FIELD PROJECT

ALLELOPATHIC EFFECTS OF CROP CROTALARIA SPS- A REVIEW

Submitted by

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SEPTEMBER 2020

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INTRODUCTION

The genus Crotalaria is a member of the Leguminosae family, and is largely made up of

Herbaceous plants. Crotalaria juncea L. or sunnhemp is native to India and Southeast Asia,

Where it is used for soil improvement, bast fiber, and forage (Rotar & Joy, 1983). Sunnhemp, one

Of the most commonly grown cover crops in the tropics (Rotar & Joy, 1983), is mentioned in

Ancient Sanskrit literature and is one of the oldest known fibers of the Indo-Pakistan subcontinent

(Duke, 1981). The fibers of sunnhemp are used to make rope, twine, rug yarn, cigarette and

Tissue papers, fishnets, sacking, and canvas (Duke, 1981). The wet fiber is stronger than the dry

Fibers and is resistant to moisture, mildew, and microorganisms in salt water.

Crotalaria species have been researched in the United States since 1909 (McKee and

Enlow, 1931). Some members of the Crotalaria genus produce seeds that are toxic to animals,

And others are considered noxious weeds in their natural habitats (USDA-NCRS, Tech. Note 10,

1999). Crotalaria juncea has very low levels of alkaloids (natural toxins) and can be safely eaten

By livestock (Reeves, 1998).

I observed at the time of collecting plant material for Experiment 1 that the weed

Population in the sunnhemp plots was dying. This provided anecdotal evidence that sunnhemp May

help control field weeds. Smooth pigweed (Amaranthus hybridus), which is known to be

Shade tolerant, was found dying in the field plots, opening the possibility that sunnhemp had anb

Effect on the weeds other than shading.

Three experiments were performed to determine the allelopathic potential of sunnhemp.

The first experiment determined the effect of sunnhemp plant material mixed with soil on seed

Germination of weeds and vegetable crops. The second experiment determined the allelopathic

Effect of various parts of the sunnhemp plant separately (stem, leaf, and root). Five species were

Used in these two experiments: two vegetable species [‘long imperator carrot’ (Daucus carota

L.), ‘black seeded Simpson’ lettuce (Lactuca sativa L.)] and three weed species [(annual ryegcarot

(Lolium multiflorum L.), smooth pigweed (Amaranthus hybridus L.), and sicklepod (Senna

Obtusifolia L.)]. A third experiment was conducted to determine the effect of sunnhemp leaf

Extracts on seed germination and seedling growth of some vegetable crops and cover crops that are

agriculturally important in Georgia and other areas of the Southeastern, USA.

OBJECTIVES

The objectives of my research were to determine whether sunnhemp has

Allelopathic potential to common weeds of the Piedmont region of Georgia, vegetable crops, and

Commonly used cover crops. In Experiment 1, the objective was to determine the effect of

Sunnhemp plant residues mixed with soil on seed germination of weeds and vegetable crops. The

Objective of Experiment 2 was to determine the allelopathic activity of the different parts of the

Sunnhemp plant. In the first two experiments, sunnhemp leaves were found to reduce

Germination in three weed and two vegetable crop species. So, the objective of Experiment 3 was

To determine the effect of sunnhemp leaf extract on seed germination and seedling growth of

Several import ant vegetable crops and cover crops. several important vegetable crops and cover crops.

EXPERIMENT-1

EFFECTS OF SUNHEMP RESIDUES MIXED WITH SOIL SEED GERMINATION AND GROWTH

Materials and methods

The objective of this experiment was to determine the effect of sunhemp plant residues mixed with soil on seed germination of weeds and vegetables growth

This experiment was conducted in a temperature controlled green house and, compared the effect of sunhemp and cereal rye residues mixed in soil the seed germination and seedling growth of various weeds and vegetables corps .The selection of weed species for the study was based on survey of the most troublesome weed species in Georgia (Webster and MacDonald's,2001)

The treatment were follows,

- Control sunhemp fresh leaf tissue
- Control Sunhemp sun-dried leaf tissue
- Control cereal rye fresh leaf tissue
- Control cereal rye sun-dried leaf tissue

The fresh tissues for both species was dried in an oven at 50°C immediately after Removing the leaves from the plant(harvest). The sun-dried sunhemp was cut and allowed to dry in the greenhouse for 16 days after harvest.The cereal rye was allowed to dry in the field for several week after

harvest,and then it was stored.Both fresh and sun-dried tissues were used to determine weather there is a decrease in allelopathic activity with time

The sunhemp for the fresh tissues treatment was grown in Watkinsville,GA at the J,phil campbell Sr,Natural Resources Conservation Research Centre ,The soil (Cecil soil sandy,clayloam ,fine, kaolinitic,thermic.,Typic Kanhapludult,with2-3% slope) Typical of the southern piedmont landscape was tilled to provide an adequate seed bed (Schomberg,2006).Whole plants ,roots and shoots,were collected 68 DAP. cereal rye 'Wrens Abruzi' was grown on the same field as sun hemp and was planted at the 84-90kg ha-1of seed rate The cerals rye leaves were harvested when they were 10-12cm long. Both sunhemp and cearal ray fresh tissue were dried in an oven(50°C) for 3 days,and then ground to a powder in a Wiley Mill

The sunnhemp for the sun-dried tissue was grown using seeds planted at therecommended rate (5 g/m^2)in flats (area = 1458 cm^2) (Rotar and Joy, 1983). Plants were grown in the greenhouse for 65 days (5 Aug. to 9 Oct.), fertilized once a week with liquid 20-20-20 fertilizer, and watered as needed. The biomass was determined at harvest in four randomly selected flats. Sunnhemp plant material was harvested, and allowed to dry in the sun for 16 days without watering. Plant shoots and roots were then separated and dried in an oven at 50 0C for one week. The remaining stems and leaves were

then allowed to dry in the greenhouse for 16 days to mimic field conditions, and to determine if slightly decomposed tissue would have a diminished effect on seed germination and growth. The leaf and flower material was removed from the stems and used for the experiment. Cereal rye was obtained from harvested bales grown at the USDA farm in Watkinsville, GA The rye was cut in the late boot stage and allowed to dry in the field before bailing. Bales were covered and stored in a barn prior to the study. The rye from the bale was then chopped in a standard food processor to reduce the tissue to a manageable size of 2-4 cm particle size. Both cereal rye and sunnhemp tissue was dried in an oven 50 0C before use and frozen at -7 0C. The sunnhemp and cereal rye tissues were mixed with sterilized soil and placed in a greenhouse under controlled conditions

A study conducted from 2001-2003 at the USDA Experiment Station in Watkinsville, GA shows that the amount of average rye biomass accumulated was 7.6 Mg ha-1 at the USDA experiment station in Watkinsville, GA (Schomberg, 2006). In another study, a July planting of sunnhemp in the same field produced 8.1 Mg ha-1 (Martini, 2004). These data on cover crop biomass production were used to determine the amount of plant tissue to be applied of each treatment. The amount of plant tissue applied per pot (styrofoam cup, containing 650 g of soil) was 9.4g(cereal ray) and 0.1g(sunhemp)

Seeds of weeds (sicklepod, smooth pigweed, annual ryegrass) and vegetables (lettuce, and carrot) were tested to determine whether seed germination and growth are reduced by the plant tissue residue in the soil. Sicklepod has been identified

as the most troublesome weed averaged across all crops surveyed including: corn (Zea mays L.), cotton (Gossypium hirsutum), forages and pastures, peanut (Arachis hypogaea), small grains, soybean (Glycine max), tobacco (Nicotiana sp.), and vegetables (Webster and Macdonald, 2001). Amaranthus species (including smooth pigweed) were ranked as third and annual ryegrass was ranked as eleventh (Webster and Macdonald, 2001). Smooth pigweed was chosen for this research because of its high germination rate. Annual ryegrass was chosen as the monocot species in this study over large crabgrass (a more troublesome weed for vegetable growers) because of low germination in the crabgrass seeds

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Proper germination temperatures and treatments were used as described by Buhler and Hoffman (1999). All seed germinated at 80 % or better in distilled water with a 16 h photoperiod and a 30 0C /20 0C cycle. A soil mixture composed of sterilized 75 % Cecil sandy loam and 25 % sand (by volume) was added to styrofoam cups (650 g of soil mixture per cup). The soil mixture was sterilized with a Progro electric soil sterilizer for 4 h at 93 0C to eliminate the possibility of microorganisms or live weed seed in the soil affecting the experiment. The amount of tissue per cup was also weighed and placed in a separate bag prior to planting (cereal rye 9.1 g per cup, sunnhemp 10.1 g per cup). The cover crops residues were incorporated into the soil mixture by shaking in a zip top plastic bag for all treatments, and then added to a styrofoam cup with small holes punched in the base. No fertilizer was used, and water was added gently with a watering can to prevent seeds from being washed from containers. The sunnhemp fresh dried tissue was only used on smooth pigweed and lettuce seeds due to insufficient sunnhemp residue available for this experiment

Germination counts were made seven days after planting. Germination percent was recorded on days 7, 14, 21, and 28. Seedling heights or leaf counts were made on days 14, 21, and 28. Seedling height was measured from the soil surface to the apex of the seedling. Seedlings were harvested 28 days after planting and dried in an oven at 50 0C for one week, after which, dry weights were determined. Only above-ground plant parts were harvested Nutrient concentration of harvested plants was determined by the University of Georgia

Soil, Plant, and Water Laboratory (2400 College Station Road, Athens, GA).

A completely randomized design with four replications was used. Each pot (experimental unit) contained 10 seeds of the species. There were only three replications each of sunnhemp fresh tissue because of lack of tissue for the experiment. The data were analyzed over time using a mixed model analysis of variance and Dunnett's multiple comparison procedure. The values for germination percent were arcsin transformed for the analysis and then untransformed for presentation of the data. The analyses were performed using the SAS for Windows 2006, version 9.1 (SAS Institute, Cary, N.C.).

RESULTS

Nutrient concentration of harvested plants was determined by the University of Georgia Soil, Plant, and Water Laboratory (2400 College Station Road, Athens, GA). The nitrogen concentrations were similar for the cereal rye fresh tissue and the sunnhemp fresh and sun-dried leaf tissues (Table 1). Sunnhemp fresh and sun-dried leaf tissues contained more calcium than any other plant part. Cereal rye fresh leaf tissue and sunnhemp sun-dried leaf tissue contained the most pottasium . All other nutrients were similar between the plant tisse types

Table 1 Mineral nutrient analysis of cereal rye and sunnhemp plant material, performed by the university of Georgia Soil, Plant, and Water Laboratory in Athens, GA

Nutrient concentration %

Plant type	Carbon	Nitrogen	Calcium	Pottasium	Magnesium	Sulfur	Phosphorus
Cereal rye fresh	44.9	3.3	0.40	2.7	0.15	0.32	0.41
Cereal rye sun dried	45.5	1.4	0.23	1.4	0.14	0.18	0.18
Sunhemp stem	41.6	41.6	1.1	0.33	1.5	0.11	0.25
Sunhemp root	44.1	1.0	0.16	1.8	0.14	0.13	0.10
Sunhemp fresh leaf	45.8	5.0	2.39	1.2	0.51	0.40	0.28
Sunhemp sin dried leaf	44.9	4.4	1.12	2.4	0.43	0.40	0.30

Experiment 1 tested the effects of sunnhemp and cereal rye plant residues on the seed germination and growth of vegetable and weed seeds. Only the germination of lettuce and pigweed was reduced by both sunnhemp and cereal rye (Table 2). The magnitude of the growth reduction was

dependent on the length of time after harvest the sunnhemp or cereal rye plant material was exposed to sunlight. The treatment vs. days to germination interaction was not significant in any of the analyses. Lettuce germination was significantly reduced by all four treatments (Table 2). The effects of sun-dried cereal rye as well as sun-dried and greenhouse grown sunnhemp tissues were not significantly different from each other for lettuce. Results were similar for smooth pigweed. Sunnhemp sun-dried and fresh tissues, and cereal rye fresh tissue caused significant germination reduction in smooth pigweed compared to the control. Annual ryegrass germination was affected only by the fresh cereal rye tissue. Neither type of sun-dried tissue had an effect on ryegrass germination. The germination of carrot and sicklepod were not affected by any treatment.

Table 2 Influence of fresh and su n-dried sunnhemp and cereal rye leaf residue on germination percent of five species grown in soil mixed with leaf residues.

Germination(%)

Weed or crop species

Cover crop residues	Lettuce	Carrot	Smooth pigweed	Annual ryegrass	Sickepod
Control (no residue)	86	45	21	75	82

Sunhemp sun dried	8*	17	5*	86	66
Sunhemp Fresh dried‡	7*	NA	1*	NA	NA
Cereal rye sun dried	36*	23	10	84	69
Cereal rye fresh dried‡	4*	16	3*	21*	58

* Indicates values within a column significantly different from the control at P ≤ 0.05 (Dunnett's multiple comparison procedure).

† Indicates values within a column significantly different from cereal rye fresh dried tissue at P ≤0.05 (Dunnett's multiple comparison procedure).

‡Fresh dried tissues were ground to a powder

. z Not enough tissue was available.

Dry weight of lettuce shoots grown in soil containing sunnhemp sun-dried and sunnhemp fresh, and cereal rye fresh residue treatments were significantly different from lettuce grown in soil with no residue (Table 3). Sunnhemp and cereal rye field and fresh tissue significantly influenced smooth pigweed compared to the control. None of the tissues

reduced dry weight of ryegrass relative to the control; however, the ryegrass dry weight was significantly lower in the sunnhemp sun-dried treatment compared to the cereal rye fresh treatment. Only the sunnhemp sun-dried tissue reduced carrot seedling dry weight. Sicklepod dry weight was not affected by any treatment

Table 3 Influence of fresh and dried sunnhemp and cereal rye leaf residue on shoot dry weight of five species grown in soil mixed with leaf residue.

Shoot dry weight (mg)

Weed or crop species

Cover crop residue	Lettuce	Carrot	Smooth	Ryegrass	Sicklepod
Control (no residue)	8.7	3.9	1.5	16.8	22.5
Sunhemp fresh dried‡	1.6*	NAz	1.6*	NAz	NAz
Sunhemp sun dried	2.6*	2.1*	0.4*	10.7†	20.1
Cereal rye sun dried	9.1†	3.3	0.5*	15.1	27.1
Cereal Rye Fresh dried‡	0.6*	2.5	0.6*	27.5	36.6

* Indicates values within a column significantly different from the control at P ≤ 0.05 (Dunnett's multiple comparison procedure).

† Indicates values within a column significantly different from cereal rye fresh dried tissue at P ≤0.05 (Dunnett's multiple compariso grown n procedure).

‡Fresh dried tissues were ground to a powder.

z Not enough tissue was available.

Seedling height in all the species (except lettuce) was unaffected by cover crop residues (Table 4). Lettuce seedling heights were not measured, instead leaf counts were taken every

week until harvest (Table 4). The number of leaves showed a significant reduction when the plant were grown in soil containing residues of either sunnhemp or cereal rye.

Table 4 Influence of sunnhemp and cereal rye leaf residue on the number of leaves or height of species in soil mixed with crop residue.

Number of leaves. Height(mm)

Weeds or crop species

Cover crop residue	Lettuce	Carrot	Smooth pigweed	Annual ryegrass	Sicklepod
Control (no residue)	3.5	12.8	9.1	107.6	22.9

Sunhemp sun dried	1.9*	8.7	5.3	85.3	24.3
Sunhemp fresh dried‡	1.9*	NAz	2.7	NAz	NAz
Cereal rye sun dried	3.5†	12.6	9.3	109.3	26.1
Cereal rye fresh dried‡	1.8*	9.3	4.9	119.4	21.6

* Indicates values within a column significantly different from the control at P ≤ 0.05 (Dunnett's multiple comparison procedure).

† Indicates values within a column significantly different from cereal rye fresh dried tissue at P ≤0.05 (Dunnett's multiple comparison procedure

‡Fresh dried tissues were ground to a powder

. z Not enough tissue was available.

EXPERIMENT 2

EFFECT OF SUNHEMP LEAF STEM OR ROOT EXTRACT ON SEED GERMINATION AND GROWTH

Materials and method

The objective of this experiment was to determine the presence of allelopathic activity ofThe different parts of the sunnhemp plant. The allelochemical potential of sunnhemp wasEvaluated using aqueous tissue extracts of the plant parts, as in White, et al. (1989).

The treatments (tissue extracts) were prepared from the following plant parts: control(water only), sunnhemp root, sunnhemp leaf, sunnhemp stem, and cereal rye leaf. The extracts of Sunnhemp and cereal rye were used at both full strength (5 g of dry tissue per 150 ml of distilled Water) and half strength. The sunnhemp and cereal rye fresh tissue and the weed and crop species Were the same as in Exp. 1.Twenty-five seeds of each species were added to a petri dish Containing a disk of filter paper, and then sealed with parafilm. This study was conduced in a Temperature (30 0C / 20 0C cycle) and photoperiod (16 h) controlled growth chamber.

Sunnhemp plants were separated into stem, root, and leaf sections. The roots were Washed to remove excess soil, and all plant parts were dried at 50 0C for 48 h. The plant tissues Were ground to a powder with a Wiley mill and frozen at -7°C until needed. Five grams of each Type of tissue

(leaves, stems, or roots of sunnhemp, and leaves of cereal rye), was placed in 150 Ml of distilled water, and the slurry was agitated at room temperature for 16 h on an orbital Shaker at 100 rpm.

The slurry was filtered through four layers of cheesecloth and vacuumsuctioned. The extract was used at full strength and half-strength in the experiment. Dilutions of The extracts were made with distilled water. Distilled water was used as a control. AfterPreparation, the extracts were stored several hours in a refrigerator (8 0C) until used.

Seeds of each species (25 seed per replicate) were placed in petri dishes, and 10 ml of Each plant extract or 10 ml of distilled water was added. Each dish contained one disk of filter Paper and was wrapped with parafilm to reduce evaporation. Petri dishes were placed in a growth Chamber with a 16 h photoperiod at 30 0C /20 0C cycle, with one species per shelf inside the Growth chamber. The experiment used a completely randomized design.

Low osmotic potential of the tissue extracts could inhibit seed germination or seedling Growth. Seeds of all five species were tested to determine their sensitivity to osmotic potential. The osmotic potential of the tissue extracts was determined by placing 1 g of plant tissue in 5 g Of water and the slurry was allowed to sit at room temperature (22 0 C) for 24 h. This slurry was Then filtered through a syringe with glass wool in the tip. The filtrate was centrifuged at 6500 Rpm for 10min and the resulting supernatant was measured on an osmometer (Osmette A, Automatic Osmometer,

Precision Systems, Inc., Tech Circle, Natick, MA). The concentrated Supernatant was used for measuring osmotic potential because the dilute solution used in the Germination experiment had an osmotic potential that was too close to zero to obtain a reading on The osmometer. However, results given have been adjusted to be the actual osmotic potential Present in the tissue extracts.

Polyethylene glycol (PEG) was used to produce solutions with osmotic potentials that Resembled those of the tissue extracts. The osmotic potentials of the PEG solutions were: 0 MPa[control (water only)], -0.03 MPa, and -0.1 MPa. The osmotic potentials at -0.03 MPa and0.1MPa were chosen because they corresponded to the actual osmotic potential of the tissueExtracts.

Ten ml of each PEG solution was added to each petri dish (replicate). Twenty five seed Of each species were placed in a petri dish and wrapped with parafilm. Then the dishes were Placed in a growth chamber with a 16 h photoperiod at 30 0C /20 0C cycle. Carrot, lettuce, Sicklepod, annual ryegrass, and pigweed were also used in this experiment. After four days, Germination data of the vegetable or weed species, length of radicle plus hypocoyl (dicots) or Radicle length only (monocots) was measured. The length of a representative sample of 10 Seedlings was averaged. After eight days, dry weights were determined.

Germination data were taken on day 4 for all species except carrot. Carrot germination was delayed and data taken on day 6. The values for germination percent

were arcsin Transformed for the analysis, and then transformed to the original values for presentation of theData. Data were analyzed using a mixed model analysis of variance and Dunnett's multiple Comparison procedure. The influence of osmotic potential could be a possible cause of Germination or dry weight reduction in addition to any allelopathic affects. Therefore, the Relationships of germination percent and seedling dry weight were tested with the osmotic

Potentials present in the extracts. The analysis for the osmotic potential germination and growth Was conducted using an analysis of variance and Dunnett's multiple comparison procedure. The Analyses were performed using SAS for Windows, 2006, version 9.1 (SAS Institute, Cary,N.C.).

RESULTS

This experiment was intended to determine which part of the sunnhemp plant contained The highest allelochemical activity, and to determine whether the allelochemicals have an effectonseed germination and seedling growth. The rye extract and the sunnhemp leaf extract reduced Germination compared to the control in every species but sicklepod (Table 5). Sicklepod Germination was not influenced by any treatment. The magnitude of the effect was dependent on The strength and type of plant extract. Sunnhemp root and stem extracts had no effect on seed Germination of any of the species. Germination of lettuce was negatively influenced by the Sunnhemp leaf treatments compared to the control

and to cereal rye. Sunnhemp full strength leaf Extract reduced lettuce germination at a percentage greater than cereal rye. For carrot, cereal rye And sunnhemp leaf extracts (both strengths) significantly reduced germination compared to the Control. Annual ryegrass germination was significantly reduced by the full strength cereal rye Extract and sunnhemp leaf extract. Smooth pigweed germination was not influenced by cereal Rye extracts, while both strengths of sunnhemp leaf extract significantly reduced germination Compared to the control

Table 5 Influence of sunnhemp leaf, stem, and root extracts and cereal rye leaf extracts onGermination percent of five species.

Average Germination (%)

Weed or crop species

Cover crop tissue extract	Strength	Lettuce	Carrot	Smooth pigweed	Annuval ryegrass	Sicklepod
Control(water)		99	70	64	88	93
Sunnhem	Full	87†	30†	71†	76†	94

p root	Half	84†	61†	77†	90†	91
Sunnhemp stem	Full	85†	25	65†	80†	98
	Half	90†	72†	63	95†	97
Sunnhemp leaf	Full	2*†	1*	0*†	0.2*†	92
	Half	57*†	10*	32*	57†	91
Cereal rye	Full	34*	1*	39	10*	98
	Half	94†	3*	67†	68†	97

*Indicates values within a column significantly different from the control at P ≤ 0.05 (Dunnett's Multiple comparison procedure). † Indicates values within a column significantly different from cereal rye fresh dried tissue at P ≤0.05 (Dunnett's multiple comparison procedure).

Compared to the control, root and stem sunnhemp extracts had no effect on seedling dry weight of any of the species (Table 6). The only treatment to significantly affect smooth pigweed or annual ryegrass dry weight was full strength sunnhemp leaf extract. Carrot seedling dry weight was

reduced by both strengths of sunnhemp leaf extract and cereal rye leaf extract. Lettuce and sicklepod seedlings were unaffected by any of the extracts.

Table 6 Influence of sunnhemp leaf, stem, and root extracts and cereal rye leaf extract on Seedling dry weight of five species.

Individual Seedling Dry Weight (milligrams)
Weed or crop species

Cover crop Tissue extract	Strength	Lettuce	Carrot	Smooth pigweed	Ryegrass	Sicklepod
Control (water)		1.1	1.7	0.3	2.6	12.3
Sunnhemp root extract	Full	1.1	1.7†	0.3	2.6	12.4
	Half	1.1	1.8†	0.3	2.8	12.3
Sunnhemp stem extract	Full	1.1	1.7†	0.3	2.5	12.4
	Half	1.1	1.7†	0.3	2.6	12.2†
Sunnhemp leaf extract	Full	0.7	0.5*	0*†	0.9*	13.1
	Half	1.2	0.2*	0.3	2.7	13.7
Cereal rye extract	Full	0.9	0.4*	0.3	2.1	14.3
	Half	0.9	0.1*	0.3	2.9	12.4

* Indicates values within a column significantly different from the control at P ≤ 0.05 (Dunnett's multiple comparison procedure). † Indicates values within a column significantly different from cereal rye fresh dried tissue at P ≤0.05 (Dunnett's multiple comparison procedure).

Length of lettuce seedlings was reduced in the sunnhemp full strength stem extract, Sunnhemp leaf extract (both strengths), and cereal rye extracts (both strengths), compared to the Control (Table 7). Lettuce seedlings in sunnhemp full strength leaf extract were shorter compared To seedlings in cereal rye full strength extract. All extracts (both strengths) reduced the length of Carrot and ryegrass seedlings compared to the control. Forcarrot and smooth pigweed, sunnhemp Leaf extracts (both strengths) reduced seedling length similarly to the cereal rye full strength extract. For ryegrass, the sunnhemp full strength root extract and half strength leaf extract Reduced seedling length similarly to cereal rye full strength extract. The sunnhemp full strength Leaf extract reduced length in ryegrass more than the reduction caused by cereal rye full strength Extract. Cereal rye and sunnhemp leaf extracts significantly reduced seedling length of smooth Pigweed. Sunnhemp and cereal

rye extracts had no effect on the length of sicklepod seedlings.

Table 7 Influence of sunnhemp leaf, stem, and root extracts and cereal rye leaf extract on length Of seedlings of five species.

Individual seedling lengthz (mm)

Weed or crop species

Cover crop tissue extract	Strength	Lettuce	Carrot	Smooth Pigweed	Rygress	Sicklepod
Control (water)		17	11	17	22	12
Sunnhemp root extract	Full	18†	4*	18†	9*	12
	Half	26*†	8*†	19†	18*†	14
Sunnhemp stem sell	Full	13*†	4*	15†	10*†	15
	Half	19†	6*†	18†	13*†	18
Sunnhemp leaf extract	Full	0.1*†	0.4*	0*	0.8*†	10
	Half	4*	1*	6*	6*	10
Cereal rye extract	Full	1*	1*	3*	1.2*	7
	Half	6*†	2*	7*	6*†	9

*Indicates values within a column significantly different from the control at P ≤ 0.05 (Dunnett's Multiple comparison procedure). † Indicates values within a column significantly different from cereal rye fresh dried tissue at P ≤0.05 (Dunnett's multiple comparison procedure). Z Length of radicle (monocots) or length of radicle plus hypocotyl (dicots).

The osmotic potential of the plant tissue extracts ranged from -0.03 to -0.11 MPa (Table 8). Among sunnhemp extracts, leaf extracts had a lower osmotic potential than root or stem Extracts. The osmotic potential of the sunnhemp leaf extract was similar to that of the cereal rye Leaf extract. A test was conducted using PEG solutions at the concentrations present in the plant tissue Extracts to determine the effect of osmotic potential on seed germination of thesame fivespecies. Within the range of the osmotic potential of the tissue extracts, the PEG solutions had no

Affect on seed germination or seedling dry weight, according to the analysis of variance and Dunnett's multiple comparison procedure.

Table 8 Osmotic potential and pH of plant tissue extracts.

Tissue source	Osmotic potential (MPa)	pH
Sunnhemp stem	-0.03	6.78
Sunnhemp root	-0.04	6.40
Sunnhemp leaf	-0.11	6.32
Cereal rye leaf	-0.11	4.34

The pH in the sunnhemp extracts of the various organs was similar among each other (mean = 6.5). The pH in the cereal rye extract was the lowest among the extracts (Table 5) and it Was noticeably below what is considered normal for vegetable crop growth. The pH of the cereal Rye extract was not adjusted in this test, as it was not adjusted in other studies (Barnes and Putnam, 1986; White, et al., 1989). The low pH of the cereal rye could contribute to its Allelopathic effect, but as cereal rye was used a control, the effect of the pH of rye extracts on Seed germination was not considered critical for evaluating sunnhemp effects.

EXPERIMENT 3

EFFECT OF SUNNHEMP LEAF EXTRACT ON SEED GERMINATION AND GROWTH

MATERIALS AND METHODS

In Experiments 1 and 2, sunnhemp leaves were found to reduce germination in three Weed and two vegetable crop species. The objective of this experiment was to determine the Effect of sunnhemp leaf extract on seed germination and seedling growth of agriculturally Important vegetable crops and cover crops. The vegetable crops were chosen based on the Georgia Farm Gate Report (Boatright and McKissick, 2005). The cover crops are commonly Used throughout the Southeastern, USA.

Seeds of the following species were evaluated: onion 'Savannah Sweet' (Allium cepa L.), Sweet corn (Zea mays L., var. rugosa), bell pepper 'California Wonder' (Capsicum annuum L.), Tomato 'Big Boy' (Lycopersicon esculentum L.), cucumber 'Long Green Improved' (Cucumis Sativus L.), collards 'Georgia Southern' (Brassica oleracea L.), turnip 'Seven Top' (Brassica Campestris L.), cowpea 'Colossus' (Vigna unguiculata L.), Austrian winter pea (Pisum sativumL.), okra 'Perkins' long green pod (Hibiscus esculentus L.), cereal rye 'Wrens Abruzzi' (Secale Cereale L.), winter wheat 'AR 494' (Triticum aestivum L.), and crimson clover 'Dixie Reseeding'

(Trifolium incarnatum L.). All seeds except onion were obtained from Athens Seed Company, Watkinsville, GA. Onion seeds were donated by Timothy Coolong (Onion Research Laboratory,Hort. Dept., University of Georgia). The onion seed had been stored in a freezer (0 0C) for 7 Years and germinated at 75 % in a pre-study germination test.

The experimental design was completely randomized and had four treatments and four Replications. The treatments (leaf extracts) were control (distilled water only), sunnhemp fresh, Sunnhemp sun-dried, and cereal rye fresh. Each extract was prepared in the same manner as in Experiment 2. Each dish contained 15 seeds, and 5 ml of the extract was added to each dish. Cowpea required more liquid to germinate, thus an additional 5 ml of plant extract was added to Cowpea dishes on day 2. There were four replications of each treatment. The temperature inside The growth chamber was 20 0C (night) and 25 0C (day). No lighting was used for this experiment.

Sunnhemp sun-dried tissue and cereal rye fresh tissue from Experiment 1 were used Again in Experiment 3. Sunnhemp fresh tissue was unavailable, and so was grown in the Greenhouse for 65 days (from 16 July to 19 September, 2006). The sunnhemp plants were grown In the same manner as in Experiment 1. The sunnhemp plants grown in the greenhouse for fresh Tissue in 2006 did not flower as did the sunnhemp plants used for sun-dried tissue grown in 2005. The leaf and stem tissues were harvested and dried at 50 0C for three days. The leaf tissue was Removed from the stems and ground to a powder in a Wiley mill.

The petri dishes were placed in growth chamber, having two species in each shelf of the Chamber in a completely randomized design. The data taken included germination value over 14 Days and total germination percent. The formula to calculate germination value was developed By Felix Czabator in 1962 to quantify germinative energy by combining speed and completeness of germination. Germination value index (GV) was calculated as follows: GV = (MDG) * (PV)where MDG (mean daily germination) is the average number of seeds germinated per day for the Total test period, and PV (peak value) is the mean daily germination rate from the initiation of the Test to the culmination of the logarithmic phase of development (Czabator 1962). Mean daily Germination is an index of total germination representing relative vigor and length of test period, And is calculated by dividing the cumulative germination percent on the final day of the test by The number of days (% day-1). Peak value indicates the speed of germination during the Logarithmic phase of germination and is represented by the maximum value obtained from the Quotient of cumulative germination percent and days since the beginning of the test (% day-1). The GV indicates both seedling vigor and speed of germination with a larger GV indicating Faster seed germination and higher total germination percent. The germination value was Developed as an empirical index value and has units of %2 Day-2, but is usually reported without Units. The values for germination percent were arcsin transformed for the statistical analysis And then were untransformed for presentation of the data. The data were

analyzed using a mixed Model analysis of variance and Dunnett's multiple comparison procedure.

RESULTS

In Experiments 1 and 2, it was determined that sunnhemp reduces germination in some Species due to the presence of the leaf tissue incorporated into the soil or leaf extract. Therefore, It was necessary to determine if the leaf extract would affect a broader group of vegetables and Cover crops, as these types of crops could follow sunnhemp in the crop rotation scheme.

The germination percent of winter wheat, rye, and Austrian winter pea was not affected By any treatment (Table 9). Onion, bell pepper, collard, turnip, okra, and cowpea seeds followed The same pattern in that germination in the control was greater than germination for all other Treatments, with no difference in the effect between sunnhemp tissue (fresh and sun-dried) and Cereal rye. Sweet corn germination was not affected by sunnhemp tissue, but was significantly Affected by cereal rye extract. The sunnhemp sun-dried tissue extract reduced germination in Cucumber, tomato, and crimson clover similarly to that produced by cereal rye. The sunnhemp Fresh tissue extract did not affect cucumber, tomato, or crimson clover germination.

Table 9 Influence of sunnhemp fresh and sun-dried extracts and cereal rye fresh extract on Germination percent of

thirteen species compared to the control (distilled water) and full strength Cereal rye extract.

	Germination Value			
	Cover crop extract			
Crop species	Control (distilled water only)	Sunnhemp fresh tissue	Sunnhemp sun-dried tissue	Cereal rye fresh tissue
Austrian Winter Pea	322.4	98.5*	66.2*	161.7*
Bell Pepper	53.3	0*	0*	0.1*
Cereal Rye	313.1	176.7	100.9*	174.4
Collard	345.2	115.9*†	31.3*	45.8*
Cowpea	181.5	46.8*	38.2*	11.6*
Crimson Clover	357.1	304.3†	129.8*	168.8*
Cucumber	357.1	345.3†	315.9*	315.9*
Okra	980	98.3*	55.2*	21.1*
Onion	69.2	0.5*	1.9*	0.4*
Sweet Corn	322.4	78.1*	100*	57.6*
Tomato	280.8	47.4*†	0.1*	0*
Turnip	357.1	124.2*	0.8*	25.9*
Winter Wheat	357.1	304.0	212.5*	271.0

Indicates values within a row significantly different from the control at P ≤ 0.05 (Dunnett's Multiple comparison procedure). Indicates values within a row significantly different from cereal rye fresh dried tissue at P ≤0.05 (Dunnett's multiple comparison procedure).

The germination values (Table 10) indicated that onion, sweet corn, bell pepper, tomato, Collard, turnip, okra, cowpea, and Austrian winter pea germination was were negatively affected By both the sunnhemp extracts and the cereal rye extract. Crimson clover and cucumber Germination values were negatively affected by the sunnhemp sun-dried tissue and the cereal rye

Tissue extracts, but not the sunnhemp fresh tissue extracts. Winter wheat and cereal rye were only Negatively impacted

by sunnhemp sun-dried tissue. The germination values for collards, crimson Clover, cucumber, and tomato were significantly different between cereal rye and the sunnhemp

Fresh tissue extract. The sunnhemp fresh tissue extract was less effective in these species Compared to cereal rye or sunnhemp field tissue extracts. Germination values for the other Species indicated that sunnhemp sun-dried tissue extract had similar effects as cereal rye extracts.

Table 10 Influence of sunnhemp fresh and sun-dried extracts and cereal rye fresh extract on Germination value of thirteen types of seed compared to the control (distilled water) and full Strength cereal rye extract.

	Germination Value			
	Cover crop extract			
Crop species	Control (distilled water only)	Sunnhemp fresh tissue	Sunnhemp sun-dried tissue	Cereal rye fresh tissue
Austrian Winter Pea	322.4	98.5*	66.2*	161.7*
Bell Pepper	53.3	0*	0*	0.1*
Cereal Rye	313.1	176.7	100.9*	174.4
Collard	345.2	115.9*†	31.3*	45.8*
Cowpea	181.5	46.8*	38.2*	11.6*
Crimson Clover	357.1	304.3†	129.8*	168.8*
Cucumber	357.1	345.3†	315.9*	315.9*
Okra	980	98.3*	55.2*	21.1*
Onion	69.2	0.5*	1.9*	0.4*
Sweet Corn	322.4	78.1*	100*	57.6*
Tomato	280.8	47.4*†	0.1*	0*
Turnip	357.1	124.2*	0.8*	25.9*
Winter Wheat	357.1	304.0	212.5*	271.0

Indicates values within a row significantly different from the control at $P \leq 0.05$ (Dunnett's Multiple comparison procedure). † Indicates values within a row significantly

different from cereal rye fresh dried tissue at P ≤0.05 (Dunnett's multiple comparison procedure).

In both sunnhemp treatments, it was observed that the radicle length in several species Was significantly smaller than in the control. Pictures were taken (Figure 3) but no data were Recorded. Only cucumber seedlings are shown as an example of the growth reduction.

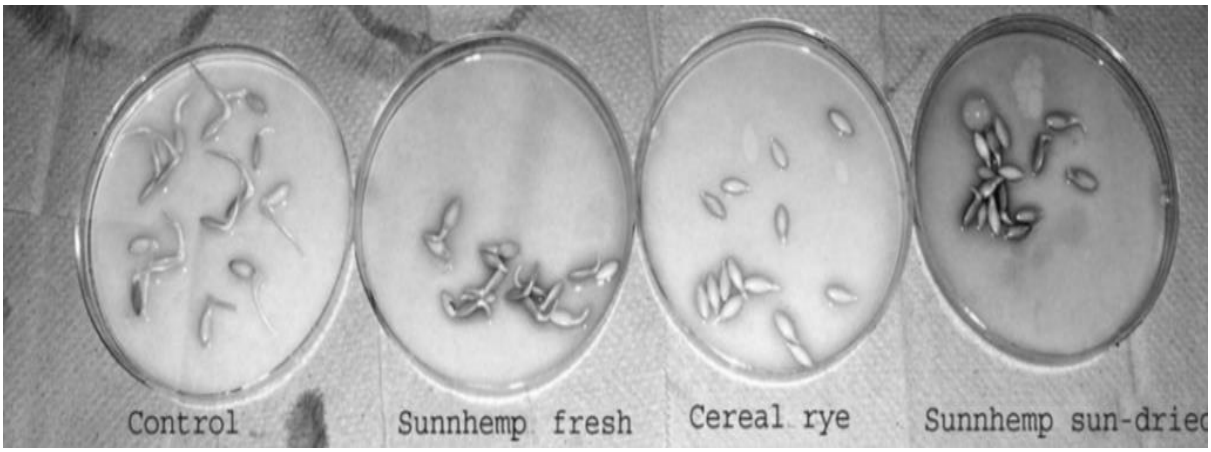

Figure 1 Cucumber seed showing radicle length reduction in sunnhemp and cereal rye leaf Extracts.

The osmotic potentials of the extracts were low, and probably had no significant effect on Germination (Table 11). The pH of the extracts was within the normal range for vegetable crop

Table 11 Osmotic potential and pH of leaf tissue extracts.

Tissue source	Osmotic Potential (MPa)	pH
Sunnhemp fresh	-0.11	5.90
Sunnhemp sun-dried	-0.02	6.05
Cereal rye leaf	-0.11	5.29

CONCLUSIONS

Putnam and Tang (1986) suggest that proving allelopathy or interference exists should be done according to a specific protocol to achieve convincing proof. The first step in this process is 33 to demonstrate interference using suitable controls; describe the symptomology, and quantitate the growth reduction (Punam and Tang, 1986). The results of the present study show that sunnhemp does appear to interfere with seed germination and seedling growth. The possible allelochemicals are produced in the leaf, and can remain chemically active up to 16 days after harvest under dry conditions. Sunnhemp has been reported to be an excellent crop for use in a vegetable crop rotation system because of its ability to suppress root-knot nematodes and reniform nematodes for the next crop (Sustainable Agriculture Network, 1998). The ability of sunnhemp to achieve a height of over 1.2m in 60 days when grown under favorable conditions, gives it a competitive advantage over smaller weed species (Rotar and Joy, 1983). The results of this study, give support to the idea that sunnhemp leaves produce allelochemicals which give it a competitive advantage over other plant species. However, because of these allelopathic effects it is necessary to determine if the next crop in rotation will be affected by the sunnhemp residues. Since sunnhemp is more likely to be grown in late summer or early autumn after a summer cash crop, a late autumn cash or cover crop is likely to be the next crop in rotation. A cash crop from the Brassicaceae family could have germination difficulties following sunnhemp as the seeds of turnip and

collard were found to be sensitive to the sunnhemp leaf extract. A cover crop of winter wheat or rye would be a good choice to follow sunnhemp in a crop rotation system because of apparent insensitivity of the seeds of these grasses to the sunnhemp leaf extracts. Other cash crops including members of the Solanaceae, Poaceae, Cucurbitaceae, Malvaceae, Fabaceae, and Alliaceae families were also found to have inhibited germination when exposed to sunnhemp extracts. Crops from these families showed at least some decrease in germination or germination value due to the presence of sunnhemp leaf extract. It is likely that after a winter 34 fallow or an insensitive cover crop planted in the crop rotation scheme after sunnhemp, the allelochemicals contained in the sunnhemp leaves would leach out, so that subsequent crops from these families would be unaffected by the sunnhemp residues. This is a question that merits further research. Putnam and Tang (1986), indicate that alleged allelochemicals represent a myriad of chemical compounds from simple hydrocarbons and aliphatic acids to polycyclic structures. These compounds include a variety of chemicals such as acids, aldehydes, flavonoids, tannins, steroids, alkaloids, and many others. To date all cases of alleged allelopathy that have been thoroughly studied appear to involve a complex of chemicals (Putnam & Tang, 1986). In no instance was one chemical produced, or responsible, for interference by a neighboring plant (Putnam & Tang, 1986). Many members of the Crotalaria genus produce alkaloids known as pyrrolizidine, which are proposed as a possible allelochemical source (Mattocks, 1986). Pyrrolizidine

alkaloids have been known to cause sickness or death in human and livestock populations (Mattocks, 1986). Sunnhemp produces low levels of pyrrolizidine alkaloids in their seeds including senecionine, integerrimine, riddelliine, trichodesmine, and junceine (Hartmann and Witte, 1995). Srungboonmee and Maskasame (1981) fed sunnhemp hay to cattle for four months. The total pyrrolizidine alkaloid content was between 0.001 to 0.01 %. The animals suffered no ill effects and their organs showed no pathological changes. The specific alkaloids found in the leaves were not given. It is possible that the alkaloids that have been found in the seeds could also be found in the leaf tissue. It is unknown if these alkaloids affect weed or crop germination and growth. Sunnhemp leaf residues and leaf extracts were found to produce allelochemicals that affect seed germination and seedling growth in weed, vegetable, and cover crop species. 35 However, more work should be done in a field setting, to confirm that leaf residues allowed to stay on the soil surface, or tilled into the soil would have a similar effect on seedling germination and growth. The hope is that more work will be done to continue the process of identifying the allelochemical(s) produced by sunnhemp.

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