the duration of incubation for decay and the level of plant material in soil. They observed phytotoxic effects on barley (cv. 'Esperanza') for these same 3 species, both when the barley was sown directly in the soil and when a barley radicle length test of an ethanolic extract of soil was used (16).

The existence of natural phytotoxins in <u>C</u>. <u>rotundus</u> tissues was established by Singh (45). He used water extracts of tubers as the imbibition liquid for seed of a number of crops. He observed a reduction in percentage germination of seed of some crops, including peanuts (<u>Arachis</u> <u>hypogaea</u> L.), sesame (<u>Sesamum</u> <u>indicum</u> L.) and cowpeas (<u>Vigna</u> <u>unguiculata</u> (L.) Walp.). In addition, the percentage emergence and seedling length of these same crops and cotton (<u>Gossypium</u> spp. L.) were reduced.

Teo et al. (52) investigated bud inhibition of <u>C</u>. <u>rotundus</u> tubers. Using methanol, they extracted an inhibitory substance, referred to as inhibitor β , from tubers in the acidic ether fraction of the methanol. This substance

inhibited spouting of excised buds of C. rotundus tubers
and wheat coleoptile elongation. Thin layer chromatography
studies suggested inhibitor β consisted primarily of pheno-
lic compounds with abscisic acid (ABA) as a minor constit-
uent. Torrey (57) confirmed the presence of ABA in the in-
hibitor complex and identified cinnamic acid and meta- and
para-coumaric acid as additional constituents. Although
ABA was considered as the most potent component of the com-
plex, not all of the inhibitory action of the complex was
attributable to ABA. Although a number of compounds present
in C. rotundus have been isolated and identified, none has
been shown to be the allelopath responsible for the afore-
mentioned growth reductions.

Komai and Ueki (19) isolated polyphenols from C. ro-
tundus and identified them as catechol tannins, consisting
primarily of leucoanthocyanin, which existed mainly as a
form of leucocyanidin. The leucocyanidin content was high
in rhizomes, seedheads, basal bulbs, and mature tubers with
the highest quantities in the basal bulbs and mature tubers
(0.83 mg/g of mature tuber on a dry weight basis).
Phenols are known to serve a regulatory role in IAA (in-
doleacetic acid) oxidation by IAA oxidase. Monophenols
serve as cofactors for the enzyme, enhance the oxidation of
IAA and result in inhibition of growth. O-diphenolics act as
inhibitors of IAA oxidase activity (20). Thus, leaching of
phenols from C. rotundus tissues could have an influence on
the IAA oxidase activity of other biological systems in the

zone of influence of these leachates. In addition to possible effects on IAA oxidase activity, phenolics, in general, are known to often be inhibitory to higher plants and microbes.

Greenhouse Studies: Experiment I

Greenhouse studies were conducted from 1978 to 1980 to determine if $\underline{C}$. $\underline{rotundus}$ plants and residues reduced the growth of selected bioassay species by release of allelopathic compounds. A glass greenhouse located at the University of Florida Horticultural Research Unit near Gainesville, Florida was employed. All $\underline{C}$. $\underline{rotundus}$ plants and residues used in this study were obtained from clonally propagated material. The experimental design was a randomized complete block with 4 replications. Each replicate consisted of a series of glazed clay crocks arranged in a stair-step fashion (43) with each upper crock draining into its respective lower crock which in turn drained into a foil covered 0.946 liter jar. The upper crocks measured 21 cm diameter x 23 cm depth. The lower crocks were 24 cm diameter x 26 cm depth. One drain hole was provided at the bottom front of each crock. A rubber stopper fitted with a foil wrapped glass tube was inserted and the interior of the drain hole was packed with glass wool. The medium employed was sterile silica sand with a particle size range of 0.4 to 0.55 mm. Nutrition was supplied by daily watering with half-strength Hoagland's No. 2 solution by applying 1 liter to each upper and lower crock. Leachate collected in the catch jar was divided into 2 equal portions and poured back into its respective upper and lower crocks to

maintain uniform nutrition. The various treatments applied to the upper crocks are listed in Tables 1 and 2. Each upper and lower crock was seeded with various bioassay crops (Table 3). Once a stand was established, all crops were thinned to a final stand of 3 plants per crock. Before each planting the sand in the crocks was screened to remove organic matter and leached thoroughly with water. The glass catch jars were thoroughly washed before reusing. Each crock received the same treatment in each successive planting. Data were collected for various growth parameters such as percent emergence, emergence rate, plant diameter, length and fresh and dry weights and leaf area (lettuce). Leaf area was measured with a Model L1-300 Li-Cor portable area meter. The average number of days required for emergence (emergence rate) was calculated by using the following equation modified from Maguire (25).

$$\frac{\sum_{n=1}^{c} X_n(n)}{z}$$

where n is the day on which emergence counts are made, X_n is the number of plants emerging on day n, c is the duration of the emergence test in days and z is the total number of seedlings emerging over the entire period. Data were analyzed statiscally using the analysis of variance with treatment means ranked by Duncan's new multiple-range test (47).

Table 1. C. rotundus material applied to upper crock seed medium for cucumber plantings, 1978-80.

Tissue treatment	Rate (g or no./crock)			
	Planting number			
	1-2	3	4	5-8
Check	0	0	0	0
Decaying tubers[z] (g)	14 28	14 28	14 28	28
Decaying leaves[z] (g)	10.5 21	10.5 21 28 56	10.5 28 56	28
Sprouting tubers[y] (no.)	40	40	--	--
Growing plants (no.)	25 50	25 50	25 50	50

[z]Decaying tubers and leaves were coarsely chopped and incorporated into the top 5 cm of the medium before planting.

[y]Sprouting tubers were not included in plantings 4-8.

Table 2. _C. rotundus_ material applied to
upper crock seed medium for lettuce
and tomato plantings, 1979-1980.

Tissue treatment	Rate/crock
Check	0
Decaying tubers[z] (g)	28
Decaying leaves[z] (g)	28
Growing plants (no.)	50

[z]Decaying tubers and leaves were coarsely
chopped and incorporated into the top 5
cm of the medium before planting.

Table 3. Planting and harvest dates and seeding rates for
bioassay crops in greenhouse experiments, 1978-1980.

Crop	Planting no.	No. of seed planted	Date planted	Date harvested
Cucumber	1	20	5-19-78	6-25-78
	2	10	5-30-78	6-29-78
	3	10	6-30-78	8-01-78
	4	10	9-19-78	12-04-78
	5	10	1-11-79	3-26-79
	6	10	9-13-79	10-23-79
	7	10	10-29-79	12-10-79
	8	10	1-18-80	3-07-80
Lettuce	1	20	1-28-79	4-03-79
	2	20	4-05-79	5-16-79
Tomato	1	25	4-09-79	5-07-79
	2	25	5-10-79	6-11-79

Field Studies: Experiments II and III

Experiment II was conducted to evaluate the effect of a solid stand of nutsedge on the no. of plants of each weed sp. present in 929 cm^2 areas. Counts were made every 1.37 m in a field infested with C. rotundus located in block 6 of the Horticultural Research Unit and beginning at the S.W. corner of the field. The soil was a Wauchula f.s. (Aeric Haplaquod). The field had been disced on March 4, 1979, and the weed counts were made April 19-21, 1979. The data were subjected to correlation analysis to determine whether there was any correlation between densities of C. rotundus and weed sp. observed.

Experiment III was conducted to evaluate tubers and CH_3OH extracts of tubers as phytotoxins under field conditions. Four treatments were replicated within 5 randomized complete blocks with each plot measuring 91.4 cm x 91.4 cm. The experimental area was prepared by double discing, applying 1210 kg/ha of 6-8-8 fertilizer with micronutrients (F.T.E. 503) and rototilling 15 to 20 cm deep on September 13, 1979. Treatments were as follows: 1) unscreened soil (Wauchula f.s. (Aeric Haplaquod)), 2) soil screened free of tubers and plant debris (123 g/plot), 3) screened soil with the removed 123 g of tubers chopped in a blender, then incorporated into the top 5-7.6 cm of the soil by raking, and 4) screened soil with the removed 123 g of tubers extracted

with CH_3OH and the extract sprayed on the soil and incorpor-
ated. The CH_3OH extract was prepared by chopping the tubers
in a blender, performing a batch extraction of 614 g of tu-
bers with 4 liters of CH_3OH and filtering through Whatman No.
3 filter paper in a Buchner funnel provided with suction.
Each sprayed plot received 800 ml of extract with a compressed
air herbicide sprayer operated at 2.8 kg/cm^2 and incorporated
by raking. The tuber and extract treatments were applied
September 23, 1979. On September 24, 1979, all plots were
raked smooth to remove any small weed seedlings, then seeded
to the following crops in rows 12.7 cm apart: 1) 50 seed of
'Poinsett' cucumber (Cucumis sativus L.), 2) 100 seed of
'Walter' tomato (Lycopersicon esculentum Mill.), 3) 40 seed
of 'Champion' radish (Raphanus sativus L.), and 4) 100 seed
of 'Grand Rapids' lettuce (Lactuca sativa L.). A fiberglass
roof was placed over the plots to reduce leaching losses from
September 25, 1979, through October 5, 1979. Irrigation was
provided by applying 2.5 cm of water weekly. Percent emer-
gence and rate of emergence were calculated daily for 8 days.
The weeds and crops on the east one-half of each plot (91.4
cm x 45.7 cm) were harvested October 12-15, 1979, with number
of individuals per species and weight recorded. This one-half
of the plots was then replanted with the same crops and seed-
ing rates. An early freeze killed this planting before har-
vest data could be collected. The west one-half of each plot
was harvested November 26-28, 1979, recording the same data
as for the east one-half.

Data were subjected to analysis of variance and means
between treatments compared with Duncan's new multiple-range
test.

Laboratory Studies

Bioassays--Experiments IV and V

Experiment IV was a series of seed germination bioassays
of CH_3OH extracts of C. rotundus tubers and leaves to deter-
mine if any allelopathic compounds were present in these tis-
sues. Each bioassay was set up as a completely random design
with 4 replications. The first bioassay was begun July 22,
1978, using freshly collected tissues with treatments as fol-
lows: 1) extract of 28 g (per replicate) fresh leaves, 2) ex-
tract of 28 g (per replicate) fresh tubers, 3) CH_3OH check,
and 4) check without CH_3OH. Batch extracts were prepared by
grinding 28 g of tissue in 100 ml of cold CH_3OH in a commer-
cial Waring blender and allowing the extract to stand for 1
hour. The extract was then vacuum filtered through Whatman
No. 1 filter paper in a Buchner funnel, and the filtrate was
flash evaporated at 37° C under vacuum to a final volume of
50 ml. Ten milliliters of this concentrate was applied to
a 9 cm disc of Whatman No. 3 filter paper in a 9 cm petri
dish and evaporated to dryness in a vacuum desiccator. The
CH_3OH check consisted of filter paper to which 10 ml of CH_3OH
was added, then dried in a vacuum desiccator. The check with-
out CH_3OH was dry filter paper. Ten 'Poinsett' cucumber seed
were placed in each dish with 4 ml distilled water for imbi-
bition and incubated at 25° C $\pm$ 2° C for 9 days. Germination

counts were made daily with germination being defined as
follows: 1) emergence of radicle greater than 3 mm, and
2) complete emergence of both radicle and plumule. The
average number of days required to germinate (germination
rate) was calculated using the equation described in Experiment I.

The second bioassay was conducted for 10 days (August
8-17, 1978) using freeze-dried tubers collected in February,
1977. The treatments were as follows: 1) 0.25 g tubers,
2) 0.50 g tubers, 3) 2.5 g tubers, 4) CH_3OH check, and 5)
check without CH_3OH. The extraction procedure was as described for the first bioassay except 25 ml of CH_3OH was used
per replicate. The extract was filtered and reduced to 5 ml
by flash evaporation at 38° C. The rest of the procedure was
the same as that used in the first bioassay. Each petri
dish was seeded with 10 'Poinsett' cucumber seeds and incubated at 25° C $\pm$ 2° C. Data collected included daily
germination counts, germination rate and radicle lengths.

The data for both bioassays were subjected to analysis
of variance with treatment means ranked by Duncan's new
multiple-range test.

Since CH_3OH extracts of tubers reduced germination and
growth of cucumber, a seed germination bioassay study
(Experiment V) was conducted on CH_3OH extracts of soil and
leachates of sand and soil in which nutsedge had grown. The
bioassay was of 1) a CH_3OH control, 2 and 3) CH_3OH leachates
of screened sand or soil, respectively, (Wauchula f.s. (Aeric

Haplaquod) with 1.7% organic matter (o.m.)) in which 40 nut-
sedge plants had grown, 4) extracts of screened Wauchula f.s.
in which nutsedge had previously grown, and 5) extracts of
screened Wauchula f.s. in which nutsedge had not previously
grown. Leachates were obtained by leaching the medium in
each crock (4 crocks per treatment) daily for 4 days with
CH_3OH beginning with 1 liter and reusing that which was re-
covered each day. The leachates were filtered and flash
evaporated to 24 ml. Four milliliter aliquots were added to
petri dishes and dried under vacuum. Soil extracts were pre-
pared by shaking 150 g of soil in 150 ml CH_3OH for 6 hours.
The extract was then filtered and flash evaporated to a final
volume of 15 ml. Ten milliliter aliquots were added to petri
dishes and dried under vacuum. Twenty-five seeds of either
'Walter' tomato or 'Grand Rapids' lettuce were added to each
dish along with 4 ml distilled water. The experimental design
was a randomized complete block with 4 replications. Seed
were incubated at 24° C $\pm$ 2^O C starting on March 28, 1979,
for 8 days (lettuce) or 11 days (tomato). Data collected
were percent germination, germination rate, radicle length
and seedling fresh weight. Data were subjected to analysis
of variance and treatment means ranked by Duncan's new mul-
tiple-range test.

Heat Stability--Experiment VI

The heat stability of tuber extract was determined by
placing 1 ml of alcohol-free extract of C. rotundus tubers

in an oven maintained at 60° C for 1 hour. The extract was obtained as described in Experiment IV, and the CH_3OH was removed by flash evaporation at 37° C. The extract was resolubilized in 10 ml CH_3OH. Two milliliter aliquots were applied to Whatman No. 3. paper discs in four 9 cm petri dishes and dried in a vacuum desiccator. Additional treatments consisited of an unheated extract of the same concentration and a control. Each petri dish received 4 ml distilled water and 10 'Poinsett' cucumber seeds on April 21, 1980. Seed were germinated for 7 days at 28° C $\pm$ 2° C with dishes (replicates) arranged in a completely random design. Data for percent germination, ADG (germination rate) and root length were subjected to analysis of variance and the treatment means ranked by Duncan's new multiple-range test.

Water Solubility--Experiment VII

Experiment VII was a water solubility study using 1 ml of alcohol-free conc. extract of $\underline{C}$. $\underline{rotundus}$ tubers, prepared as described in Experiment VI. Twenty milliliters water were added to the 1 ml of extract, then the solution was agitated, allowed to stand for 24 hours, thoroughly agitated again, filtered through Whatman No. 3 filter paper, and 5 ml aliquots added to each of 4 petri dishes fitted with No. 3 Whatman filter paper discs. A check treatment was included. The discs were dried in a vacuum desiccator. Four milliliters distilled water were added, and each dish seeded with 10 'Poinsett' cucumber seed on March 13, 1980. Seed were germinated for 7

days at 27° C $\pm$ 2° C with dishes arranged in a completely random design. Data for percent germination, germination rate and radicle lengths were subjected to analysis of variance and the treatment means ranked by Duncan's new multiple-range test.

Gel Chromatography--Experiment VIII

Tuber extracts, obtained as described in Experiment IV, were gel chromatographed to determine the approximate molecular weight (m.w.) of the unknown growth inhibitor. Sephadex LH-20 gel was solvated with absolute CH_3OH and placed in an 11 mm x 90 cm Pharmacia glass column with a volume of 137 ml. A number of preliminary chromatographs were performed. In these preliminary experiments, maximum inhibitory activity was found in the 96 to 125 ml portions of the elution volume, varying somewhat with flow rate. In all chromatographs, CH_3OH was the solvent and eluant. The gel column was set up with a CH_3OH reservoir attached so as to provide a flow rate of 0.060 ml/min. A solution of standards was prepared by combining 5 ml aliquots of solutions of varying concentrations of polyethylene glycol (PEG) 6000, PEG 1000, PEG 400 and $CaCl_2$ (m.w.= 110.99 amu (atomic mass units)). Peg 6000 was used because it is well beyond the molecular exclusion limit of the LH-20 gel in CH_3OH (4000 amu) and should elute in the void volume (V_o) of the column. The remaining compounds provide 3 additional molecular weight points. One ml of the prepared extract or 1 ml of the previously mentioned solution of standards of known molecular

weight was introduced to the top of the column and a Bio-Rad
Laboratories automatic fraction collector was attached to
the outlet tube. The eluant was collected as 2.4 ml frac-
tions until 135 fractions had been collected. If the intro-
duced sample was tuber extract, a bioassay was conducted by
pouring each fraction into a petri dish, drying under vacuum,
adding 4 ml distilled water and 10 'Poinsett' cucumber seeds
and germinating for 7 days at 27° C $\pm$ 2° C. Datum collected
was radicle length. If the sample was a solution of stan-
dards, the refractive index of each fraction was determined,
using a Bausch and Lomb refractometer. Graphs were con-
structed of elution volume (V_e) vs. log molecular weight
with elution peaks determined by refractive index (R.I.)
and V_e vs. percent inhibition of cucumber root growth (rela-
tive to control), using average values obtained in 2 chroma-
tographic separations. These graphs were used to approxi-
mate the molecular weight of the unknown.

Mass Spectrometric Analysis--Experiment IX

A fresh sample of C. rotundus tuber extract was obtained
as described in Experiment IV and was chromatographed on the
gel column. The 3 consecutive fractions which had shown the
most inhibitory activity were analyzed by mass spectrometry
in order to determine the identity of the unknown inhibitor.
These fractions were combined, reduced in volume to 2 ml by
vacuum desiccation, and methylated with diazomethane. The
methylated sample was chromatographed on a gas liquid

chromatograph to further separate the inhibitor from additional compounds. The sample was then passed through a mass spectrometer, and the results analyzed.

Mass spectrometric analysis suggested 2 possible compounds in the inhibitory fraction: protocatechuic acid (3, 4-dihydroxy benzoic acid) and linoleic acid. Therefore, a cucumber seed bioassay with 4 replicates arranged in a completely random design was conducted using the following concentrations and combinations of compounds: 100, 500, 1000 and 5000 ppm of a) protocatechuic acid, b) linoleic acid, and c) linoleic and protocatechuic acids. Ten 'Poinsett' cucumber seeds imbibed with 4 ml of water were germinated at 27° C $\pm$ 2° C for 7 days. Germination counts were recorded daily. Data subjected to analysis of variance with treatment means ranked by Duncan's new multiple-range test included percent germination, germination rate and root length.

High Pressure Liquid Chromatography--Experiment X

Experiment X was conducted to improve chemical separation of the allelopaths from associated compounds. An extract of 20 g of fresh $\underline{C}$. $\underline{rotundus}$ tubers in 100 ml CH_3OH was prepared and filtered in the usual manner. The CH_3OH was removed by flash evaporation at 37° C until a dry residue remained. This residue was resolubilized with 2 ml methylene chloride (CH_2Cl_2). One milliliter of the resulting extract was loaded (by using a glass syringe) onto a silica

cartridge (Sep-pak (Waters Associates, Framington, Mass.))
prewashed with 5 ml CH_2Cl_2. A series of 5 ml aliquots of
organic solvent mixtures were used to remove contaminating
compounds from the silica cartridge (Sep-pak) by washing.
A flow rate of approximately 5 ml/min of aliquot was used
throughout. Five aliquots of CH_2Cl_2 were followed sequen-
tially by 5% (v/v) ether in CH_2Cl_2, 5% ethyl acetate in
CH_2Cl_2, 5% acetone in CH_2Cl_2, 4% CH_3OH in CH_2Cl_2, 10, 20 and
60% CH_3OH in CH_2Cl_2 and 2 aliquots of 100% CH_3OH. Thus,
compounds were eluted by a solvent sequence of increasing
polarity. The resulting fractions were bioassayed as pre-
viously described by collecting them in petri dishes as they
eluted. Also included were a control and the complete un-
fractionated extract. Each dish was seeded with 10 seeds of
'Poinsett' cucumber and root lengths were measured after 6
days. The data were subjected to analysis of variance with
the model based on a randomized complete block design with 3
replications (3 Sep-paks) and means ranked by Duncan's new
multiple-range test.

The above separation procedure was repeated. The 3
replicates for each active fraction were pooled, then evap-
orated to dryness in a vacuum desiccator, resolubilized in
1 ml CH_3OH and filtered through a 20 μ (pore diameter)
Metricel filter disc. Two 200 μl samples of each fraction
which had shown activity were injected into a high pressure
liquid chromatograph (HPLC) fitted with a u Bondapak C18
column. The HPLC, a Waters Associates Model 660 solvent

programmer, was operated in the reverse phase mode with a linear curve. The mobile phase was water and CH_3OH which eluted into a Waters Associates Model 450 variable wavelength detector set at 254 nm with an attenuation range of 0.02 AUFS. Detector response was recorded with a Waters Associates Data Module. Fractions were collected from the column eluant at 1 min intervals with a Gilson Microfractionator. The HPLC program took 20 minutes to go from initial conditions of 10% CH_3OH and 90% water to final conditions of 90% CH_3OH and 10% water. The resultant fractions were bioassayed for inhibition by seed germination in the manner previously described. Ten 'Poinsett' cucumber seeds were used in each petri dish. Data for radicle lengths were analyzed for significance by analysis of variance. The treatment means were ranked by Duncan's new multiple-range test.

Greenhouse Studies: Experiment I

The stairstep arrangement of crocks employed in the greenhouse studies allows separation of allelopathic effects from those of competition. Growth reductions of cucumber growing in upper crocks receiving the nutsedge tissue treatments reflect the effects of competition and/or allelopathy, while reductions in lower crocks receiving leachates from upper crocks result from allelopathic effects of the treatments. In these studies, allelopathic effects varied with season.

First Cucumber Planting: May 19, 1978

Addition of nutsedge material to the sand of upper crocks significantly influenced emergence percentage, emergence rate and fresh weight of cucumber (Table 4). Cucumber emergence (%) was higher in upper crocks receiving the various nutsedge treatments than in the control treatment (Table 5). Emergence rate of cucumber plants grown in upper crocks was significantly decreased by the addition of both rates of growing nutsedge plants and the highest rates of decaying tubers and leaves. Fresh weight of cucumber plants grown in upper crocks was significantly reduced by both rates of growing nutsedge plants and sprouting nutsedge tubers. The greatest weight reduction over the control was 4.6 g, produced by the addition of 25 growing nutsedge plants to the

Table 4. Mean squares for the analyses of variance of the
effect of C. rotundus material in upper crock medium on
growth of cucumber planted on May 19, 1978, in upper and
lower crocks (planting number 1).

Source of variation	df	Plant emergence				Fresh wt (g/plant)	
		Total (%)		Rate (days)			
		Upper	Lower	Upper	Lower	Upper	Lower
Treatments	7	37.5**	17.4	0.32*	0.17	12.3*	3.9
Blocks	3	27.1	26.0	0.17	0.32	8.5	13.7
Error	21	11.6	36.8	0.11	0.08	4.0	3.4

*,**Significant at 5% (*) or 1% (**) probability level.

Table 5. Effect of C. rotundus material in upper crock medium on growth of cucumber planted on May 19, 1978, in upper and lower crocks (planting number 1).

| Treatment | | Plant emergence | | | | Fresh wt (g/plant) | |
| | | Total (%) | | Rate (days) | | | |
Nutsedge tissue	Rate per crock	Upper	Lower	Upper	Lower	Upper	Lower
Control	0	90.0B^z	96.2	5.2a	4.4	6.52a	5.91
Decaying tubers	14g	100.0A	98.8	4.9abc	4.6	4.43abc	3.94
	28g	96.2A	97.5	4.5c	4.7	6.54a	4.17
Growing plants	25(no.)	98.8A	92.5	4.6bc	4.4	1.92bc	5.39
	50(no.)	96.2A	97.5	4.6bc	4.3	2.85bc	6.17
Sprouting tubers	40(no.)	97.5A	98.8	4.8abc	4.4	3.21bc	5.21
Decaying leaves	10.5g	98.8A	95.0	5.1ab	4.9	3.42abc	3.64
	21.0g	97.5A	96.2	4.5c	4.6	5.64ab	5.85

^{z}Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

seed medium. Leachates from the various treatments had no effect on percent emergence, emergence rate or fresh weight of cucumber plants grown in lower crocks.

Second Cucumber Planting: May 30, 1978

Emergence of cucumbers in upper and lower crocks of the May 30, 1978, planting was not significantly affected by any treatment (Table 6). Although there were significant changes in diameter and length of cucumber plants grown in upper crocks, there were no significant differences in these param- eters for cucumber plants in lower crocks. Diameter of cucumber plants in upper crocks was increased by incorporation of 28 g of decaying tubers, but was decreased by both rates of growing nutsedge plants and sprouting tubers as compared to the control (Table 7). Cucumber shoot length was de- creased where grown with 50 growing nutsedge plants in the seed medium of upper crocks, but increased in the presence of 21 g of decaying nutsedge leaves. Dry weight of cucumber plants in upper crocks was significantly reduced over the control by the addition of 14 g decaying nutsedge tubers, both rates of growing nutsedge plants and sprouting tubers to the sand medium. None of the treatments significantly in- fluenced dry weight of cucumber plants grown in lower crocks as compared to the control; however, 10.5 g decaying leaves did result in weights higher than any of the other nutsedge treatments.

Table 6. Mean squares for the analyses of variance of the effect of C. rotundus material in upper crock medium on growth of cucumber planted on May 30, 1978, in upper and lower crocks (planting number 2).

| Source of variation | df | Plant emergence | | | | Diameter (mm/plant) | | Plant size | | | |
| | | Total (%) | | Rate (days) | | | | Length (cm/plant) | | Dry wt (g/plant) | |
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Treatments	7	56.7	33.9	0.10	0.05	5.19**	0.38	374.5**	100.0	1.40**	0.19*
Blocks	3	53.1	12.5	0.10	0.51**	0.12	0.08	227.2**	48.4	0.08	0.02
Error	21	46.1	19.6	0.12	0.09	0.19	0.18	51.4	91.0	0.17	0.07

*, ** Significant at 5% (*) or 1% (**) probability level.

Table 7. Effect of C. rotundus material in upper crock medium on growth of cucumber planted on May 30, 1978, in upper and lower crocks (planting number 2).

| Treatment | | Plant emergence | | | | Plant size | | | | | |
| Nutsedge tissue | Rate per crock | Total (%) | | Rate (days) | | Diameter (mm/plant) | | Length (cm/plant) | | Dry wt (g/plant) | |
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Control	0	90.0	100.0	4.2	3.5	5.1B^z	4.6	52.1B	49.9	1.57A	0.90ab
Decaying tubers	14g	100.0	100.0	3.9	3.7	4.8B	3.9	42.0BC	44.2	0.94B	0.62b
	28g	97.5	100.0	3.7	3.8	5.9A	4.1	50.5B	46.6	1.75A	0.67b
Growing plants	25(no.)	100.0	100.0	3.8	3.8	3.4C	4.0	43.8BC	55.2	0.69B	0.72b
	50(no.)	97.5	100.0	3.8	3.7	3.0C	3.7	34.6C	50.8	0.40B	0.58b
Sprouting tubers	40(no.)	95.0	97.5	4.0	3.6	3.3C	4.1	44.8BC	51.6	0.66B	0.75b
Decaying leaves	10.5g	92.5	95.0	3.9	3.6	5.4AB	4.5	51.0B	60.0	1.61A	1.26a
	21.0g	100.0	92.5	3.8	3.7	5.2B	3.9	67.5A	54.5	1.95A	0.67b

^{z}Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

Third Cucumber Planting: June 30, 1978

Cucumber seedling emergence (%) was not affected by treatment in either upper or lower crocks (Table 8). Emergence rate, diameter, length and dry weight of cucumber plants grown in upper crocks were significantly altered by the treatments, but the same growth parameters for cucumber plants grown in lower crocks were not significantly influenced by the treatments. Emergence rate and diameter of cucumber plants in upper crocks were reduced by the 2 rates of growing nutsedge plants in the medium (Table 9). Where cucumber plants were grown in the presence of growing nutsedge plants (both rates) and 28 and 56 g of decaying nutsedge leaves, cucumber plant length was reduced by as much as 63 cm compared to the control. Plant dry weight was also reduced by these 4 treatments and by the addition of sprouting tubers to the seed medium.

Fourth Cucumber Planting: September 19, 1978

Emergence (%) of cucumbers planted in upper crocks on September 19, 1978, was significantly influenced by the treatments (Table 10). Emergence (%) and emergence rate of cucumbers planted in lower crocks and emergence rate of cucumbers planted in upper crocks were not significantly affected by the treatments. Incorporation of 28 g of decaying nutsedge tubers into the sand of upper crocks significantly reduced emergence (%) of cucumber plants growing in them (Table 11). The remaining treatments did not significantly affect percent

Table 8. Mean squares for the analyses of variance of the effect of C. rotundus material in upper crock medium on growth of cucumber planted on June 30, 1978, in upper and lower crocks (planting number 3).

Source of variation	df	Plant emergence				Plant size					
		Total (%)		Rate (days)		Diameter (mm/plant)		Length (cm/plant)		Dry wt (g/plant)	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Treatments	9	200.3	51.1	1.01*	0.06	9.58**	0.23	2766.8**	288.9	24.10**	0.45
Blocks	3	1342.5**	100.0	2.60**	1.29**	0.89	0.75*	2555.8**	1552.5*	4.46	0.61
Error	27	127.7	51.8	0.37	0.09	0.38	0.22	525.7	389.4	3.72	0.91

*, ** Significant at 5% (*) or 1% (**) probability level.

Table 9. Effect of C. rotundus material in upper crock medium on growth of cucumber planted on June 30, 1978, in upper and lower crocks (planting number 3).

Treatment		Plant emergence				Plant size					
		Total (%)		Rate (days)		Diameter (mm/plant)		Length (cm/plant)		Dry wt (g/plant)	
Nutsedge tissue	Rate per crock	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Control	0	95.0	100.0	5.0ab^z	4.0	7.4A	6.3	125.2AB	65.5	7.90A	2.02
Decaying tubers	14g	100.0	100.0	4.6abc	4.2	6.8A	6.1	110.8ABC	54.5	5.87AB	1.59
	28g	95.0	100.0	4.2bc	4.2	7.4A	5.9	122.0AB	62.0	8.18A	1.65
Growing plants	25(no.)	95.0	97.5	3.8c	4.2	3.8C	5.7	94.8CDE	44.0	2.05CD	1.08
	50(no.)	90.0	95.0	3.9c	4.0	3.4C	5.9	75.0CDE	44.2	1.45D	1.10
Sprouting tubers	40(no.)	95.0	97.5	4.7abc	4.1	5.1B	6.0	100.5BCD	56.2	3.59BCD	1.39
Decaying leaves	10.5g	87.5	92.5	4.6abc	4.2	6.8A	5.6	114.2AB	49.8	5.64AB	1.31
	21g	90.0	95.0	4.4abc	4.1	7.5A	5.9	141.8A	58.0	7.86A	1.54
	28g	75.0	92.5	5.3a	4.1	6.6A	5.6	69.5DE	60.1	4.80BC	1.99
	56g	85.0	90.0	5.2ab	4.4	7.2A	5.8	61.5E	69.2	3.26BCD	1.82

^{z}Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

Table 10. Mean squares for the analyses of variance of the effect of C. rotundus material in upper crock medium on growth of cucumber planted on September 19, 1978, in upper and lower crocks (planting number 4).

Source of variation	df	Plant emergence				Plant size					
		Total (%)		Rate (days)		Diameter (mm/plant)		Length (cm/plant)		Dry wt (g/plant)	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Treatments	7	48.2*	10.3	0.16	0.02	5.70**	0.67*	1819.4**	1244.5	20.64**	10.87**
Blocks	3	20.8	28.1	0.37	0.03	0.04	0.82*	1234.6*	1628.0	4.73	10.32*
Error	21	13.7	18.6	0.15	0.03	0.18	0.21	295.7	592.2	4.51	2.71

*, ** Significant at 5% (*) or 1% (**) probability level.

Table 11. Effect of C. rotundus material in upper crock medium on growth of cucumber planted on September 19, 1978, in upper and lower crocks (planting number 4).

Treatment		Plant emergence				Plant size					
		Total (%)		Rate (days)		Diameter (mm/plant)		Length (cm/plant)		Dry wt (g/plant)	
Nutsedge tissue	Rate per crock	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Control	0	97.5A	97.5	4.2	4.2	6.3A	6.1a	148.8A	135.5	19.2A	16.6A
Decaying tubers	14g	100.0A	97.5	4.2	4.1	6.4A	5.7a	139.6A	120.8	18.3A	15.8A
	28g	90.0B	97.5	4.4	4.2	6.6A	5.7a	145.4A	114.8	19.4A	15.0A
Growing plants	25(no.)	100.0A	95.0	4.2	4.0	4.2B	6.0a	135.4A	126.8	17.2A	16.1A
	50(no.)	100.0A	97.5	4.0	4.2	3.5C	4.8b	93.6B	88.4	13.0B	11.6B
Decaying leaves	10.5g	100.0A	100.0	4.2	4.2	6.6A	5.6a	146.8A	147.9	19.4A	16.6A
	28g	97.5A	97.5	4.7	4.1	6.4A	5.5a	160.8A	112.5	20.0A	14.5A
	56g	100.0A	100.0	4.2	4.0	6.2A	5.5a	161.1A	127.5	19.3A	15.4A

[z] Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

emergence of cucumber plants. Diameter and dry weight of
cucumber plants grown in upper and lower crocks were signi-
ficantly influenced by treatments. Length of cucumber plants
grown in upper crocks was significantly affected by treat-
ments, but length of plants in lower crocks was not. Diam-
eter of cucumber plants grown in upper crocks was reduced by
both rates of growing plants, while diameter of plants in
lower crocks was reduced only by the high rate of nutsedge
plants. Leachates from upper crocks containing 50 growing
nutsedge plants reduced dry weight of cucumber plants grown
in lower crocks. Length of cucumber plants grown in upper
crocks was reduced by 55 cm over the control where 50 nut-
sedge plants were growing in the same crocks. Although
length of cucumber plants grown in lower crocks was not
significantly different, plants which received leachate from
50 nutsedge plants tended to be smaller.

Fifth Cucumber Planting: January 11, 1979

No significant differences were found for emergence per-
centage and rate and dry weight of cucumber plants grown in
either upper or lower crocks (Table 12). The addition of 50
growing nutsedge plants to the seed medium of upper crocks
significantly reduced diameter and length of cucumber plants
grown in upper crocks (Table 13). Diameter and length of cu-
cumber plants grown in lower crocks were not significantly
influenced by the treatments.

Table 12. Mean squares for the analyses of variance of the effect of C. rotundus material in upper crock medium on growth of cucumber planted on January 11, 1979, in upper and lower crocks (planting number 5).

Source of variation	df	Plant emergence				Diameter (mm/plant)		Plant size			
		Total (%)		Rate (days)				Length (cm/plant)		Dry wt (g/plant)	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Treatments	3	100.0	6.3	0.128	0.031	1.96**	0.07	963.1*	183.7	2.78	0.19
Blocks	3	33.3	22.9	0.002	0.188	0.59	0.44**	68.7	424.6	4.89	2.74
Error	9	66.6	17.4	0.080	0.055	0.18	0.05	249.4	185.0	1.47	2.66

*,** Significant at 5% (*) or 1% (**) probability level.

Table 13. Effect of C. rotundus material in upper crock medium on growth of cucumber planted on January 11, 1979, in upper and lower crocks (planting number 5).

| Treatment | | Plant emergence | | | | Plant size | | | | | |
| Nutsedge tissue | Rate per crock | Total (%) | | Rate (days) | | Diameter (mm/plant) | | Length (cm/plant) | | Dry wt (g/plant) | |
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Control	0	97.5	97.5	7.6	7.4	4.6A^Z	4.8	127.9a	107.9	15.4	13.6
Growing plants	50(no.)	87.5	97.5	7.3	7.2	3.2B	4.5	92.5b	93.6	13.4	13.3
Decaying tubers	28g	97.5	100.0	7.6	7.2	4.5A	4.8	115.6ab	107.3	14.3	13.7
Decaying leaves	28g	97.5	97.5	7.4	7.3	4.6A	4.8	122.2a	106.2	14.6	13.2

^{Z}Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

Sixth Cucumber Planting: September 13, 1979

Diameter, length and fresh and dry weights of cucumber plants grown in upper and lower crocks were significantly influenced by treatment, but treatment had no effect on emergence of cucumber plants grown in upper and lower crocks (Table 14). Fifty nutsedge plants growing in the medium of upper crocks and leachate from these reduced diameter, length and fresh and dry weights of cucumber plants grown in upper and lower crocks (Table 15). Leachates from upper crocks containing decaying nutsedge leaves and tubers significantly reduced fresh and dry weights of cucumber plants grown in lower crocks. Leachates from upper crocks containing decaying nutsedge leaves also reduced length of cucumber plants grown in lower crocks.

Seventh Cucumber Planting: October 29, 1979

Addition of nutsedge materials to the medium of upper crocks and leachates from these entering the lower crocks significantly influenced diameter, length and fresh and dry weights of cucumber plants planted on October 29, 1979 (Table 16). Cucumber emergence in upper and lower crocks was not influenced by treatment. The presence of growing nutsedge plants in the medium reduced diameter, length and fresh and dry weights of cucumber plants grown in upper crocks (Table 17). Leachates from growing plants and decaying leaves of purple nutsedge significantly reduced length of cucumber plants grown in lower crocks with the

Table 14. Mean squares for the analyses of variance of the effect of $\underline{C}$. rotundus material in upper crock medium on growth of cucumber planted on September 13, 1979, in upper and lower crocks (planting number 6).

Source of variation	df	Plant emergence				Plant size							
		Total (%)		Rate (days)		Diameter (mm/plant)		Length (cm/plant)		Fresh wt (g/plant)		Dry wt (g/plant)	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Treatments	3	50.0	6.2	0.17	0.004	10.1**	0.71**	1585.3**	573.2**	2669.4**	661.9**	34.1**	9.9**
Blocks	3	83.3	6.2	0.04	0.094	0.4	0.33*	71.7	165.9*	103.1	253.1**	1.0	1.4*
Error	9	44.4	6.2	0.05	0.067	0.2	0.08	87.4	30.1	29.4	25.4	0.4	0.2

*, ** Significant at 5% (*) or 1% (**) probability level.

Table 15. Effect of C. rotundus material in upper crock medium on growth of cucumber plant planted on September 13, 1979, in upper and lower crocks (planting number 6).

Treatment		Plant emergence						Plant size								
		Total (%)		Rate (days)		Diameter (mm/plant)		Length (cm/plant)		Fresh wt (g/plant)		Dry wt (g/plant)				
Nutsedge tissue	Rate per crock	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower			
Control	0	100.0	100.0	4.0	3.8	6.7A[z]	6.7A	97.7A	102.4A	76.1A	78.9A	7.2A	7.4A			
Growing plants	50(no.)	95.0	97.5	3.6	3.8	3.7B	5.6B	56.4B	74.7C	26.3B	47.5C	1.4B	3.5C			
Decaying tubers	28g	92.5	100.0	4.1	3.9	6.6A	6.2A	95.9A	97.0AB	77.4A	65.5B	7.0A	5.6B			
Decaying leaves	28g	92.5	100.0	3.9	3.8	7.2A	6.3A	94.8A	91.4B	80.1A	65.3B	7.6A	5.7B			

[z]Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

Table 16. Mean squares for analyses of variance of the effect of C. rotundus material in upper crock medium on growth of cucumber planted on October 29, 1979, in upper and lower crocks (planting number 7).

Source of variation	df	Plant emergence				Plant size							
		Total (%)		Rate (days)		Diameter (mm/plant)		Length (cm/plant)		Fresh wt (g/plant)		Dry wt (g/plant)	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Treatments	3	122.9	6.2	0.008	0.020	9.89**	1.48**	1687.6**	1070.4**	1578.3**	510.8**	16.8**	5.0**
Blocks	3	72.9	6.2	0.074	0.022	0.15	0.23	132.7	34.5	18.4	139.1	0.7	1.0
Error	9	111.8	6.2	0.050	0.026	0.11	0.06	92.0	59.5	59.9	63.4	0.8	0.7

*, **, Significant at 5% (*) or 1% (**) probability level.

Table 17. Effect of C. rotundus material in upper crock medium on growth of cucumber planted on October 29, 1979, in upper and lower crocks (planting number 7).

Treatment		Plant emergence				Plant size							
Nutsedge tissue	Rate per crock	Total (%)		Rate (days)		Diameter (mm/plant)		Length (cm/plant)		Fresh wt (g/plant)		Dry wt (g/plant)	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Control	0	100.0	100.0	4.3	4.2	$6.0A^Z$	5.6A	76.9A	72.4A	56.8A	51.7A	4.87A	4.01A
Growing plants	50(no.)	97.5	100.0	4.2	4.2	3.0B	4.2C	38.8B	32.5C	18.4B	24.2B	0.92B	1.31B
Decaying tubers	28g	87.5	100.0	4.3	4.2	6.4A	5.2B	83.0A	56.0A	63.0A	38.7A	5.53A	2.73A
Decaying leaves	28g	97.5	97.5	4.3	4.0	5.9A	5.2B	78.8A	54.0B	50.8A	40.6A	4.30A	2.98A

^{Z}Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

greatest reduction due to the leachate from nutsedge plants.
The only treatment to significantly reduce fresh and dry
weights of cucumber plants grown in lower crocks was the
leachate from growing nutsedge plants; however, there was a
trend for reduction in fresh and dry weights where receiving
leachates from decaying leaves and tubers.

Eighth Cucumber Planting: January 18, 1980

Treatment effects in the eighth planting were similar
to those in the sixth planting. Emergence of cucumbers
planted on January 18, 1980, in upper and lower crocks was
not significantly influenced by any of the nutsedge treat-
ments (Table 18). Plant diameter, length and fresh and dry
weights of cucumbers planted in both upper and lower crocks
were significantly influenced by the treatments. The pres-
ence of growing nutsedge plants in the seed medium of upper
crocks reduced cucumber plant diameter, length and fresh and
dry weights (Table 19). Leachates from growing nutsedge
plants and decaying tubers reduced diameter, length and fresh
and dry weights of cucumber plants growing in lower crocks,
while leachates from decaying nutsedge leaves only reduced
fresh and dry weights of cucumber plants.

First Lettuce Planting: January 28, 1979

Since leachates from the various nutsedge tissues did
not reduce cucumber growth in lower crocks in spring or

Table 18. Mean squares for the analyses of variance of the effect of C. rotundus material in upper crock medium on growth of cucumber planted on January 18, 1980, in upper and lower crocks (planting number 8).

Source of variation	df	Plant emergence				Plant size							
		Total (%)		Rate (days)		Diameter (mm/plant)		Length (cm/plant)		Fresh wt (g/plant)		Dry wt (g/plant)	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Treatments	3	39.6	6.2	0.11	0.10	12.46**	0.70**	1110.5**	750.2**	2215.3**	648.7**	22.83**	6.95**
Blocks	3	39.6	6.2	0.18	0.33	0.44	0.31*	69.8	53.2	74.0	25.0	1.05	0.49
Error	9	34.0	6.2	0.07	0.09	0.22	0.07	48.4	71.7	43.1	37.3	0.51	0.32

*, ** Significant at 5% (*) or 1% (**) probability level.

Table 19. Effect of C. rotundus material in upper crock medium on growth of cucumber planted on January 18, 1980, in upper and lower crocks (planting number 8).

| Treatment | | Plant emergence | | | | Plant size | | | | | | | |
| Nutsedge tissue | Rate per crock | Total (%) | | Rate (days) | | Diameter (mm/plant) | | Length (cm/plant) | | Fresh wt (g/plant) | | Dry wt (g/plant) | |
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Control	0	97.5	100.0	5.3	5.2	6.3A^z	5.9A	77.6A	79.3A	57.0A	56.2A	5.92A	5.49A
Growing plants	50(no.)	92.5	97.5	5.5	5.5	2.6B	5.1B	41.7B	48.1C	8.7B	29.2C	1.12B	2.64C
Decaying tubers	28g	100.0	100.0	5.4	5.6	6.2A	5.1B	68.6A	54.7BC	52.1A	30.0C	5.50A	2.77C
Decaying leaves	28g	97.5	100.0	5.7	5.4	5.8A	5.5A	76.0A	66.0AB	57.4A	43.2B	6.17A	3.88B

^{z}Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

summer, lettuce and tomato were tried as bioassay or indi-
cator plants.

Addition of nutsedge materials to the growing medium
significantly influenced percent emergence of lettuce seeded
in upper crocks on January 28, 1979, but had no significant
effect on either emergence rate in upper and lower crocks or
percent emergence in lower crocks (Table 20). Leaf area and
fresh weight of lettuce grown in upper and lower crocks were
influenced by the treatments. Nutsedge plants growing in the
seed medium of upper crocks reduced percent emergence of
lettuce seedlings by nearly 40% over the control, but neither
decaying tubers not leaves significantly affected percent
emergence of lettuce (Table 21). All nutsedge treatments re-
duced leaf area and fresh weight of lettuce grown in upper
crocks compared to the control, with the greatest effect pro-
duced by growing nutsedge plants in the seed medium of upper
crocks. Leachate from growing nutsedge plants significantly
reduced leaf area and fresh weight of lettuce grown in lower
crocks, but leachates from decaying tubers and leaves of nut-
sedge did not.

Second Lettuce Planting: April 5, 1979

Emergence percentage of lettuce planted in the spring of
1979 in upper and lower crocks was not significantly affected
by the treatments; however, the rate of emergence was (Table
22). Lettuce emerged faster in those upper and lower crocks
receiving the growing nutsedge plants treatment, while

Table 20. Mean squares for the analyses of variance of the effect of C. rotundus material in upper crock medium on growth of lettuce planted on January 28, 1979, in upper and lower crocks (planting number 1).

| Source of variation | df | Plant emergence | | | | Plant size | | | |
| | | Total (%) | | Rate (days) | | Leaf area (cm²/plant) | | Fresh wt (g/plant) | |
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Treatments	3	1102.1*	43.2	0.22	0.11	34876.3**	984.4*	19938813.2**	453630.3*
Blocks	3	35.4	80.7	0.21	0.06	567.0	170.6	200740.1	56065.8
Error	9	197.9	54.3	0.11	0.03	267.0	255.8	527906.6	100744.7

*, ** Significant at 5% (*) or 1% (**) probability level.

Table 21. Effect of C. rotundus material in upper crock medium on growth of lettuce planted on January 28, 1979, in upper and lower crocks (planting number 1).

| Treatment | | Plant emergence | | | | Plant size | | | |
| Nutsedge tissue | Rate per crock | Total (%) | | Rate (days) | | Leaf area (cm²/plant) | | Fresh wt (g/plant) | |
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Control	0	91.2a[z]	88.8	5.6	5.3	5340.2A	2587.4a	244.9A	117.0a
Growing plants	50(no.)	52.5b	87.5	5.3	5.6	62.7C	1773.0b	21.2C	83.8b
Decaying tubers	28g	82.5a	86.2	5.7	5.2	3079.2B	2293.4ab	154.4B	116.1a
Decaying leaves	28g	76.2a	81.2	5.8	5.4	3942.4B	2206.0ab	173.4B	111.4a

[z]Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

Table 22. Mean squares for the analyses of variance of the effect of C. rotundus material in upper crock medium on growth of lettuce planted on April 5, 1979, in upper and lower crocks (planting number 2).

Source of variation	df	Plant emergence				Plant size							
		Total (%)		Rate (days)		Leaf area (cm²/plant)		Fresh wt (g/plant)		Root length (cm/plant)		Dry wt (g/plant)	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Treatments	3	270.8	329.2	7.8**	1.5**	308025.5**	6103.8	400.6**	5.6	44.7**	8.7	1.67**	0.02
Blocks	3	612.5	250.0	0.6	0.7*	20215.8	3471.8	36.9	4.2	0.6	6.7	0.20	0.02
Error	9	169.4	176.4	0.8	0.1	22779.2	3158.6	30.8	3.2	2.5	3.7	0.12	0.01

*, ** Significant at 5% (*) or 1% (**) probability level.

leachate from decaying tubers reduced emergence rate of lettuce grown in lower crocks (Table 23). Although treatment significantly influenced leaf area, primary root length and fresh and dry weights of lettuce grown in upper crocks, these differences were not found in lettuce grown in lower crocks. The presence of growing nutsedge plants and decaying tubers in the seed medium of upper crocks reduced leaf area and fresh and dry weights of lettuce grown in upper crocks. The greatest reductions in leaf area and dry weight of lettuce were due to the growing nutsedge plants treatment. The length of the primary or tap root of lettuce grown in upper crocks was decreased by the presence of decaying leaves and growing plants of nutsedge with the greatest reduction resulting from the growing nutsedge plants treatment.

First Tomato Planting: April 9, 1979

Emergence and length of tomato plants grown in both upper and lower crocks were not significantly influenced by the various treatments; however, significant differences did occur in plant diameter, root length and fresh weight of tomato plants grown in upper and lower crocks (Table 24). Nutsedge plants growing in the seed medium of upper crocks and leachates from these plants reduced root length and diameter of tomato plants grown in upper and lower crocks below those of the control and decaying tissue treatments (Table 25). Although the presence of nutsedge plants in the seed medium reduced fresh weight of tomato plants grown in

Table 23. Effect of C. rotundus material in upper crock medium on growth of lettuce planted on April 5, 1979, in upper and lower crocks (planting number 2).

| Treatment | | Plant emergence | | | | Plant size | | | | | | | |
| Nutsedge tissue | Rate per crock | Total (%) | | Rate (days) | | Leaf area (cm²/plant) | | Fresh wt (g/plant) | | Root length (cm/plant) | | Dry wt (g/plant) | |
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Control	0	38.8	55.0	5.3A^Z	6.2B	680.6A	143.9	24.2A	4.0	9.2A	7.4	1.59A	0.27
Growing plants	50(no.)	52.5	46.2	3.1B	5.4C	29.1C	61.5	0.8C	1.6	1.4C	4.2	0.05C	0.13
Decaying tubers	28g	35.0	33.8	6.4A	6.9A	278.2B	115.3	9.5BC	3.4	7.4AB	5.3	0.67B	0.23
Decaying leaves	28g	48.8	40.0	5.0A	6.4AB	470.3AB	144.7	16.8AB	4.2	6.6B	7.0	1.60AB	0.28

^{Z}Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

Table 24. Mean squares for the analyses of variance of the effect of *C. rotundus* material in upper crock medium on growth of tomato planted on April 9, 1979, in upper and lower crocks (planting number 1).

Source of variation	df	Plant emergence				Plant size							
		Total (%)		Rate (days)		Diameter (mm/plant)		Length (cm/plant)		Root length (cm/plant)		Fresh wt (g/plant)	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Treatments	3	177.0	110.3	0.56	0.02	4.55**	0.60**	45.5	33.8	26.4**	4.6*	9.0*	6.1*
Blocks	3	505.0	27.7	0.41	0.01	0.37	1.16**	24.9	86.0*	0.5	2.4	1.4	8.3**
Error	9	217.0	111.2	0.59	0.04	0.79	0.09	25.2	15.6	1.8	1.1	2.4	1.1

*, ** Significant at 5% (*) or 1% (**) probability level.

Table 25. Effect of C. rotundus material in upper crock medium on growth of tomato planted on April 9, 1979, in upper and lower crocks (planting number 1).

Treatment		Plant emergence				Plant size							
		Total (%)		Rate (days)		Diameter (mm/plant)		Length (cm/plant)		Root length (cm/plant)		Fresh wt (g/plant)	
Nutsedge tissue	Rate per crock	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Control	0	80.0	86.0	7.5	6.3	3.0^z	4.3A	21.4	30.2	7.0A	8.5a	1.9ab	5.8a
Growing plants	50(no.)	74.0	76.0	7.1	6.4	1.6b	3.4B	17.4	23.5	2.4B	6.4b	0.7b	2.9b
Decaying tubers	28g	86.0	88.0	6.6	6.3	3.9a	4.1A	24.7	29.0	7.5A	8.3a	4.0a	4.7a
Decaying leaves	28g	89.0	83.0	7.0	6.2	3.7a	3.9A	24.4	27.8	8.0A	8.8a	3.3a	4.7a

[z] Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

upper crocks relative to the 2 decaying tissue treatments, there were no significant differences between any of the nutsedge treatments and the control. Fresh weight of tomato plants grown in lower crocks which received leachate ¦from growing nutsedge plants was also reduced compared to the other treatments. Plant weight was approximately one-half that of the control.

Second Tomato Planting: May 10, 1979

As in previous spring and summer plantings of cucumber, some growth parameters of tomatoes grown in upper crocks were significantly influenced by treatments, while growth of tomato plants grown in lower crocks was not significantly affected (Table 26). Percent emergence and length of entire plant and shoot of tomatoes grown in upper crocks were not significantly affected by any treatment. Emergence rate of tomato plants grown in upper crocks was increased by decaying tubers and growing plants of nutsedge relative to the control (Table 27). Although the presence of growing nutsedge plants in the seed medium of upper crocks reduced tomato plant diameter over that with the decaying tissue treatments, the differences in plant diameter between the nutsedge treatments and the control were not significant. Growing nutsedge plants in the seed medium of the upper crocks reduced tomato root length to approximately one-third the length of tomato roots in the control. Dry weight of tomato plants in upper crocks that received decaying tubers was over 3 times

Table 26. Mean squares for analyses of variance of the effect of C. rotundus material in upper crock medium on growth of tomato planted on May 10, 1979, in upper and lower crocks (planting number 2).

Source of variation	df	Plant emergence				Plant size												
		Total (%)		Rate (days)		Diameter (mm/plant)		Length (cm/plant)		Root length (cm/plant)		Shoot length (cm/plant)		Fresh wt (g/plant)		Dry wt (g/plant)	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Treatments	3	162.7	82.7	0.96*	0.09	7.10*	0.94	124.9	110.0	33.4**	5.8	81.9	65.5	84.1*	60.7	1.10**	0.53
Blocks	3	18.7	152.0	0.10	0.19	0.27	2.14	73.3	150.4	1.8	5.1	65.6	101.1	18.7	68.9	0.19	0.49
Error	9	227.6	65.8	0.21	0.05	1.71	0.57	108.6	53.7	2.8	3.2	76.8	34.4	14.6	25.9	0.15	0.22

*, ** Significant at 5% (*) or 1% (**) probability level.

Table 27. Effect of C. rotundus material in upper crock medium on growth of toma-
to planted on May 10, 1979, in upper and lower crocks (planting number 2).

Treatment		Plant emergence				Plant size										
Nutsedge tissue	Rate per crock	Total (%)		Rate (days)		Diameter (mm/plant)		Length (cm/plant)		Root length (cm/plant)		Shoot length (cm/plant)		Fresh wt (g/plant)		Dry wt (g/plant)	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Control	0	65.0	90.0	7.2a[z]	6.2	3.6ab	5.2	25.8	44.7	7.8A	11.5	18.0	33.2	4.1b	14.6	0.4B	1.4
Growing plants	50 (no.)	72.0	85.0	6.1b	6.6	1.7b	4.0	26.2	32.6	2.8B	8.7	22.7	23.9	1.9b	5.4	0.2B	0.5
Decaying tubers	28g	76.0	86.0	6.3b	6.3	4.8a	4.5	37.8	36.7	9.2A	9.6	28.6	27.1	12.5a	10.9	1.4A	1.1
Decaying leaves	28g	77.0	79.0	6.6ab	6.4	4.1a	4.6	29.2	35.1	8.6A	9.4	20.7	25.7	5.7b	8.3	0.6B	0.9

[z]Mean separation within columns by Duncan's new multiple-range test, 5% level
(lower case), 1% level (upper case) or nonsignificant (no letters).

greater than that of the control and growing nutsedge plants treatments and more than twice the weight of tomato plants grown in the presence of decaying leaves.

Field Studies

Since allelopathic reductions in growth of cucumber, lettuce and tomato were demonstrated in the greenhouse studies, field experiments were conducted to determine if an allelopathic response to natural nutsedge populations and tuber treatments occurred.

Experiment II

Since negative correlations between species are suggestive of allelopathic interactions, the predominant broadleaved and grass species were counted and their population densities (no.) compared by correlation analysis. The major broadleaved weeds were Richardia scabra L., Raphanus raphanistrum L., Oenothera laciniata Hill, Euphorbia hirta L., Geranium carolinianum L. and Mollugo verticillata L., while the predominant grasses were Digitaria sanguinalis (L.) Scop. and Eleusine indica (L.) Gaertn. Purple nutsedge was almost exclusively the only sedge present. Correlation analysis between each weed species and nutsedge indicated that significant correlations occurred between densities of nutsedge and R. scabra, E. hirta and G. carolinianum with correlation coefficients (r values) of -0.41, 0.39 and 0.29, respectively (Table 28). Regression analyses were then performed for quadratic and logarithmic models. R^2 values for these 3 species based on the quadratic model were 0.18, 0.16 and 0.09, respectively. The logarithmic model gave R^2 values

Table 28. Correlations between C. rotundus densities and densities of other weed species.

Sp.	C. rotundus density
	Correlation coefficient, r
Digitaria sanguinalis	-0.28492
Cynodon dactylon	-0.23246
Richardia scabra	-0.41470**[z]
Raphanus raphanistrum	-0.04357
Linaria canadensis	-0.21103
Cerastium vulgatum	-0.02444
Oenothera laciniata	0.08356
Eleusine indica	0.21373
Gnaphalium sp.	0.05207
Euphorbia hirta	0.39287**
Lepidium virginicum	0.20198
Geranium carolinianum	0.28689*
Oxalis stricta	-0.12998
Mollugo verticillata	0.15188
Amaranthus sp.	-0.08448
Plantago major	0.00386
Solanum americanum	-0.09653

[z]Significant at the 5% (*) or 1% (**) probability level.

of 0.181, 0.090 and 0.049, respectively. Thus, the linear
model did not describe the relationship any better than the
other models. The negative correlation between densities of
nutsedge and $\underline{R}$. scabra suggested allelopathic effects of
nutsedge may have reduced the density of $\underline{R}$. scabra.

Experiment III

The effects of incorporation of mascerated tubers and a
CH_3OH extract of nutsedge tubers into a natural field soil
were determined in experiment III. Number of plants of cu-
cumber and tomato and percent emergence of tomato were sig-
nificantly affected by the various treatments, but signifi-
cant differences were not obtained for the remaining growth
parameters of these 2 species or the growth of radish and
lettuce plants (Tables 29 and 30). Chopped tubers added to
the soil reduced the no. of cucumber plants harvested to 2.8
compared to 10.2 in the unscreened control treatment. There
were no differences between the screened and unscreened con-
trols or between the screened control and the 2 tuber treat-
ments.

Percent emergence and no. of tomato plants harvested
October 15, 1979, were reduced by screening the soil
(screened vs. unscreened control) but were not significantly
influenced by the tuber treatments. The predominant 7 weed
spp. present in these plots were also harvested October 15,
1979. Populations of $\underline{R}$. scabra and $\underline{R}$. raphanistrum were the
only ones significantly altered by treatment (Table 31).

Table 29. Mean squares for the analyses of variance of the
effects of <u>C</u>. <u>rotundus</u> tubers and tuber extract on num-
ber and growth of radish, cucumber, tomato and lettuce
plants in field plots, October 15, 1979.

Source of variation	df	Plant emergence		No. per 46 cm row	Plant dry wt (g/plant)
		Total(%)	Rate(days)		
			Radish		
Treatments	3	431.2	0.117	34.33	0.006
Blocks	4	670.6	0.182	37.88	0.006
Error	12	287.5	0.150	13.04	0.003
			Cucumber		
Treatments	3	961.8	0.21$_*$	47.8*	0.013
Blocks	4	767.3	0.27	35.8	0.031
Error	12	302.0	0.08	11.5	0.032
			Tomato		
Treatments	3	2775.3**	0.96	493.4*	0.017
Blocks	4	79.9	0.64	37.7	0.003
Error	12	279.2	0.42	104.1	0.006
			Lettuce		
Treatment	3	147.0	0.04	27.5	0.07
Blocks	4	101.9	0.37	14.9	0.05
Error	12	49.5	0.12	11.7	0.04

*, ** Significant at 5% (*) or 1% (**) probability level.

Table 30. Effects of C. rotundus tubers and tuber extract on number and growth of radish, cucumber, tomato and lettuce in field plots, October 15, 1979.

Treatment	Plant emergence			Plant dry wt (g/plant)
	Total (%)	Rate (days)	No./46 cm row	
		Radish		
Unscreened control	71.5	3.7	14.8	0.17
Screened control	57.5	3.8	11.2	0.23
Chopped tubers	50.5	3.8	8.4	0.21
Tuber extract	53.5	4.0	11.6	0.15
		Cucumber		
Unscreened control	55.2	4.2	10.2a^z	0.20
Screened control	44.4	3.9	7.2ab	0.23
Chopped tubers	24.0	4.4	2.8b	0.21
Tuber extract	31.2	4.2	5.6ab	0.31
		Tomato		
Unscreened control	71.6A	5.2	29.2a	0.05
Screened control	20.2B	5.3	9.4b	0.15
Chopped tubers	24.4B	5.5	7.4b	0.18
Tuber extract	31.8B	6.2	12.6b	0.08
		Lettuce		
Unscreened control	24.0	4.3	8.2	0.13
Screened control	26.6	4.3	9.2	0.12
Chopped tubers	15.8	4.2	7.0	0.36
Tuber extract	16.4	4.4	3.8	0.18

^{z}Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

Table 31. Mean squares for the analyses of variance of the effects of C. rotundus tubers and tuber extract on number and weight of plants of 7 weed species in field plots, October 15, 1979.

Source of variation	df	Richardia scabra	Eleusine indica	Digitaria sanguinalis	Mollugo verticillata	Oenothera laciniata	Cyperus[z] spp.	Raphanus raphanistrum
				Plants (no./4177 cm^2)				
Treatments	3	22892.4**	109.6	81.3	14.6	1260.3	13833.9	17.2*
Blocks	4	4065.3	10.7	56.5	7.3	46.3	2746.2	35.4**
Error	12	3217.3	56.7	33.8	7.9	523.8	10308.4	3.4
				Dry wt (g/plant)				
Treatments	3	0.0004*	0.004	0.04	0.04	0.00046	0.00030	0.05
Blocks	4	0.0009	0.001	0.09	0.04	0.00005	0.00019	0.13
Error	12	0.0002	0.002	0.03	0.13	0.00017	0.00025	0.06

[z]Mixture of Cyperus spp. other than C. rotundus.

*, ** Significant at 5% (*) or 1% (**) probability level.

Dry weights of harvested weed spp. were not significantly influenced by treatment. Although screening the soil reduced the no. of R. scabra and R. raphanistrum plants, the tuber treatments had no significant effect on population sizes relative to the screened control treatment (Table 32).

Treatment significantly influenced the no. of cucumber plants, but not the no. of radish, tomato or lettuce plants, or dry weight of the 4 crops harvested November 28, 1979 (Table 33). The no. of cucumber plants was almost 10 times less in plots sprayed with tuber extract than in plots which received control treatments (Table 34). Incorporation of chopped nutsedge tubers had no significant effect on the no. of cucumber plants.

The no. of R. scabra, E. indica and D. sanguinalis plants harvested on November 28, 1979, were significantly influenced by the treatments (Table 35). Screening the soil reduced the no. of R. scabra plants to one-fourth the no. in the unscreened control, whereas there were no significant differences in no. of R. scabra plants between the tuber treatments and the screened control (Table 36). The no. of E. indica plants in plots sprayed with tuber extract was significantly lower than in the screened control. Incorporation of chopped tubers in the soil did not significantly affect the no. of E. indica plants that emerged. Screening the soil significantly increased the no. of D. sanguinalis plants that emerged over those in the unscreened control. Incorporation of tuber extract in the soil reduced the no. of D. sanguinalis plants

Table 32. Effect of C. rotundus tubers and tuber extract on number and weight of plants of 7 weed species in field plots, October 15, 1979.

Treatment	Richardia scabra	Eleusine indica	Digitaria sanguinalis	Mollugo verticillata	Oenothera laciniata	Cyperus[z] spp.	Raphanus raphanistrum
			Plants (no./4177 cm^2)				
Unscreened control	180.0A[y]	14.2	14.2	4.8	52.0	226.2	5.6a
Screened control	50.0B	20.2	12.6	6.4	63.8	203.6	2.6b
Chopped tubers	42.0B	19.0	5.8	2.4	34.2	212.0	2.6b
Tuber extract	42.0B	10.0	7.4	3.6	29.6	110.4	1.2b
			Dry wt (g/plant)				
Unscreened control	0.02	0.08	0.09	0.35	0.03	0.007	0.33
Screened control	0.04	0.06	0.16	0.18	0.02	0.007	0.20
Chopped tubers	0.04	0.07	0.32	0.37	0.04	0.007	0.40
Tuber extract	0.05	0.13	0.16	0.32	0.04	0.022	0.19

[z]Mixture of Cyperus spp. other than C. rotundus.

[y]Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

Table 33. Mean squares for the analyses of variance of the effect of C. rotundus tubers and tuber extract on number and weight of plants of radish, cucumber, tomato and lettuce in field plots, November 28, 1979.

Source of variation	df	Radish	Cucumber	Tomato	Lettuce
			Plants (no./4177 cm^2)		
Treatments	3	16.9	53.6*	128.2	81.7
Blocks	4	14.6	27.4	32.3	39.9
Error	12	24.5	14.2	52.6	26.0
			Dry wt (g/plant)		
Treatments	3	1.24*	1.71	0.08	0.10
Blocks	4	2.81	1.30	0.10	0.14
Error	12	0.68	1.13	0.04	0.08

*Significant at 5% level of probability.

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Table 34. Effect of C. rotundus tubers and tuber extract on number and weight of plants of radish, cucumber, tomato and lettuce in field plots, November 28, 1979.

Treatment	Radish	Cucumber	Tomato	Lettuce
		Plants (no./4177 cm^2)		
Unscreened control	12.2	7.2a^z	16.0	12.0
Screened control	9.6	7.8a	3.8	11.2
Chopped tubers	8.2	3.6ab	8.2	3.4
Tuber extract	8.4	0.8b	10.2	6.6
		Dry wt (g/plant)		
Unscreened control	1.29	1.92	0.156	0.182
Screened control	2.48	0.49	0.387	0.181
Chopped tubers	2.04	1.22	0.283	0.395
Tuber extract	2.06	1.07	0.452	0.446

^{z}Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

Table 35. Mean squares for the analyses of variance of the effect of C. rotundus tubers and tuber extract on number and weight of plants of 7 weed species in field plots, November 28, 1979.

Source of variation	df	Richardia scabra	Eleusine indica	Digitaria sanguinalis	Mullugo verticillata	Oenothera laciniata	Cyperus[z] spp.	Raphanus raphanistrum
				Plants (no./4177 cm^2)				
Treatments	3	27718.0**	156.9*	101.2*	3.0	396.1	7132.1	5.9*
Blocks	4	3234.9	6.0	59.4	3.2	317.3	231.4	19.4
Error	12	2258.0	33.2	27.2	4.7	1173.3	3023.9	4.8
				Dry wt (g/plant)				
Treatments	3	0.00007**	0.085*	0.047*	0.072	0.0192	0.0027	55.7**
Blocks	4	0.00662*	0.178	0.062	0.025	0.0011	0.0009	167.2
Error	12	0.00172	0.053	0.015	0.179	0.0093	0.0012	12.8

[z]Mixture of Cyperus spp. other than C. rotundus.

*, **Significant at 5% (*) or 1% (**) probability level.

Table 36. Effect of C. rotundus tubers and tuber extract on number and weight of plants of 7 weed species in field plots, November 28, 1979.

Treatment	Richardia scabra	Eleusine indica	Digitaria sanguinalis	Mollugo verticillata	Oenothera laciniata	Cyperus[z] spp.	Raphanus raphanistrum
	Plants (no./4177 cm²)						
Unscreened control	184.8A[y]	18.0ab	10.4b	2.0	65.4	114.0	4.4
Screened control	42.0B	24.6a	20.4a	3.8	76.0	150.2	2.2
Chopped tubers	32.0B	16.4ab	13.8ab	2.4	55.2	192.4	2.8
Tuber extract	34.4B	11.0b	11.4b	2.6	60.2	112.2	2.0
	Dry wt (g/plant)						
Unscreened control	0.110	0.472	0.164	0.651	0.206	0.019	15.0a
Screened control	0.105	0.263	0.315	0.424	0.322	0.021	14.2a
Chopped tubers	0.104	0.169	0.127	0.703	0.255	0.038	7.1b
Tuber extract	0.112	0.236	o.307	0.631	0.341	0.070	9.2ab

[z]Mixture of Cyperus spp. other than C. rotundus.

[y]Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

over the screened control, but incorporation of chopped tu-
bers did not. Plant dry weights of the 7 weed spp. were not
affected by any treatment, except R. raphanistrum dry weights
were reduced over the controls by incorporation of chopped
tubers. Although the tuber extract treatment reduced dry
weight of this species, the reduction was not significant
relative to the control.

Laboratory Studies

Bioassays--Experiment IV

First bioassay

Extracts of nutsedge leaves and tubers were bioassayed
for biological activity by cucumber seed germination (Tables
37 and 38). Four milliliters of extracts of 28 g of leaves
and tubers of nutsedge in 100 ml CH_3OH completely inhibited
cucumber seed germination. Percent and rate of germination
(based on radicle emergence) of cucumber seed in the CH_3OH
and water control treatments were similar. When germination
was defined as emergence of both radicle and plumule, cucum-
ber seed in the CH_3OH control had a significantly slower
germination rate than in the water control.

Second bioassay

Extracts of 3 quantities of nutsedge tubers were com-
pared for biological activity. These tuber extracts signifi-
cantly decreased germination percentages, germination rates

Table 37. Mean squares for the analyses of variance of the
  influence of C. rotundus tissue extracts on cucumber
  seed germination.

Source of variation	df	Germination ($\%$)^Z		Germination rate (days)^Z	
		A	B	A	B
Treatments	3	12691.7**	12691.7**	5.4006**	27.12**
Error	12	25.0	25.0	0.0006	0.05

^{Z}Germination was defined as A) radicle emergence greater
 than 3 mm, or B) radicle and plumule emergence.

*, **Significant at 5% (*) or 1%(**) probability level.

Table 38. Influence of C. rotundus tissue extracts
on cucumber seed germination.

Treatment	Germination (%)[z]		Germination rate (days)[z]	
	A	B	A	B
Leaves (28g)	0B[y]	0B	0B	0B
Tubers (28g)	0B	0B	0B	0C
Control (CH$_3$OH)	100A	100A	2A	4.9A
Control	95A	95A	2A	4.1B

[z]Germination was defined as A) radicle emergence
greater than 3 mm, or B) radicle and plumule emer-
gence.

[y]Mean separation within columns by Duncan's new mul-
tiple-range test, 5% level (lower case), 1% level
(upper case) or nonsignificant (no letters).

(based on radicle emergence) and root lengths of cucumber
(Table 39). Germination, based on radicle emergence, was
reduced from 100% with the CH_3OH control to 70% with the 2.5
g tuber extract (Table 40). Extracts of the lower rates of
tubers had no effect on germination percentage. When germin-
ation was defined as emergence of radicle and plumule, all 3
rates of tuber extracts reduced germination with the greatest
reduction resulting from the 2.5 g tuber extract. Percent
germination and germination rate (both definitions) were
similar for the 2 control treatments. Extracts of 0.5 and
2.5 g of tubers increased the time required for cucumber
germination (radicle emergence). Germination took signifi-
cantly longer with the 2.5 g tuber extract than with the 0.5
g rate. Cucumber root length was significantly less with
the 3 tuber extracts than the 2 control treatments with the
shortest roots occurring in the 2.5 g tuber extract. Also,
the water control produced roots significantly shorter than
those in the CH_3OH control.

Experiment V

CH$_3$OH extracts of soil and leachates of sand and soil
in which nutsedge had previously grown were bioassayed by
seed germination to determine if residual quantities of
allelopath existed in the media. Along with the usual con-
trol treatment, an extract control, consisting of an extract
of Wauchula f.s. in which nutsedge had not grown, was in-
cluded for comparison purposes. Thus, the extract of

Table 39. Mean squares for the analyses of variance of the influence of C. rotundus tuber extract on cucumber seed germination and growth.

Source of variation	df	Germination (%)z		Germination rate (days)z		Root length (mm/plant)
		A	B	A	B	
Treatments	4	650.0**	3680.0**	1.01**	1.06	6435.1**
Error	15	38.3	131.7	0.02	1.68	181.4

zGermination was defined as A) radicle emergence greater than 3 mm, or B) radicle and plumule emergence.

*,** Significant at 5% (*) or 1% (**) probability level.

Table 40. Influence of C. rotundus tuber extract on cucumber seed germination and growth.

Treatment	Germination (%)z		Germination rate (days)z		Root length (mm/plant)
	A	B	A	B	
Tubers (0.25g)	100Ay	75B	2.1C	4.5	54.2C
Tubers (0.50g)	95A	65B	2.5B	4.9	42.2C
Tubers (2.50g)	70B	20C	3.2A	3.8	6.0D
Control (CH₃OH)	100A	92A	2.0C	3.9	109.0A
Control	98A	95A	2.0C	3.7	87.8B

zGermination was defined as A) radicle emergence greater than 3 mm, or B) radicle and plumule emergence.

yMean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

nutsedge infested soil was compared with its own control,
and the extract control could be compared with the CH_3OH
control to determine if there were any deleterious extract-
able substances in noninfested soil. Since differences did
exist between the 2 controls, the extract of nutsedge in-
fested soil was only compared to the extract control.

Percent germination, rate of germination (radicle and
plumule emergence basis only), root length and fresh weight
of tomato and all parameters of lettuce growth, except fresh
weight, were significantly affected by the treatments (Table
41). All treatments reduced percent germination (radicle
emergence) of tomato over the control (Table 42). Leachates
of both soil and sand previously infested with nutsedge
significantly reduced germination percentages of tomato.
The greatest reduction over the control was in response to
the sand leachate. No significant differences in percent
germination of tomato were noted between the extract of in-
fested soil and the extract of noninfested soil, regardless
of how germination was defined. When defined as emergence
of radicle and plumule, germination percentage of tomato was
not significantly affected by any treatment relative to that
treatment's respective control, even though the leachates of
sand and soil infested with nutsedge completely inhibited
germination. Leachate of nutsedge infested sand significantly
reduced root length of tomato, but leachate of infested
soil did not. No significant differences in germination rate
or root length of tomato existed between the extracts of

Table 41. Mean squares for analyses of variance of the effect of leachates and extract of media infested with $\underline{C}$. $\underline{rotundus}$ on seed germination and growth of tomato and lettuce.

Source of variation	df	Germination (%)z		Germination rate (days)z		Root length (mm/plant)	Fresh wt (g/plant)
		A	B	A	B		
				Tomato			
Treatments	4	2885.2**	170.0**	1.12	103.94**	882.3**	0.000148*
Blocks	3	235.7	22.4	3.34	0.01	177.2	0.000043
Error	12	91.1	16.4	3.16	0.13	115.8	0.000043
				Lettuce			
Treatments	4	6562.0**	2534.8**	13.44**	26.70**	1030.7**	0.0017
Blocks	3	15.7	17.9	0.07	0.53	19.5	0.0011
Error	12	53.7	45.2	0.08	0.23	24.9	0.0011

zGermination was defined as A) radicle emergence greater than 3 mm, or B) radicle and plumule emergence.

*, **, Significant at 5% (*) or 1% (**) probability level.

Table 42. Effect of leachates and extract of media infested with C. rotundus on seed germination and growth of tomato and lettuce.

Treatment	Germination (%) A	B	Germination rate (days)[z] A	B	Root length (mm/plant)	Fresh wt (g/plant)
Tomato						
Leachate						
Control	76.0A[y]	5.0BC	4.5	9.8A	39.5A	0.024ab
Soil	61.0B	0C	5.2	0C	32.1A	0.024ab
Sand	6.0C	0C	5.7	0C	2.1B	0.016b
Extract						
Noninfested soil	59.0B	15.0A	4.5	9.1B	36.0A	0.031a
Infested soil	60.0B	10.0AB	4.7	9.0B	28.1A	0.029a
Lettuce						
Leachate						
Control	94.0A	62.0A	2.0C	4.3B	43.3A	0.016
Soil	75.0B	9.0CD	4.4A	6.1A	13.3C	0.012
Sand	0C	0D	0D	0C	0D	
Extract						
Noninfested soil	95.0A	41.0B	3.1B	5.8A	30.8B	0.018
Infested soil	91.0A	19.0C	4.2A	5.9A	20.9C	0.054

[z] Germination was defined as A) radicle emergence greater than 3 mm, or B) radicle and plumule emergence.

[y] Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

infested and noninfested soil. Leachate of sand in which
nutsedge had grown reduced tomato plant fresh weight com-
pared to the extracts of infested and noninfested soil.

Leachates of sand and soil infested with nutsedge sig-
nificantly reduced germination percentage and rate and root
length of lettuce. Leachate of sand completely inhibited
germination. Although no significant differences in percent
germination of lettuce existed between the extracts of nut-
sedge infested and noninfested soil when based on radicle
emergence, when germination was defined as emergence of both
radicle and plumule, the extract of soil in which nutsedge had
grown significantly reduced germination percentage of lettuce
over the extract of soil not infested with nutsedge. In the
case of germination rate, the opposite was true, that is,
when germination was defined as radicle emergence, the in-
fested soil extract significantly reduced the rate of germin-
ation, but when based on emergence of radicle and plumule,
there was no significant difference between the extracts
of infested and noninfested soil. Extract of nutsedge in-
fested soil significantly reduced seedling root length of
lettuce compared to both control treatments.

Heat Stability--Experiment VI

Since most proteins are denatured by temperatures in
excess of 50° C, a 60° C heat treatment was applied to an
extract of nutsedge tubers for 1 hour, then the heated ex-
tract was bioassayed for biological activity by a cucumber

seed germination bioassay. This heated extract was compared to an unheated tuber extract and a control treatment.

Emergence of both radicle and plumule was significantly influenced by treatment, but emergence of radicle alone was not (Table 43). Both the heated and unheated extract treatments reduced radicle length and emergence of radicle and plumule of cucumber (Table 44). Cucumber seed germinated slower in the heated and unheated extracts than in the control when germination was defined as emergence of both radicle and plumule. Where germination was based on emergence of radicle alone, the unheated extract reduced the rate of germination over the control, but was not significantly different from the unheated extract treatment. In no case was there any significant difference between the heated and unheated extract treatments, thus indicating that the inhibitor is heat stable and most probably not proteinaceous.

Water Solubility--Experiment VII

Since the solubility of growth regulating compounds in water can be an important characteristic, a cucumber seed germination bioassay was conducted to determine the water solubility of the allelopath contained in a CH_3OH extract of nutsedge tubers. Germination and growth of cucumber were significantly influenced by treatment (Table 45). Although the water soluble fraction of tuber extract significantly reduced percent germination of cucumber when germination was based on emergence of radicle and plumule, no significant

Table 43. Mean squares for the analyses of variance of the influence of heat on biological activity of C. rotundus tuber extract as measured by cucumber seed bioassay.

Source of variation	df	Germination (%)[z]		Germination rate (days)[z]		Root length (mm/plant)
		A	B	A	B	
Treatments	2	25.0	1255.0**	0.13*	1.22**	6891.4**
Error	9	19.4	86.1	0.03	0.05	71.3

[z]Germination was defined as A) radicle emergence greater than 3 mm, or B) radicle and plumule emergence.

*, ** Significant at 5% (*) or 1% (**) probability level.

Table 44. Influence of heat on biological activity of C. rotundus tuber extract as measured by cucumber seed bioassay.

Treatment	Germination (%)^z		Germination rate (days)^z		Root length (mm/plant)
	A	B	A	B	
Control	100.0	97.5A^y	1.46b	4.59B	124.3A
Extract	95.0	70.0B	1.78a	5.42A	50.2B
Heated extract	97.5	65.0B	1.72ab	5.64A	54.9B

^{z}Germination was defined as A) radicle emergence greater than 3 mm, or B) radicle and plumule emergence.

^{y}Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

Table 45. Mean squares for the analyses of variance of the influence of the water soluble fraction of C. rotundus tuber extract on cucumber seed germination and growth.

Source of variation	df	Germination (%)^z		Germination rate (days)^z		Root length (mm/plant)
		A	B	A	B	
Treatments	1	0	2812.5	0.080*	2.25*	9367.4**
Error	6	0	112.5	0.0009	0.22	73.3

^{z}Germination was defined as A) radicle emergence greater than 3 mm, or B) radicle and plumule emergence.

*,** Significant at 5% (*) or 1% (**) probability level.

differences occurred between treatments when germination was defined as radicle emergence only (Table 46). The water soluble fraction also increased the time required to germinate and reduced the length of cucumber seedling roots to less than half that of the control. Thus, the inhibitor is at least partially water soluble.

Gel Chromatography--Experiment VIII

Gel chromatography was employed to determine the approximate molecular weight (m.w.) of the allelopath. The column calibration curve, established by chromatography of several compounds of known molecular weight is presented in Fig. 1. From this calibration curve, a regression equation was derived relating elution volume (V_e) in milliliters to molecular weight as follows: $Log^{-1}((V_e-413.8)/127.7)$. The inhibitor elution profile (Fig. 2), constructed from average values of 2 chromatographic separations, showed little inhibition of cucumber root growth before 90 ml of solvent had eluted. A large increase in inhibitory activity was observed after 94 ml of eluant with a peak of maximum inhibition at 110 ml. Inhibition of root growth then dropped off rapidly to less than 10% by the time 128 ml had eluted from the column. Since a broad peak was obtained, the elution volume at which maximum inhibition of cucumber root growth occurred was determined from the inhibitor elution profile using the arithmetic average of the elution volumes of the 5 most inhibitory fractions. This average elution volume was calculated to be

Table 46. Influence of the water soluble fraction of C. rotundus
tuber extract on cucumber seed germination and growth.

Treatment	Germination (%)^z		Germination rate (days)^z		Root length (mm/plant)
	A	B	A	B	
Control	100	100A^y	1.48b	4.22b	123.7A
Extract	100	62.5B	1.68a	5.29a	55.2B

^{z}Germination was defined as A) radicle emergence greater than 3 mm, or
B) radicle and plumule emergence.

^{y}Mean separation within columns by Duncan's new multiple-range test,
5% level (lower case), 1% level (upper case) or nonsignificant (no
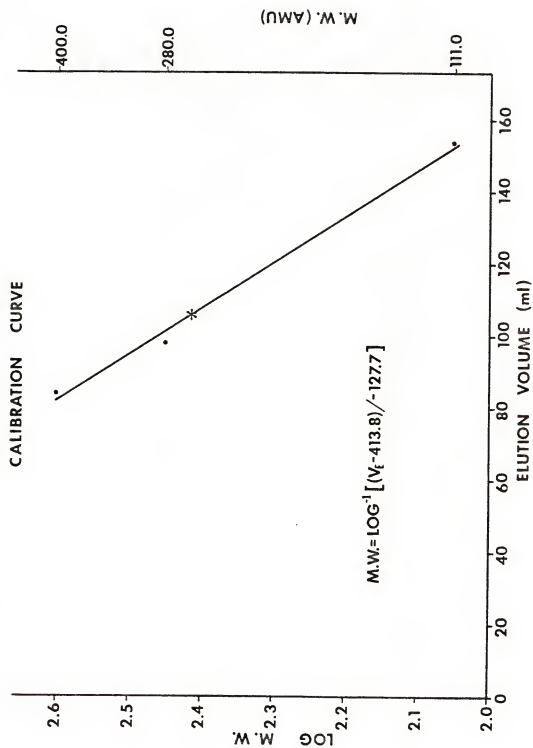letters).

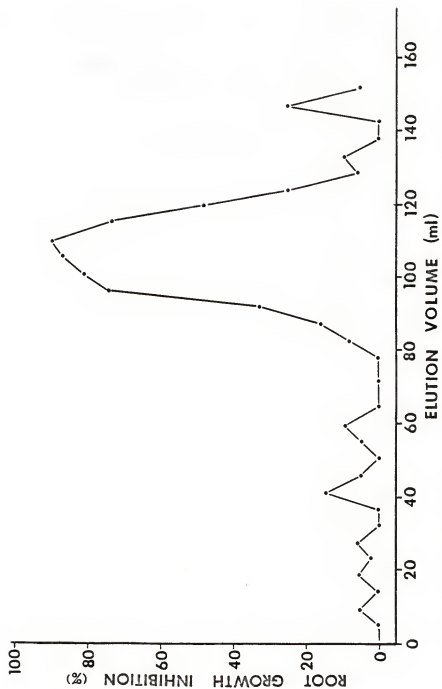Fig. 1. Molecular weight calibration curve for Sephadex LH-20 gel column.

Fig. 2. Elution profile for inhibitors eluted from Sephadex LH-20 gel column (average of 2 separations).

105.7 ml. The approximate molecular weight was then calculated from elution volume by using the regression equation. It was thus determined that the inhibitor(s) had molecular weight(s) of approximately 250 atomic mass units (amu) or daltons.

Mass Spectrometric Analysis--Experiment IX

Analysis of the combined sample of the 3 most inhibitory fractions from the gel column by mass spectrometry indicated a mixture of compounds. Two of these compounds were identified as protocatechuic acid and linoleic acid. In addition to these 2 compounds, there were several more compounds which were not identified.

Bioassay of acids

Determination of linoleic and protocatechuic acids as inhibitors and their effective concentrations required bioassay of pure samples of them, alone and in combination, at various concentrations. The bioassay employed was a cucumber seedling root growth bioassay.

Significant differences in root length of cucumber seedlings occurred between treatments at concentrations of 100, 500 and 5000 ppm, but not at 1000 ppm (Table 47). At 100 ppm the combination treatment increased cucumber root length to a level above that of the 2 acids alone, but was not significantly different from the control (Table 48). Protocatechuic acid at 500 ppm significantly decreased cucumber root length, but neither linoleic acid alone nor in combination with protocatechuic acid had a significant effect

Table 47. Mean squares for the analyses of variance of the ef-
fect of four concentrations of protocatechuic and linoleic
acids, alone and in combination, on cucumber seedling root growth.

Root length (mm/plant)

Source of variation	df	Concentration			
		100ppm	500ppm	1000ppm	5000ppm
Treatments	3	849.0 *	1177.8 **	454.5	12038.6 **
Error	12	250.4	117.7	141.1	162.7

*, ** , Significant at 5% (*) or 1% (**) probability level.

Table 48. Effect of four concentrations of protocatechuic and linoleic acids, alone and in combination, on cucumber seedling root growth.

| | Root length (mm/plant) | | | |
| | Concentration | | | |
Treatment	100ppm	500ppm	1000ppm	5000ppm
Control	128.4ab[z]	128.4A	128.4	128.4A
Linoleic	104.9b	136.2A	102.3	23.2BC
Protocatechuic	104.9b	101.6B	114.2	7.8C
Combination	131.7a	139.3A	115.6	29.7B

[z]Mean separation within columns by Duncan's new multiple-range test, 5% level (lower case), 1% level (upper case) or nonsignificant (no letters).

on root length. At concentrations of 5000 ppm, all 3 acid treatments significantly reduced cucumber root growth with the greatest reduction resulting from protocatechuic acid alone.

High Pressure Liquid Chromatography -- Experiment X

High pressure liquid chromatography (HPLC) was chosen as a means of separating the allelopath(s) in tuber extracts from any associated compounds. Nutsedge tuber extract was fractionated on a silica cartridge (Sep-pak) with solvents of increasing polarity prior to liquid chromatographic separation to improve the separation by HPLC. Cucumber root length bioassay of the polarity group fractions, unfraction- ated tuber extract and a control treatment demonstrated sig- nificant treatment effects (Table 49). The unfractionated tuber extract, first CH_2Cl_2 fraction, 60% CH_3OH fraction and first 100% CH_3OH fraction significantly reduced cucumber root length over the control (Table 50). There was no signifi- cant difference in root lengths between the 60% and first 100% CH_3OH fractions; however, the first CH_2Cl_2 fraction significantly reduced cucumber root length over these 2 CH_3OH fractions. There was no significant difference in seedling root length between the first CH_2Cl_2 fraction and the un- fractionated tuber extract. These findings suggested the existence of 2 or more allelopathic compounds in nutsedge tuber extracts.

Table 49. Mean squares for the analysis of
variance of the effect of fractions of C.
rotundus tuber extract eluted from a sil-
ica cartridge (Sep-pak) on cucumber seed-
ling root length.

Source of variation	df	Root length (mm/plant)
Treatments	15	2635.4**
Blocks	2	352.9
Error	30	245.5

*, **, Significant at 5% (*) or 1% (**) proba-
bility level.

Table 50. Effect of fractions of C. rotun-
dus tuber extract eluted from a silica
cartridge (Sep-pak) on cucumber seedling
root length.

Eluting solvent	Root length (mm/plant)
Control	96.2A[z]
None (tuber extract)	3.1D
Methylene chloride-1	4.3D
Methylene chloride-2	74.2ABC
Methylene chloride-3	80.4AB
Methylene chloride-4	89.4AB
Methylene chloride-5	96.0A
5% diethyl ether	91.4AB
5% ethyl acetate	81.2AB
5% acetone	84.2AB
4% methanol	84.0AB
10% methanol	89.5AB
20% methanol	91.1AB
60% methanol	61.9BC
100% methanol-1	47.8C
100% methanol-2	86.2AB

[z]Mean separation by Duncan's new multiple-
range test, 5% level (lower case), 1%
level (upper case) or nonsignificant (no
letters).

Each inhibitory silica cartridge fraction was evaporated to dryness, resolubilized in 1 ml CH_3OH, chromatographed on an HPLC and the resultant eluant fractions bioassayed. None of the HPLC fractions significantly altered cucumber seedling root length compared to the control treatment (Tables 51 and 52).

Since the concentration of the compounds in the silica cartridge eluants was approximately 1.66 times that in the sample loaded onto the HPLC, a cucumber root length bioassay was conducted comparing the control treatment and an unfractionated tuber extract with 1 ml of each inhibitory silica cartridge fraction diluted to the equivalent concentration by bringing 1 ml to a final volume of 1.66 ml with the appropriate solvent. Comparison of root growth in the control treatment with that in the diluted silica cartridge fractions would determine whether the lack of inhibition of root growth by the HPLC fractions was due to a concentration effect or not. The first CH_2Cl_2 and 100% CH_3OH and the 60% CH_3OH significantly reduced cucumber root growth when compared with the control (Tables 53 and 54). The first CH_2Cl_2 fraction was as inhibitory as the unfractionated tuber extract and significantly more inhibitory than the 60% and first 100% CH_3OH fractions. Thus, the lack of significance between the various HPLC fractions and the control treatment was most likely not due to a concentration effect.

Table 51. Mean squares for the analyses of variance of the effect of fractions of C. rotundus tuber extract separated by liquid chromatography on cucumber seedling root length.

Source of variation	df	Root length (mm/plant)
Methylene chloride fraction-1[z]		
Treatments (fractions)	20	113.29
Blocks	1	10.25
Error	20	104.88
60% Methanol fraction[z]		
Treatments (fractions)	20	86.33
Blocks	1	2.50
Error	20	105.23
100% Methanol fraction[z]		
Treatments (fractions)	20	253.27
Blocks	1	76.42
Error	20	563.52

[z]Fraction eluted from silica cartridge (Sep-pak) with specified solvent.

*,** Significant at 5% (*) or 1% (**) probability level.

Table 52. Effect of fractions of C. rotundus tuber ex-
       tract separated by liquid chromatography on cucumber
       seedling root length.

	Root length (mm/plant)		
	Silica cartridge (Sep-pak) fraction		
Fraction no.	Methylene chloride-1	60% Methanol	100% Methanol-1
1	115.2	113.6	116.4
2	114.4	123.9	112.5
3	124.1	128.9	111.8
4	126.1	117.1	125.5
5	126.8	122.6	117.0
6	116.5	128.6	121.0
7	107.5	114.5	114.8
8	115.6	118.2	121.4
9	119.8	126.1	115.1
10	125.9	126.8	123.8
11	123.6	117.4	120.8
12	115.4	117.0	124.4
13	111.6	111.8	121.1
14	109.4	115.0	123.5
15	100.0	117.9	108.9
16	117.8	121.5	114.1
17	112.6	120.0	130.4
18	125.9	114.1	118.2
19	113.1	120.8	123.9
20	112.8	107.5	83.0
Control	103.6	103.6	103.6

Table 53. Mean squares for the analyses of variance of
the effect of diluted fractions of C. rotundus tuber
extract eluted from a silica cartridge (Sep-pak) on
cucumber seedling root length.

Source of variation	df	Root length (mm/plant)
Treatments (fractions)	4	6155.8[**]
Blocks	3	25.4
Error	12	46.6

[*], [**] Significant at 5% (*) or 1% (**) probability level.

Table 54. Effect of diluted fractions of C.
rotundus tuber extract eluted from a sil-
ica cartridge (Sep-pak) on cucumber seed-
ling root length.

Eluting solvent	Root length (mm/plant)
Control	98.0A[z]
None (tuber extract)	4.2C
Methylene chloride	11.6C
60% Methanol	65.4B
100% Methanol	58.8B

[z]Mean separation by Duncan's new multiple-
range test, 1% level.

Greenhouse Studies: Experiment I

Cucumber, lettuce and tomato plants are very sensitive to a wide range of growth regulating compounds and, as such, often make good bioassay or indicator species. In these experiments growth of plants of these 3 species demonstrated that purple nutsedge was capable of growth interference by both competitive and allelopathic means. Any attempt to determine if allelopathic effects were involved in growth reduction required removal of competition as a component of interference. In these studies, growth reductions in upper crocks reflect total interference that consisted of both competition and allelopathy. Reductions in growth of plants in lower crocks should have been due to allelopathy alone.

Cucumber Plantings

Growth of cucumber in the various plantings varied considerably. Allelopathic growth reductions were found and the level of reduction varied with season. Reductions of cucumber plant growth in upper crocks containing growing $\underline{C}$. rotundus plants were products of competition and possibly allelopathy. Growth reductions in upper crocks due to decaying tubers and leaves may have been the result of immobilization of nitrogen by associated microorganisms, microbial pathogens or an allelopathic compound. Decreases in cucumber growth in lower crocks could only be due to allelopathic

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effects or nutritional deficiencies. Nutritional problems
can be ruled out since there were no reductions in cucumber
growth in lower crocks corresponding to reductions in growth
of cucumbers in upper crocks in the first 3 plantings (spring
and summer planting dates). If reductions in cucumber growth
in lower crocks were due to nutritional disorders, then cu-
cumbers receiving leachate from growing plants and decaying
tissues of nutsedge would have had their growth decreased.
Since this was not the case in the first 3 plantings and
reductions in cucumber growth in lower crocks in plantings
6, 7 and 8 (fall and winter planting dates) were not accom-
panied by reductions in cucumber growth in upper crocks, it
can be assumed that nutrition supplied to the plants was
adequate, and cucumber growth reductions in lower crocks were
due to a leachable allelopathic substance.

The improbability that microbial pathogens were respon-
sible for reductions in cucumber plant growth is evidenced
by the fact that in the last 3 plantings there were growth
reductions in lower crocks receiving leachates from decaying
tubers and leaves of nutsedge without corresponding reductions
in cucumber growth in upper crocks. Thus, these reductions
must have been the product of a leachable growth inhibitor.
Lack of growth inhibition of cucumber in upper crocks of
these 3 plantings as a result of the decaying tissue treat-
ments may have been due to 1) the presence of a growth pro-
moter which is insoluble in water and masking the effect
of any inhibitors, or 2) masking effects due to improved

physical aspects of the medium as a result of organic mate-
rial additions. The latter seems to be the most plausible
explanation, especially since it was only in the last 4
plantings (January 11, September 13 and October 29, 1979,
and January 18, 1980) that this occurred. Organic debris
in the medium would have increased with time even though
attempts were made to remove these materials before adding
fresh tissues for the next planting. Decreases in growth
of cucumbers in lower crocks were not observed for the
first 3 plantings. This may have been due to a low con-
centration of inhibitor in C. rotundus during that season
of the year coupled with a low solubility coefficient in
water. Thus, the amount of inhibitor present in lower
crocks would have been too low to reduce growth. Seasonal
variation in inhibitor content of many perennial species
exists with concentrations increasing in fall in response
to decreasing photoperiod and temperature. If this is the
case with C. rotundus, maximum growth reductions in cucum-
bers should be in the fall and winter months. Such a trend
is obvious from the data with the exceptions of plantings
4 and 5 (fall and winter). The lack of growth reductions
due to leachates from decaying tissues in planting 4 (fall
1978) may have been a result of 1) low concentrations of
allelopath in these tissues at the time the treatments were
applied, or 2) low solubility of the allelopath caused by
lowered temperatures. A decrease in allelopathic activity in
planting 5 (winter 1979) may have been related to low greenhouse

temperatures as there were several prolonged periods of no heat during this time. This hypothesis is further supported by the fact that maximum tuberization of C. rotundus had been shown to occur in the fall and winter, dropping to a minimum in spring and summer (3). Number of tubers associated with growing plants would thus increase in fall and winter, providing a greater inhibitor concentration. This increased concentration should be and was accompanied by a greater decrease in cucumber plant growth where leachates originated from growing plants than where the leachate was obtained from decaying tissue.

Lettuce Plantings

Lettuce growth reductions in upper crocks were recorded for the growing nutsedge plants and decaying tubers and leaves treatments in both plantings. The fact that lettuce in the January 28, 1979, planting failed to demonstrate allelopathic decreases in growth in lower crocks receiving leachates from tubers and leaves of nutsedge may be explained on the basis of 1) temperature effects as discussed for the fifth cucumber planting, or 2) inhibitor concentration was inadequate to produce significant decreases. Concentration of inhibitor is probably the explanation for the lack of growth reduction in lower crocks receiving leachates from decaying tissues in the April 5, 1979, planting, since these same treatments reduced growth in the upper crock series. A leachable allelopath was obviously present in leachates

entering the lower crocks of the growing nutsedge plants
treatment in both plantings. The fact that weight and leaf
area of lettuce were affected in the first planting and
only emergence rate was decreased in the second suggests a
decrease in concentration and/or activity as season changed
from winter to spring.

Tomato Plantings

Decreased tomato plant growth in lower crocks receiving
leachate from growing nutsedge plants in the April 9, 1979,
planting demonstrates the presence of an allelopathic com-
pound. Absence of corresponding decreases in the May 10,
1979 planting suggests a drop in inhibitor concentration or
activity as the season progressed from spring into summer.
The general lack of activity in both upper and lower crocks
due to decaying leaves and tubers may also be related to
low inhibitor concentration in these tissues at the time in
question. Another distinct possibility for tomato and
lettuce plantings is that these species are not as sensitive
to the allelopathic compound as are cucumber plants.

The data presented herein indicate that a leachable
allelopathic substance is released by decaying tubers and
leaves and growing plants of C. rotundus. The observed
growth reductions due to decaying tubers lend further sup-
port to the findings of Horowitz and Friedman (10, 16).
Furthermore, the data suggest that seasonal variations exist
in the concentration and importance of this compound with

concentration increasing in fall and winter and decreasing in spring and summer. The greater reduction in growth where receiving leachates from growing plants over that from decaying tissues suggests that 1) there are more tubers produced by nutsedge plants in upper crocks and thus more inhibitor and/or 2) the allelopathic substance is also released as a root exudate.

Field Studies

Experiment II

Allelopathic effects of one plant upon another may be manifest as a negative correlation between the number of plants of one species versus those of another. Thus, the significant negative correlation between density of Richardia scabra and density of C. rotundus suggests C. rotundus has an allelopathic effect upon emergence of R. scabra under field conditions. The positive correlations between C. rotundus and densities of E. hirta and G. carolinianum suggest that allelopathy is not involved in determining densities of these species.

Experiment III

Since the plots for the 2 tuber treatments and the screened control were screened to remove C. rotundus tubers while those for the unscreened control were not, the 2 tuber treatments can be compared only to the screened control. Differences between the 2 controls could possibly reflect

differences due to screening or incidence of C. rotundus; whereas, a reduction in growth of the bioassay plants as a result of either of the tuber treatments relative to their control would indicate allelopathic effects of the treatment. The only differences noted for the 4 crops on October 15, 1979 were between screened and unscreened controls. The significantly lower number of plants of cucumber and tomato and the reduced percent germination of tomato seed due to screening suggests these reductions are due to changes in the physical and biological properties of the soil. If the growth reduction was due to C. rotundus, then one would expect better growth where C. rotundus was absent. Such was not the case. Most probably, screening the soil resulted in dividing the soil into particle size separates which could have the observed effects upon plant growth. Also, screening would tend to redistribute seeds and microbes in the soil, which could influence plant growth and the no. of weeds present. This same effect was noted for number of plants of R. scabra and R. raphanistrum in the field plots on October 15, 1979. Reduction in number of cucumber plants on November 28, 1979, by tuber extract indicates the presence of an allelopathic inhibitor in tubers which was potent enough to produce the effects under field conditions. The absence of a corresponding decrease in cucumber plant dry weight suggests either the compound degrades rapidly or is easily leached under field conditions. Decrease in number of R. scabra plants on November 28, 1979, in plots where soil was screened is suggestive

of the aforementioned changes in the physical and biological properties of the soil. Increase in number of D. sanguinalis plants for the same treatment and decrease for the tuber extract treatment indicate C. rotundus tubers contain an allelopathic compound and strongly suggest the increase in number of plants in the screened control was due to removal of C. rotundus plants and tubers as a source of this allelopath. Decreased plant weight of R. raphanistrum on November 28, 1979, due to incorporation of chopped nutsedge tubers into the soil demonstrates the presence of an allelopathic compound in tubers which is capable of expressing itself under natural field conditions. This experiment did not substantiate the negative correlation noted in Experiment II between densities of C. rotundus and R. scabra.

Laboratory Studies: Experiments IV-X

Inhibition of cucumber seed germination by extracts of 28 g of leaves and tubers of nutsedge and reductions in cucumber growth produced by extracts of 0.25, 0.50 and 2.5 g of nutsedge tubers demonstrate the presence of a CH_3OH soluble growth inhibitor in these tissues. The significant decrease in germination and growth of cucumber as quantity of tuber increases illustrates that as concentration increases so does growth inhibition. Lack of a significant difference in percent germination of cucumber between the CH_3OH control and water control treatments shows there are no harmful residues from the extracting solvent.

Decreased percent germination of lettuce and tomato seed and increased time required for germination of tomato seed by sand and soil leachates demonstrates the presence of a leachable allelopath. Also, reduction in percent germination of tomato and lettuce by sand leachate relative to soil leachate suggests that the inhibitor may be retained by soil to a degree. Reductions in tomato root length by sand leachate and lettuce root length by both leachates further demonstrates the presence of a leachable inhibitor. Reduction in germination and root length of lettuce by extract of nutsedge infested soil further substantiates the conclusions of the first laboratory bioassay.

Reduction in percent germination and root length and increase in time required for germination of cucumber seed by heated and unheated extracts of nutsedge tubers and the absence of any significant difference between the 2 extracts for these parameters indicate the inhibitor is heat stable and most probably not protein-based. This would rule out those proteins which have biological activity based on shape or conformation, but not necessarily those whose activity is dependent on a specific amino acid sequence.

Reductions in cucumber seed germination percentage and root length and increase in time required for germination by the water soluble fraction of tuber extract indicates the inhibitor present in C. rotundus tubers is at least partially water soluble. Linoleic and protocatechuic acids are both water soluble, thus the results of the water solubility

experiment lends support to the presence of these acids in the inhibitory fraction.

The approximate molecular weight of the fraction of maximum inhibition was 250 amu as determined by gel chromatography. It should be noted that this value is for the peak and does not reflect the remainder of the elution curve. Thus, the active fractions could contain compounds with weights varying from the approximation by roughly $\pm$ 100 amu. This molecular weight range encompasses many fatty acids, including linoleic acid (280 amu), phenolic acids, such as protocatechuic acid (154 amu), abscisic acid and many other growth inhibitors. Most enzymes and other proteins are considerably larger molecules than this; therefore, most probably proteins are not involved in the observed inhibition as noted in the heat stability experiment.

Although protocatechuic acid and linoleic acid were suggested as constituents of the inhibitory fractions, there were several other component compounds for which suggested identities were not offered. Also, there were some inorganic ionic species in the sample which were complicating or confusing the analysis.

Since reduction in cucumber root length by protocatechuic acid at a concentration of 500 ppm was not followed by reduction for the 1000 ppm rate, the results of the 500 ppm rate must be viewed with skepticism. The significant reduction in root length by the 2 acids, alone and in combination, at a concentration of 5000 ppm indicates these acids

are capable of reductions in growth at this concentration; however, 5000 ppm is equivalent to 0.5% by weight and it does not seem likely that either acid, alone or in combination, would normally constitute such a large percentage of the weight of C. rotundus tubers. Therefore, it is doubtful that the allelopath in C. rotundus tubers is composed of only these 2 acids. It seems more logical to assume that the allelopath is actually several compounds, 2 of which may be protocatechuic and linoleic acid.

Data from bioassay of silica cartradge (Sep-pak) eluants indicates the allelopathic compound is actually 2 or more compounds. One compound has low polarity, while the other is fairly polar. Linoleic acid is low in polarity and protocatechuic acid is relatively polar, thus the polarity description fits these 2 acids.

Lack of root length reductions in the bioassay of HPLC separations of the active fractions eluted from the silica cartridge was not due to a concentration effect. Data suggest that either the inhibitors were retained on the column or each silica cartridge fraction consisted of several compounds which were responsible for the inhibitory activity. The latter seems quite plausible for the following reasons: 1) mass spectrometric analysis suggested the presence of several unidentified compounds and 2) the previously mentioned problems associated with the concentration of the 2 identified compounds, protocatechuic and linoleic acids. If the inhibitor was a complex of compounds, separation from one another

might produce results such as those obtained, assuming no single compound was capable of reducing growth by a statistically significant amount.

Protocatechuic acid and other phenolic compounds have been isolated from C. rotundus tubers (19) along with inhibitor β (52). Any of a number of these compounds have properties similar to those of the unidentified allelopaths. In addition, many of these same compounds are capable of producing similar growth responses when present in sufficient concentrations. Therefore, it is possible that some of these compounds may be involved in the growth reductions noted. Most probably the allelopaths are not proteinaceous, considering the low molecular weight and heat and alcohol stability of the inhibitor.

SUMMARY AND CONCLUSIONS

Greenhouse studies were conducted to determine if C. rotundus plants and residues reduced growth of selected bioassay species by release of an allelopathic compound into the growth medium. Treatments included growing C. rotundus plants and decaying residues of leaves and tubers of C. rotundus.

Growing plants and decaying leaves and tubers of C. rotundus reduced growth of bioassay species with growing nutsedge plants producing the greatest growth reduction. A seasonal effect was observed with maximum growth reduction of bioassay plants occurring in fall and winter, a time that coincides with the period of maximum nutsedge tuberization.

Two field experiments were conducted to determine if the presence of C. rotundus reduced the frequency of occurrence of other weed species, and to evaluate tubers and CH_3OH extracts of tubers as phytotoxins under field conditions. The first experiment was a field weed survey subjected to correlation analysis. In the second experiment, treatments were 1) unscreened soil control, 2) screened soil control, 3) chopped tubers incorporated into screened soil, and 4) CH_3OH tuber extract incorporated into screened soil. Bioassay crops employed were cucumber, lettuce, tomato and radish. Weed populations were also identified and counted. The presence of C. rotundus was negatively correlated with Richardia scabra and positively correlated with Euphorbia

hirta and Geranium carolinianum. Data from the second field
experiment indicate the presence of an allelopathic compound
in C. rotundus tubers as evidenced by the reduction in number
of cucumber, Eleusine indica and Digitaria sanguinalis plants
when grown in soil treated with nutsedge tuber extract and the
decrease in Raphanus raphanistrum plant weight where chopped
tubers were incorporated into the soil.

Laboratory experiments were conducted to evaluate tubers
as the source of the allelopath. Cucumber seed germination
bioassays of CH_3OH tuber extracts demonstrated the presence
of a potent growth inhibitor. An allelopathic compound was
shown to be present in soil in which C. rotundus had pre-
viously grown. Also, the allelopath was leachable and data
suggested the soil was somewhat retentive of it.

Additional laboratory studies were conducted to chemi-
cally separate and characterize the inhibitor obtained from
tubers. The allelopath was shown to be heat stable and at
least partially water soluble. Gel chromatography of tuber
extract determined it had a molecular weight of approximately
250 amu or daltons.

Mass spectrometric analysis of inhibitory fractions of
tuber extract obtained by gel chromatography suggested con-
stituents included protocatechuic and linoleic acids. A
number of other compounds and some inorganic ions were also
present, but were not identified. A cucumber seed germina-
tion bioassay was performed on these 2 acids alone and in

combination at concentrations of 100, 500, 1000 and 5000 ppm. Both acids and the combination were inhibitory at 5000 ppm.

Further separation of allelopathic constituents of tuber extract was attempted by employing high pressure liquid chromatography preceded by a separation procedure based on elution by a solvent sequence of increasing polarity. The allelopath consisted of 2 or more compounds, one of which was low in polarity, while the other was fairly polar. Attempts to further separate these components by high pressure liquid chromatography were not successful. It is suggested that the allelopath may actually be a complex of compounds that are water soluble, heat stable and have molecular weights of approximately 250 ± 100 amu.

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BIOGRAPHICAL SKETCH

James Preston Gilreath was born on September 20, 1947, in Greenville, South Carolina, where he received his primary and secondary education. He attended Clemson University from June 1965, to July 1966, majoring in chemical engineering. After leaving Clemson, he served in the United States Army Reserves and worked for a paint manufacturing firm in Greenville, South Carolina. In August 1970, he re-enrolled at Clemson University and was awarded the degree of Bachelor of Science in Plant Sciences--Agronomy in May 1974. In May 1976, he received the degree of Master of Science in Horticulture from Clemson University. He entered the University of Florida in June 1976, and was awarded the degree of Doctor of Philosophy in March 1981, with a major in horticultural science (vegetable crops).

He was honorably discharged from the United States Army Reserves in June 1972.
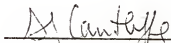
He married Phyllis Anne Robinson of Knoxville, Tennessee, on June 21, 1975.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

S. J. Locascio, Chairman
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

D. J. Cantliffe
Associate Professor of
Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.
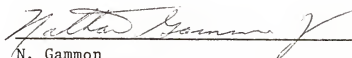
C. B. Hall
Professor of Horticultural Science

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S. R. Kostewicz
Associate Professor of
Horticultural Science

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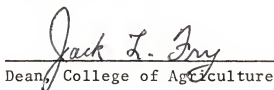N. Gammon
Professor Emeritus of Soil Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

March, 1981

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Dean for Graduate Studies and Research