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Evaluation of Crop Seed Powders as Amendments for Purple Nutsedge (*Cyperus rotundus*) Control Compared to the Traditional Herbicide, Roundup

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

EVALUATION OF CROP SEED POWDERS AS AMENDMENTS FOR PURPLE
NUTSEDGE (*CYPERUS ROTUNDUS*) CONTROL COMPARED TO THE
TRADITIONAL HERBICIDE, ROUNDUP

A thesis submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

in

ENVIRONMENTAL STUDIES

by

Eric Betancourt

2015

To: Dean Michael R. Heithaus
College of Arts and Sciences

This thesis, written by Eric Betancourt, and entitled Evaluation of Crop Seed Powders as Amendments for Purple Nutsedge (*Cyperus rotundus*) Control Compared to the Traditional Herbicide, Roundup, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

DeEtta Mills

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Date of Defense: June 19, 2015

The thesis of Eric Betancourt is approved.

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University Graduate School

Florida International University, 2015

DEDICATION

I dedicate this thesis to my parents Gilbert Betancourt and Daisy Spence. Their love and support lightened the mental burden that accompanied this process. To my brother Andrew and my friends, whose company and encouragement kept me motivated. And to Fury and Grizzly who were always there to brighten my day during the times when it was hard to see the light.

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ABSTRACT OF THE THESIS

EVALUATION OF CROP SEED POWDERS AS AMENDMENTS FOR PURPLE
NUTSEDGE (*CYPERUS ROTUNDUS*) CONTROL COMPARED TO THE
TRADITIONAL HERBICIDE, ROUNDUP

by

Eric Betancourt

Florida International University, 2015

Miami, Florida

Professor Krishnaswamy Jayachandran, Major Professor

Purple nutsedge (*Cyperus rotundus*) is a troublesome weed that outcompetes crops and contributes to poor yields. In the past, agriculturalists controlled purple nutsedge by fumigating soil with methyl bromide but the fumigant has since been classified as a controlled substance under the Montreal Protocol. This study evaluated the effectiveness of several alternative purple nutsedge control techniques and compared them with results obtained from the application of Roundup. Concentration treatment effects for the allelopathic seed powders of watercress and turnip were tested in a field trial while seed powders of yellow mustard and sunflower were tested in a potted trial. The allelopathic amendments significantly delayed weed emergence but several factors interfered with long-term effectiveness. Roundup was determined to be the most effective season-long weed control among the treatments consistently leaving the least amount of surviving weeds and underground organs.

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ABBREVIATIONS AND ACRONYMS

AP	Active principle
BCE	Before the Common Era
c.	Circa
CE	Common Era
cm	Centimeter
cm ²	Square centimeter
CO ₂	Carbon dioxide
d	Day
EPA	Environmental Protection Agency
EU	Experimental unit
FIU	Florida International University
g	Gram
h	Hour
ha	Hectare
IARC	International Agency for Research on Cancer
ITC	Isothiocyanate
L	Liter
LC50	50% Lethal Concentration
lb	Pound
kg	Kilogram
kPa	Kilopascal

m	Meter
m ²	Square meter
mg	Milligram
ml	Milliliter
ppb	Parts per billion
ppm	Parts per million
USDA	United States Department of Agriculture
w/w	Weight percent mixture

1. INTRODUCTION

1.1 Significance of Research

A widespread distribution and significant resistance to various control measures has led to the portrayal of purple nutsedge as the world's worst weed (Holm *et al.*, 1991). Purple nutsedge competition adversely affects 52 crops worldwide and presents a serious problem for agriculturalists and landscapers in more than 90 countries. Within the United States, notable plants that produce reduced yields in the presence of the weed include corn (*Zea mays*), peanut (*Arachis hypogaea*), soybean (*Glycine max*), cotton (*Gossypium* spp.), sugarcane (*Saccharum* spp.), strawberry (*Fragaria* × *ananassa*), other assorted fruiting vegetables and agronomic crops, and turf grasses. Purple nutsedge is a nuisance to nearly every crop grown in Florida and acts as the primary culprit behind vegetable production losses in the state which average over \$100 million per year (Kadir and Charudattan, 1997). Studies have shown that purple nutsedge interference causes substantial declines in output for tomato (*Solanum lycopersicum*) (53%), lettuce (*Lactuca sativa*) (54%), garlic (*Allium sativum*) (89%), okra (*Abelmoschus esculentus*) (62%), cabbage (*Brassica oleracea*) (35%), bell pepper (*Capsicum annuum*) (73%), carrot (*Daucus carota* subsp. *sativus*) (39 to 50%), cucumber (*Cucumis sativus*) (43%), radish (*Raphanus raphanistrum*) (70%), beans (*Phaseolus vulgaris*) (81%), and rice (*Oryza sativa*) (43%) (William and Warren, 1975; Okafor and De Datta, 1974, 1976; Keeley, 1987; Santos *et al.*, 1996; Morales-Payan *et al.*, 1996, 1998).

In addition to the weed's considerable capacity to reduce yields of valuable crops, purple nutsedge is also responsible for poor crop quality, impaired irrigation efficiency, and harvesting interference (Swiader *et al.*, 1992; Rao, 2000). The weed can cause further

complications for farmers in the form of mulch deterioration (Kadir and Charudattan, 1997). Purple nutsedge may also act as an alternate host for other deleterious crop pests which could potentially threaten the bulk of harvest (Thomas *et al.*, 1997, 2004, 2005; Martinez-Ochoa *et al.*, 2004; Davis and Webster, 2005).

The spread of purple nutsedge has prompted producers to intensify their focus on weed management and increase the use of traditional chemical herbicides. Chemical herbicides applied to agricultural lands often runoff into surrounding natural ecosystems, poisoning wildlife and polluting waterways. Overexploitation of synthetic herbicides has resulted in the development of weed populations displaying higher levels of resistance to chemical treatment (Puwain, 1982). The assistance of new detection methods has led researchers to find that many pesticides persist in the environment for far longer than previously thought. Particularly alarming is an EPA report affirming that approximately 100,000 of the 1.3 million wells in the United States are contaminated with pesticides, especially considering that pesticides have been linked to cancer epidemics in children and young adults (Fleming, 1987; Weisskopf, 1988).

1.2 Statement of Research

Utilizing the scientific method, my thesis project assesses the performance of alternative control techniques for the eradication of purple nutsedge infestations. The purpose of the study is two-fold: first, to reveal which of three weed control techniques allows for the fewest purple nutsedge plants to persist under field conditions and second, to compare the treatment concentration effects of two potential nutsedge control methods with herbicide application. The three field tested techniques include soil amendment with watercress (*Eruca sativa*) seed powder, soil amendment with turnip (*Brassica rapa*) seed

powder, and application of Roundup. Treatment concentration effects for soil mixture with yellow mustard (*Sinapis alba*) seed powder and soil mixture with sunflower (*Helianthus annuus*) seed powder were evaluated and compared against application of Roundup. Each of the aforementioned techniques was applied to separate experimental units (EUs) infested by purple nutsedge and the results were compared against those of untreated controls.

1.3 Hypotheses

1.3.1 Hypothesis A: Field Trial

I hypothesize that if Roundup is applied to field plots three times then the herbicide treatment should demonstrate the greatest capacity for eliminating purple nutsedge but that the highest concentration (200 g/plot) of the watercress and turnip seed powder amendments should control the weed to a nearly commensurate degree because they contain high concentrations of phytotoxins. I expect purple nutsedge to demonstrate some resilience against the lower concentration treatments with a few weeds persisting in the plots treated with two or fewer applications of Roundup, a modest amount surviving in the plots amended with 150 g of either plant residue amendment, a slightly larger number of weeds surviving in the plots treated with 100 g of the watercress amendment, and the highest weed populations remaining in plots receiving 100 g of the turnip amendment.

1.3.2 Hypothesis B: Potted Trial

I hypothesize that if Roundup is applied to potted purple nutsedge populations then the herbicide treatments will exhibit the highest efficacy for eliminating the weed with all application amounts approaching complete control. As a consequence of

phytotoxic action, I expect low purple nutsedge persistence in pots treated with 150 g of either seed powder or 100 g of yellow mustard amendment, a modest number of weeds to survive in pots treated with 100 g of the sunflower treatment or 50 g of yellow mustard seed powder, and the poorest weed control to be exhibited by pots amended with 50 g of the sunflower treatment.

1.4 Objectives

The objectives of my thesis were to:

- Determine the effectiveness of currently researched alternative methods for controlling purple nutsedge populations in the field.
- Ascertain whether or not field treatments with watercress and turnip seed powder provide outcomes analogous to those achieved in previous potted experiments.
- Find out if the application of Roundup contributes to a greater degree of weed mortality in the field than watercress or turnip plant residue treatments.
- Discover if smaller populations of purple nutsedge endure following the incorporation of ground yellow mustard or sunflower seed into potted soil.
- Produce novel information pertaining to the treatment concentration effects of alternative purple nutsedge controls in a potted experiment.
- Compare the level of weed control afforded by yellow mustard and sunflower seed powder treatments with application of Roundup in a potted trial.

2. LITERATURE REVIEW

2.1 Purple Nutsedge

2.1.1 Biology

Purple nutsedge is a perennial weed with a wide range spanning throughout the tropical and subtropical regions of the world. The weed is able to survive in most regions where the average minimum air temperature is greater than -1 °C (Bendixon and Nandihalli, 1987). Antarctica is the only continent with a climate harsh enough to prohibit purple nutsedge growth. Purple nutsedge has a grass-like appearance but is actually a true member of the sedge family (Cyperaceae). The leaves are dark green, grow close to ground level, and become abruptly tapered at the tips. The leaves are also thicker and stiffer than most grasses and protrude outward in a triangular arrangement from three vascular strands originating from the basal bulb (Figure 1); as the plant matures, additional leaves will continue to develop from the three vascular strands. Purple nutsedge is most easily identifiable by the reddish-brown or purple tinged inflorescence which grows on a central stalk and can reach a height of about 75 cm (Figure 2). The inflorescence is comprised of an umbel of spikes with small dark brown or black seeds and typically develops 7-8 weeks following plant emergence. The weed forms chains of tubers with fibrous roots (Figure 3) which are connected together by an extensive underground network of thin vascular tissues known as rhizomes. Greater than 95% of purple nutsedge tubers form within 45 cm of the soil surface (Stoller and Sweet, 1987). At temperatures averaging above 20 °C and under the influence of light, purple nutsedge buds sprout from nodes along the ends of the tubers and generate one or two

rhizomes which then make their way upward through the soil to produce the basal bulbs (Horowitz, 1972; Groenendaal and Habekotte, 1988).



Figure 1. Triangular arrangement of nutsedge leaves



Figure 2. Purple nutsedge inflorescence



Figure 3. Purple nutsedge tubers, rhizomes, and roots

2.1.2 Tuber Hardiness

Purple nutsedge proliferates effectively because of an ability to propagate asexually via tubers. Seed reproduction is minimally important to the spread of the weed with individual populations generally being descended from only very few plants (Thullen and Keeley, 1979; Horak and Holt, 1986). Purple nutsedge tubers have been known to sprout shoots up to seven times before energy supply depletion making mechanical control of the weed a tedious activity (Kemble *et al.*, 2004). Mechanical control may even contribute to the translocation of tubers to areas where there previously were none (Rotteveel, 1993). Under favorable environmental conditions and in the absence of interspecies competition, one purple nutsedge plant can generate between 10 million and 30 million tubers per ha in a single growing season (Horowitz, 1972). Tubers can persist in a dormant state within the soil for an average of 3-4 years but have been known to remain viable for as long as 10 years (Schonbeck, 2014). Tubers can be made nonviable upon being dried out to a water content of 15% or less, however, the task is reliant on dry weather conditions over potentially unachievable time scales and fatal temperatures may not permeate to tubers located deeper within the soil (Stoller and Sweet, 1987; Webster, 2003). Purple nutsedge populations are commonly suppressed in India before the planting of rice by allowing pigs to uproot and devour the succulent tubers. About 60-75 pigs are reportedly enough to eradicate weed infestation in a 1 ha field (OSWALD, 1997). Ironically, pig control is less applicable on USDA-certified organic farms where free roaming animals must be excluded from fields at least 120 d prior to the harvest crops coming into contact with the soil (Schonbeck, 2014).

2.1.3 Management Issues

Purple nutsedge has earned an egregious reputation for causing yield deficits in a multitude of crop plants because of a unique set of traits which allow the weed to aggressively outcompete most other plants. Purple nutsedge shoots and tubers produce phytotoxins released through root exudation, volatilization, and decaying of plant residues which assist the weed in outcompeting crop plants such as cotton, mustards, barley (*Hordeum vulgare*), and sweet potato (*Ipomoea batatas*) for light, moisture, nutrients, and space (Friedman and Horowitz, 1971; Horowitz and Friedman, 1971; Peterson and Harrison, 1995; Quayyum *et al.*, 2000). Phytotoxins identified in purple nutsedge tissues include ferulic, caffeic, hydroxyl benzoic, syringic, chlorogenic, and *p*-coumaric acids (Alsaadawi and Salih, 2009). The weed converts carbon dioxide into glucose through the C₄ photosynthetic pathway and demonstrates an incredible capacity to withstand unfavorable environmental conditions such as saturated soil and high temperatures (Bendixon and Nandihalli, 1987). As a C₄ plant, purple nutsedge has a higher photosynthetic efficiency compared to C₃ weeds but is remarkably shade intolerant and suffers from diminished growth under closed canopies (Lati, Filin, and Eizenberg, 2011). Nonetheless, shade only compels tubers to enter into a state of dormancy until the canopy dies back or is removed and new shoots can be produced (Holm *et al.*, 1991). A greenhouse trial conducted with 60% shading showed an 80% reduction in purple nutsedge combined shoot and leaf dry weight with a 97% reduction in tuber dry weight (Santos *et al.*, 1997). However, a previous field experiment found that the weed was able to successfully offset the effects of up to 63% shading by producing

taller shoots and doubling leaf length while experiencing no loss in biomass (William and Warren, 1975).

2.2 Methyl Bromide

Historically, agriculturalists controlled the spread of purple nutsedge by fumigating the soil with methyl bromide. Methyl bromide is a broad spectrum preplant fumigant which became popular in the southern United States for its ability to control many of the weed species that infiltrate polyethylene-mulched crop production systems (Duniway, 2002). Unfortunately, methyl bromide was added as a controlled substance to the Montreal Protocol in 1992 as a consequence of its ozone-depleting attributes. The fumigant was gradually phased out of use in developed countries before becoming prohibited in January, 2005 (Riemens *et al.*, 2008). In the absence of the fumigant, purple nutsedge control presents a particularly frustrating challenge as the weed has been shown to readily penetrate polyethylene mulch (Patterson, 1998). For the time being, methyl bromide is still available to some agriculturalists in the U.S. whom have been granted critical use exemptions but it has become imperative that a more economical alternative be identified (U.S. EPA, 2008). The difficulty involved in obtaining methyl bromide with the dearth of supply has driven up the price which in turn has increased the cost of vegetable production in recent years (Bangarwa *et al.*, 2011).

2.3 Glyphosate

2.3.1 Popularity

The spread of purple nutsedge has prompted producers to intensify their focus on weed management and increase the use of traditional chemical herbicides. While most chemical herbicides have been deemed inadequate for purple nutsedge management as a

consequence of either lack of uptake by the weed or potential harm to surrounding crop plants, research indicates that glyphosate is a suitable means for controlling the weed. Glyphosate is an amino acid derivative used as a foliar applied post-emergence herbicide whose isopropylamine salt serves as the active ingredient in Roundup (Wilén, 2010). Roundup is one of the most commonly used herbicides partially because of its broad spectrum and relatively benign reputation compared to other synthetic herbicides. First introduced by Monsanto in 1974, Roundup promptly became one of the world's premier pesticides following the engineering of glyphosate-resistant genetically modified crops (Szekacs and Darvas, 2012). Worldwide, glyphosate is used in more than 160 countries and ranks among the top 15 most widely used pesticides by weight with 1.4 billion lbs applied per year (Grossman, 2015). By 2012, annual use of glyphosate in U.S. agriculture climbed to 300 million lbs with nearly all corn, soy, and cotton receiving treatment (Fernandez-Cornejo *et al.*, 2014; Grossman, 2015). Manufacture of glyphosate is a particularly lucrative industry with global sales of glyphosate-based products exceeding U.S. \$3 billion in 2002 alone (Copping, 2002).

2.3.2 Mode of Action and Efficacy

Upon application to purple nutsedge leaves, glyphosate is absorbed and translocated through the phloem to the primary tuber where the chemical is then transferred to the connected chain of subordinate tubers (Doll and Piedrahita, 1982; Zandstra *et al.*, 1974). The herbicide inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase and impedes the shikimic acid pathway, an important amino acid biosynthesis process for plants and some microorganisms (DellaCioppa *et al.*, 1986). Killmer, Widholm, and Slife believe that upon becoming exposed to glyphosate, plants

allocate a substantial amount of carbon to the shikimic acid pathway which leads to the exhaustion of respiratory substrate and the build-up of ammonia (1981). The rapid accumulation of shikimic acid and ammonia debilitates the weed and ultimately culminates in death. Glyphosate has also been shown to breach chloroplasts, cell membranes, and cell walls, impede photosynthesis, reduce chlorophyll production, and interfere with protein and nucleic acid synthesis (Tymonko, 1979; Lee, 1981). Visual confirmation of damage to purple nutsedge comes within a week and plant death is frequently observed inside of a year. Perceptible impacts of glyphosate toxicity are gradual wilting and yellowing of the leaves and stems (Figure 4) which develops into complete browning of above-ground growth and atrophy of underground roots and tubers (Webster *et al.*, 2008). It is interesting to note that purple nutsedge is recognized as one of few plants that can actively increase its tolerance to glyphosate by rapidly metabolizing the chemical into less toxic α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (Wang, 2001).

Several studies have been published describing the level of purple nutsedge management achieved by the application of glyphosate at varying rates. Zandstra *et al.* (1974) reported that glyphosate at 2 kg/ha decreased weed population density by 26% relative to the untreated control while a 66% decrease was achieved with 4 kg/ha. A 3 year study found that glyphosate at 1.1 kg/ha controlled purple nutsedge foliage 93% and tuber population densities 60% after the first year (Edenfield *et al.*, 2005). The same rate applied during the second and third year controlled foliage by 92% and 100% and tuber population densities by 86 and 100% relative to the nontreated control, respectively. Multiple applications of glyphosate at lower rates (0.42 kg/ha succeeded by 0.28 kg/ha

two weeks later) saw purple nutsedge foliage decline by 74% but tuber population density shrank by only 42% (Akin and Shaw, 2001). Webster *et al.* (2008) tested a range



Figure 4. Browning and wilting of purple nutsedge stalk as the result of glyphosate toxicity

of application rates on mature purple nutsedges and reported that glyphosate administered at a rate of 0.59 kg/ha reduced tuber biomass by 48% more than the untreated control while a rate of 2.57 kg/ha reduced tuber biomass by 75%. All tuber production beyond the third-order was restricted by glyphosate at rates of 0.74 kg/ha or higher.

2.3.3 Pros and Cons of Use

As is the case with the majority of synthetic herbicides, glyphosate is convenient and deficient for addressing the problem of weed extirpation. A single application of

glyphosate is enough to partially mitigate the impact of weeds for an entire growing season. The herbicide is easy to use and can be accurately applied to target plants with a CO₂-pressurized backpack sprayer. The chemical is inexpensive to synthesize allowing market prices to stay low and extending accessibility to poor farmers. However, the tendency for glyphosate to adsorb strongly to soil severely hampers its preemergence herbicidal activity (Schuette, 1998). Use of glyphosate is thus limited to controlling purple nutsedge populations that have already begun to germinate and cause damage to nearby plants. A large profusion of consumers erroneously believe that glyphosate applied to fully established purple nutsedges will immediately kill the tubers. In reality, it may take years of glyphosate applications to eradicate an established purple nutsedge population because the weed significantly reduces translocation of the herbicide from foliage to tubers upon reaching maturity at the eight-leaf stage (Wilén, 2010). The chemical works optimally for suppressing purple nutsedge when the weed has first germinated or entered the four-leaf stage before attaining subsequent life stages. In addition, glyphosate should not be used if precipitation is expected within 24 h as this may wash off the formulation. Overexploitation of this herbicide as a control measure may also contribute to the development of the pesticide treadmill in the purple nutsedge species. The process starts when a naturally resistant weed survives exposure to the herbicide and passes on its traits to the next generation allowing future populations to make an unencumbered resurgence. Furthermore, secondary pests, whose populations are generally kept in control by other species, may become a significant crop pest if their natural enemies are eliminated by the toxicity of glyphosate (Gliessman, 2007).

2.3.4 Environmental Impacts

Glyphosate possesses myriad unique properties that distinguish it from other synthetic herbicides and allegedly make use of the chemical more favorable for the environment. Although glyphosate is typically applied by spraying the isopropylamine salt, the herbicide will not vaporize and is not harmful to the atmosphere because particles settle gravitationally. Glyphosate accumulates principally in sediments. The chemical strongly adsorbs to mineral clays and organic matter within upper soil layers bestowing a low propensity for leaching or running-off (Schuette, 1998). The herbicide is resistant to chemical degradation but readily and completely decomposes in soil and water under a range of temperatures via microbial degradation (Franz *et al.*, 1997). The average half-life of glyphosate in soil is about 60 d but the chemical has been known to linger for several years in a bound but inactive form (U.S. EPA, 1990). The herbicide may infiltrate waterways through accidental spraying, aerial drift, or less frequently by surface runoff but dissipates quickly from the water column, usually within a week. Glyphosate is minimally retained and rapidly eliminated in the bodies of larger mammals and birds with no concrete evidence to suggest that the chemical bioaccumulates in the food web (Schuette, 1998). Careful application of the chemical onto the leaves of target plants can prevent detrimental effects on surrounding vegetation as less than 1% of glyphosate in the soil is absorbed through plant roots (Ghassemi *et al.*, 1981). In terms of human health impact, Roundup owns a reputation as one of the most benign herbicides on the market because the formulation disrupts activity of an enzyme found only in plants.

Although glyphosate has been touted as an environmentally friendly solution to the purple nutsedge problem, there are still many negative aspects to its use. One of the biggest obstacles to overcome is the inherent non-selectivity of the herbicide. Glyphosate must be applied manually and with great care as the chemical will kill almost any small plant in the vicinity. Glyphosate has been known to leach through sites containing high levels of inorganic phosphates as these compounds exclude the chemical from soil adsorption (Schuette, 1998). The chemical and its formulations have indirectly led to population declines in some species of birds and amphibians through the deterioration and decimation of habitats and food sources (Carlisle, 1988). In fact, destruction of habitat by glyphosate has led to the listing of the Houston toad (*Bufo houstonensis*) as an endangered species (U.S. EPA, 1993). Although uncommon, when the herbicide is applied to agricultural lands in large enough quantities runoff may enter into surrounding natural ecosystems and directly poison small mammals, waterfowl, aquatic wildlife, insects, and microorganisms. Aquatic organisms are more susceptible to glyphosate poisoning because of toxicity amplification in the presence of elevated pH and water temperatures (“Active ingredient fact sheet: glyphosate,” 1996). An experiment conducted to test the toxicity of nine herbicides to soil microorganisms revealed that glyphosate was the second most pernicious to various bacteria, fungi, actinomycetes, and yeasts (Carlisle, 1988). Recent research indicates that glyphosate may also play a role in enhancing the antibiotics resistance of harmful bacteria such as *Escherichia coli* and *Salmonella enterica* serovar *Typhimurium* (Kurenbach, 2015).

2.3.5 Effects on Human Health

Exposure to glyphosate has been known to cause numerous afflictions to manifest in humans. Lower concentrations regularly provoke eye irritation, skin inflammation, and mouth, throat, and upper abdominal pain as well as dysphagia. Disruption of hepatic and renal activities is common as a result of reduced blood flow to the liver and kidneys. Higher concentrations may induce respiratory problems, impaired consciousness, pulmonary edema, ventricular arrhythmia, and metabolic acidosis. An adult will typically succumb to glyphosate toxicity upon ingestion of 85 ml or greater of the chemical (Talbot *et al.*, 1991). Until recently, very little information existed pertaining to how much the general public is exposed to and affected by glyphosate because the U.S. has never required testing for the presence of the chemical on food or in human blood and tissues (Grossman, 2015).

New research is starting to divulge how glyphosate invades human systems and the long-term detrimental health effects of the chemical on humans. Detection of glyphosate residue on produce is common with traces of the chemical being found on 100% of sampled genetically modified soybeans (Bohn *et al.*, 2014). The EPA reported that approximately 100,000 of the 1.3 million wells in the United States are contaminated with pesticides (Fleming, 1987). Two U.S. Geological Survey studies consistently found glyphosate in streams and rain around agricultural areas while a 2012 investigation confirmed that glyphosate is represented among pesticides contaminating groundwater (Roseboro, 2011; Sanchis *et al.*, 2012). Another study found significant concentrations of glyphosate in urine taken from residents of Berlin. All of the samples indicated concentrations of glyphosate for drinking water at between 5 to 20 times the legal limit

(Brandli and Reinacher, 2012). The detection of glyphosate at such high concentrations is disturbing considering that pesticides have been linked to cancer epidemics in children and young adults (Weisskopf, 1988).

Although the EPA classified glyphosate as a Group E oncogen, a substance which is non-carcinogenic for humans, new information indicates that this may likely have been a premature attribution (U.S. EPA, 1993; Seralini *et al.*, 2012). The World Health Organization's International Agency for Research on Cancer (IARC) recently classified glyphosate as a probable carcinogen (Guyton *et al.*, 2015). The IARC uncovered strong evidence to suggest a link between glyphosate and several forms of cancer in animals with limited evidence linking the chemical to non-Hodgkin's lymphoma in humans. Laboratory rats, which have long been used as model test subjects because of their similarities to human biology, experienced interference with an enzyme involved in testosterone production as well as a heightened prevalence of tumors, organ deterioration, and mortality after consuming glyphosate (Walsh *et al.*, 2000; Antoniou *et al.*, 2012). Exposure to glyphosate also promotes the development of functional abnormalities within pregnant rats and birth defects in their offspring (Daruich, 2001; Seralini *et al.*, 2012). Glyphosate is known to be lethal to human chorionic placental JAr cells responsible for synthesizing the hormone progesterone at a concentration of only 1 mg/L in water (Young *et al.*, 2015). Lastly, a correlation has been drawn between exposure to Roundup and the incidence of Parkinson's disease (Negga *et al.*, 2012).

Predictably, several studies have found Roundup to be far more toxic than glyphosate alone. Prolonged exposure is reported to strengthen Roundup toxicity. Benachour *et al.* (2007) revealed that after 72 h of exposure, the differential toxicity

between glyphosate and Roundup increased by a factor of 5. The LC₅₀ for Roundup on human cells after 24 h of exposure was measured at approximately 63 ppm (Mesnage *et al.*, 2014). Another study found that rats exposed to Roundup at a concentration of only 0.1 ppb exhibited a high incidence of negative health effects and mortality after 2 years (Seralini *et al.*, 2013). Mesnage *et al.* (2014) theorized that the adjuvants used in Roundup formulations may be responsible for bioaccumulation of the herbicide within living tissues.

2.3.6 The Role of Adjuvants

Critical to our understanding of the toxicity and effects of prolonged exposure to herbicides is the role of adjuvants within their formulations. Adjuvants are essential to the proper function of an herbicide's active principle (AP) for several purposes such as improving solubility, inhibiting deterioration, and breaching cell membranes (Marutani and Edirveerasingam, 2006). Adjuvants used in pesticide formulations are classified as inert and are generally kept confidential by the manufacturers. Adjuvants are not subject to the same long-term toxicological regulatory experimentation as the AP because of the assumption that the AP should be the most toxic compound within a pesticide formulation. The acceptable daily intake, or maximum amount of pesticide residue which is considered safe in contact with organisms and the environment, is determined through toxicological testing of the AP. Nonetheless, many pesticide formulations have been proven to be several times more toxic than the AP alone (Mesnage *et al.*, 2014). Although glyphosate has a low toxicity compared with the APs of other traditional herbicides, the widely used formulation of glyphosate with the surfactant polyoxyethylene amine is considerably more toxic ("Active ingredient fact sheet: glyphosate," 1996). Many

glyphosate-based herbicides contain ethoxylated adjuvants which are reported to be 10,000 times more toxic than glyphosate (Mesnage, Bernay, and Seralini, 2013). The authors attribute the augmented toxicity to alterations in membrane and mitochondrial respiration brought on by adjuvants. Monsanto consistently insists that Roundup is among the most benign pesticides on the market. However, testing the cytotoxicity of several pesticides on embryonic, placental, and hepatic human cell lines showed Roundup to be 125 times more toxic than glyphosate alone (Mesnage *et al.*, 2014). Furthermore, the study found Roundup to be the most toxic among the six herbicides and insecticides used.

2.4 Allelopathic Compounds

2.4.1 History of Allelopathy

Some of the most promising currently researched alternative controls for purple nutsedge involve the utilization of naturally occurring allelopathic compounds. Allelopathy is best defined as any positive or negative impact wrought on the growth characteristics or development of an organism as a consequence of secondary metabolite interference originating in separate plants, algae, bacteria, or fungi (Mallik, 2005). The general notion that a plant can produce chemicals which directly influence the growth characteristics of surrounding plants has been understood since c. 370 BCE with evidence of Greeks and Romans applying rudimentary knowledge of the phenomenon in their agricultural practices as early as 64 CE (Willis, 1985; Willis, 1997). Over time, horticulturalists observed that repeatedly cultivating specific crops in proximity to one another would result in the production of inferior yields, an anomaly attributed to “soil sickness” (Mallik, 2005). Centuries of experimentation and subsequent adjustment in

cultivation strategies allowed horticulturalists to build an understanding of how specific plants, and more recently algae, bacteria, and fungi, interact with each other. The term “allelopathy” was coined in 1937 by Austrian plant physiologist, Hans Molisch, to describe this process (Molisch, 1937). The utilization of allelopathy in agricultural systems presents an intriguing alternative to the use of environmentally detrimental synthetic herbicides. Allelopathic compounds can be exploited for biologically based weed control systems in a variety of ways including surface mulching, incorporation of plant residues into soil, application of aqueous extracts, crop rotation, smothering, and intercropping (Cheema and Khaliq, 2000; Narwal, 2000; Singh *et al.*, 2003; Sati *et al.*, 2004; Iqbal and Cheema, 2007a; Iqbal and Cheema, 2007b).

2.4.2 Brassicaceae Plants

Several allelopathic controls for purple nutsedge have been studied in the last two decades but there is no consensus as to which can be regarded as the most effective. Plants of the Brassicaceae, or crucifer family, have been utilized as cover crops and green manures for decades because of their ability to suppress crop pests such as weeds, nematodes, insects, and soil-borne pathogens (Earlywine *et al.*, 2010). Incorporation of Brassicaceae tissues into the soil also provides other ecological services such as nutrient recycling, moisture retention, improvement of soil tilth, and mitigation of erosion (Bangarwa, 2011). Brassicaceae plants generate secondary metabolites called glucosinolates which are biologically inactive within living plant tissues under normal conditions because they are compartmentalized in vacuoles (Vaughn and Boydston, 1997). When plant tissues become damaged, as through herbivory, glucosinolate degradation is catalyzed by enzymes of the family myrosinase upon coming into contact

with moisture. The process breaks glucosinolates down into glucose and an unstable aglucone, which is then subjected to Lossen rearrangement, producing several allelopathic compounds including isothiocyanates (ITCs), nitriles, ionic thiocyanates, epithionitriles, and oxazolidinethiones (Brown and Morra, 1997; Bones and Rossiter, 2006). Phytotoxic ITCs are both the most prevalent and the most important of the compounds produced in the hydrolyzation process (Messiha *et al.*, 2013). Isothiocyanates are known to not only stymie weed growth and delay emergence but also restrict germination while seeds are still dormant (Vaughan and Boydston, 1995). Studies have shown that glucosinolates produced by Brassicaceae plants are most highly concentrated within the seeds suggesting that utilization of seed powders would confer the greatest effectiveness as a natural herbicide (Fahey, Zalcmann, and Talalay, 2001; Velasco *et al.*, 2008).

2.4.3 Crop Injury

As with many other forms of biocontrol, the utilization of glucosinolate-derived allelochemicals is not yet an exact science and there is still much to be learned about specific ecological interactions. Negative impacts on crop growth characteristics and development present a major limitation with the exploitation of these phytotoxins for pest control. A 1997 study by Vaughn and Boydston found that wheat (*Triticum aestivum*) germination rates were impaired when the seeds were sown into pots amended with chopped yellow mustard, black mustard (*Brassica nigra*), or garden cress (*Lepidium sativum*). Furthermore, the same study reported that allyl-ITC, the primary volatile generated by black mustard and brown mustard (*Brassica juncea*), inhibited seed germination of wheat, corn, soybeans, cucumber, rapeseed (*Brassica napus*), alfalfa

(*Medicago sativa*), and dandelion (*Taraxacum officinale*) to the same degree as the commercial soil fumigant methyl-ITC (Vaughn and Boydston, 1997). The crushed leaves of black mustard and brown mustard plants are also known to produce volatiles that hinder seed germination of lettuce (Oleszek, 1987). Ionic thiocyanates originating from yellow mustard tissues severely impede hydroponic growth of bean and tobacco (*Nicotiana tabacum*) (Ju *et al.*, 1983).

2.4.4 Weed Control

The exploitation of Brassicaceae plant-derived allelochemicals has produced many positive results as well. Greenhouse trials found that yellow mustard green manure was capable of suppressing the emergence of various weeds by up to 97% (Al-Khatib *et al.*, 1997). The same study showed that yellow mustard green manure satisfactorily controlled weeds competing with green pea (*Pisum sativum*) up to one month following soil amendment in field trials. The use of chopped tissues of yellow mustard, brown mustard, black mustard, rapeseed, garden cress, or leafy turnip (*Brassica campestris*) as green manures successfully inhibited germination of hemp sesbania (*Sesbania exaltata*) and reduced fresh weight by greater than 95% (Vaughn and Boydston, 1997). A greenhouse study by Peterson *et al.* (2001) showed that ITCs released by turnip-rape mulch strongly suppress seed germination of wheat, barnyardgrass (*Echinochloa crusgalli*), spiny sowthistle (*Sonchus asper*), scentless mayweed (*Matricaria inodora*), smooth pigweed (*Amaranthus hybridus*), and blackgrass (*Alopecurus myosuroides*).

Teasdale and Taylorson (1986) explained that sufficient weed control is correlated with both ITC concentrations and length of exposure to the target weed species. A greenhouse study conducted by Bangarwa *et al.* (2010) showed that applying a high

concentration of phenyl ITC (676 ppm) to purple nutsedge grown within sealed containers reduced tuber viability by 97% after 3 d. Complete weed suppression was then achieved with both high (676 ppm) and low (68 ppm) concentrations of phenyl ITC after 7 d and 14 d exposure, respectively. Although several factors can interfere with effectiveness, allelochemicals have provided adequate suppression of purple nutsedge in field trials as well. Purple nutsedge populations under virtually impermeable film mulch treated with 1,500 kg/ha phenyl ITC experienced at least 66% reduced shoot density four weeks after treatment while tuber viability was reduced 72% over the same time frame (Bangarwa *et al.*, 2010).

Allelopathic plant residues have been successfully utilized for weed control while minimizing crop injury. A study investigating the weed control performance of seven Brassicaceae cover crops found that brown mustard residues provided up to 79% control of large crabgrass (*Digitaria sanguinalis*) four weeks after the transplanting of organically grown bell peppers (Norsworthy *et al.*, 2007). The study also found that turnip residues reduced Palmer amaranth (*Amaranthus palmeri*) numbers by up to 48% within the same time frame. None of the glucosinolate-producing cover crops caused more than 5% crop damage to bell pepper. A similar study reported no injury to tomatoes or bell peppers transplanted into soils two weeks after amendment with Brassicaceae cover crops (Bangarwa, 2011). Finally, the incorporation of wild radish into soils infested with yellow nutsedge (*Cyperus esculentus*) drastically reduced tuber weight, cut tuber production by as much as 88%, and actually improved the competitiveness of tomato and bell pepper crops (Norsworthy, 2005).

2.4.5 Watercress, Turnip, and Mustard

Recent research suggests that watercress and turnip residues may present promising controls for purple nutsedge. Messiha *et al.* (2013) conducted two experiments in which seed powder of watercress and turnip were mixed into potted soil at varying rates in order to discover their effects on the growth characteristics and final dry weights of purple nutsedge and maize plants grown together. Both watercress and turnip seed powders were found to substantially inhibit purple nutsedge shoot and tuber growth while minimizing final dry weights. Watercress was found to have slightly better allelopathic potential because of higher glucosinolate and total phenolic content levels measured in the seeds. Low and medium concentrations (25 g/kg soil, 50g/kg soil) of watercress and turnip actually enhanced maize growth and increased total carbohydrate contents within leaves.

Other Brassicaceae plants that may have potential bioherbicidal use for purple nutsedge are the mustards which are high in glucosinolate content (Rice *et al.*, 2006). One study showed that soil amendment with brown mustard seed meal successfully suppressed the emergence of several weeds species including large crabgrass, annual bluegrass (*Poa annua*), buckhorn plantain (*Plantago lanceolata*), white clover (*Trifolium repens*), and common chickweed (*Stellaria media*) by 63% or more (Earlywine, 2010). Brown mustard meal applications at 3% (w/w) proved to be highly effective for early season weed control of redroot pigweed (*Amaranthus retroflexus*) and common lambsquarters (*Chenopodium album*) (Rice *et al.*, 2006). Until now, no studies have been performed investigating the effects of yellow mustard residues on purple nutsedge growth and survival.

2.4.6 Sunflower

Although not of the Brassicaceae family, sunflower produces many allelochemicals such as chlorogenic acid, isochlorogenic acid, α -naphthol, scopolin, annuionones, and most importantly the highly phytotoxic phenolic acids thus conferring intriguing potential as an alternative control for purple nutsedge (Macias *et al.*, 2002; Anjum and Bajwa, 2005). Phenolic acids are known to interfere with plant growth by inhibiting nutrient uptake and moisture absorption through the roots (Blum, Shafer, and Lehman, 1999). One study measuring the total phenolic compounds contained by various fruits, vegetables, and grain products found that sunflower seed produced 1,601 mg of total phenolics per 100 g of seed (Velioglu *et al.*, 1998). Sunflower seed displayed the eighth highest concentration of phenolics among the 28 crop tissues tested and is among the easiest of crops for agriculturalists to acquire. The allelopathic effect of sunflower on other plants has been demonstrated with guar (*Cyamopsis tertragonoloba*), broomcorn (*Sorghum vulgare*), and pearl millet (*Pennisetum americanum*) all experiencing reduced height, biomass, and crop density in the presence of sunflower residues (Batish *et al.*, 2002). Furthermore, a more recent study reported that incorporation of sunflower plant residues into the soil resulted in 40% reduction of purple nutsedge shoot length along with 72% reduction in shoot dry weight and 66% reduction in root dry weight (Matloob *et al.*, 2010).

3. METHODS

3.1 Materials and Set Up

Two experiments were conducted simultaneously during the Fall of 2014 to elucidate which of several treatments would provide the greatest degree of purple nutsedge control. The experiments were performed at the Florida International University (FIU) Organic Garden in Miami, Florida. The first experiment was accomplished under field conditions while the second was a potted experiment located within the shadehouse. Each experiment was organized in a completely randomized design with EUs arranged using a random number generator. In addition, each experiment received three replications to ensure accuracy of data and provide statistically relevant results. Purple nutsedge tubers were harvested from a naturally occurring infestation in a field adjacent to the FIU Nature Preserve. Timberline Soil brand topsoil was chosen as the soil medium for the experiments. Watercress seeds were obtained from Blue Mountain Organics, turnip seeds were purchased from the Hancock Seed Company, yellow mustard seeds were acquired from Food to Live, and Wagner's brand sunflower seeds were procured.

3.2 Field Trial

To accomplish the field experiment, forty 30 cm² plots were exhumed to a depth of 50 cm and then filled with topsoil at a location away from all organic garden plots. Each experiment plot was designed as a raised bed surrounded by a 15 cm buffer zone to provide separation from other plots and prevent possible cross-contamination of treatments (Figure 5). After being cleaned, watercress seeds were ground to a fine powder and immediately mixed into the top 2 cm of soil in twelve plots. Seed powder incorporation techniques were adapted from Messiha *et al.* (2013) and occurred at rates



Figure 5. Pictured above is the site of the field experiment following incorporation of the watercress and turnip seed powder amendments. Each raised mound constituted one EU. EUs were arranged in a completely randomized design with three replications.

of 100 g, 150 g, and 200 g per plot with each concentration receiving three replications.

The entire process was repeated using turnip seeds for twelve of the remaining untreated plots. Next, five dormant purple nutsedge tubers weighing between 0.1 and 0.2 g were sown equidistant from one another 2 cm deep in each of the forty total plots. On day 15 of the experiment, Roundup was applied to the leaves of emerging weeds within twelve untreated plots at a rate of 0.1 g/m². The amount of Roundup administered per application was adapted from Edenfield *et al.* (2005). Another Roundup application was consummated on day 29 to eight of the herbicide treated plots with a third application

occurring on day 43 to four of the same plots. The final four untreated plots served as the control group.

3.3 Potted Trial

In order to carry out the shadehouse experiment, forty plastic pots were each filled with 2 kg topsoil (Figure 6). After being cleaned, yellow mustard seeds were ground to a fine powder and immediately mixed into the top 2 cm of soil in twelve pots. Seed powder incorporation occurred at rates of 50 g, 100 g, and 150 g per pot with each concentration receiving three replications. The entire process was repeated using sunflower seeds for twelve of the remaining untreated pots. Then five dormant purple nutsedge tubers weighing between 0.1 and 0.2 g were planted equidistant from one another 2 cm deep in each of the forty total pots. Holes located at the bases of the twelve pots to receive



Figure 6. Pictured above is the potted experiment prior to treatment (left) and following incorporation of the yellow mustard and sunflower seed powder amendments. Each pot constituted one EU. EUs were arranged in a completely randomized design with three replications.

herbicide treatment were carefully covered with plastic garbage bags to prevent potential leaching to the surrounding organic garden. On day 15 of the experiment, Roundup was applied to emerging weeds within twelve untreated pots at a rate of 0.1 g/m². Roundup was applied again on day 29 to eight of the herbicide treated pots with a third application occurring on day 43 to four of the same pots. The final four untreated pots served as the control group.

3.4 Experimental Maintenance and Data Collection

Water was applied directly to the soil every other day throughout the duration of the experiments to meet optimal requirements for purple nutsedge growth and to avoid potentially washing off treatment in herbicide treated EUs. Foreign plants observed intruding into EUs were removed twice per week. The sites were visited daily to take note of the first day of purple nutsedge emergence within each EU. Final plant counts were tallied following completion of the experiments on day 90 with the longest shoot of each plant being identified, measured, and recorded. Next, the tubers were recovered from the soil and final counts of viable tubers were documented. Techniques for testing tuber viability were adapted from Bangarwa *et al.* (2010). Firmness and pulp color were used as measures to determine the viability of non-sprouted tubers. Rotten tubers and those which were firm yet lacked a white inner pulp were labeled as nonviable. The sprouted tubers and viable non-sprouted tubers were then quantified together to accurately determine the total number of viable tubers per treatment. The tubers were pruned of root hairs and then thoroughly washed before being oven-dried at 70 °C for 72 hours. All tuber dry weights were recorded and averaged per EU. For each of the experiments, treatment replication data was pooled for number of plants and number of

viable tubers in order to calculate the emergence percentages for each group.

Climatological reports for daily and monthly temperature and precipitation for the duration of the experiments were recorded by the National Oceanic and Atmospheric Administration's Miami NWSFO, FL U.S. station and obtained upon request from the National Climatic Data Center.

3.5 Statistical Analysis

All datasets were analyzed using SPSS software. One-way ANOVA tests were performed to compare statistical means between groups and independent-samples T tests were used to assess dataset treatment significance compared against corresponding controls; data was considered significant when $p < 0.05$ and marginally significant when $0.05 \leq p < 0.1$.

4. RESULTS

4.1 Weed Emergence

4.1.1 Field Trial

All of the seed powder treatments tested in the field experiment delayed initial purple nutsedge emergence. A one-way ANOVA between groups revealed a highly significant difference in the average day of weed emergence among plots at the $p < 0.05$ level [$F(9,30) = 91.512$, $p < 0.001$]. Weed emergence was delayed most by the highest concentration of turnip seed powder (200 g/plot) which caused purple nutsedge plants to emerge an average of approximately 15 d later than corresponding control plots. Amendment with the highest concentration of watercress seed powder (200 g/plot) also provided impressive postponement of weed emergence, averaging about 14 d later than controls. Independent-samples T tests run between treatment data and that of controls found that all allelopathic amendments significantly delayed initial weed emergence (Table 1). The average day of weed emergence became later in correlation with increasing concentrations of either turnip or watercress seed powder treatments (Figure 7). No difference was detected for average day of purple nutsedge emergence in plots to be treated with Roundup (a post-emergence treatment) as compared with the controls.

Table 1. Independent-samples T test results comparing field trial allelopathic treatment data for average day of emergence to control data.

Field Trial Delay of Emergence Independent-Samples T Test Results	
Treatment	p-Value
Watercress 100 g/plot	<0.001
Watercress 150 g/plot	<0.002
Watercress 200 g/plot	<0.001
Turnip 100 g/plot	<0.023
Turnip 150 g/plot	<0.001
Turnip 200 g/plot	<0.001

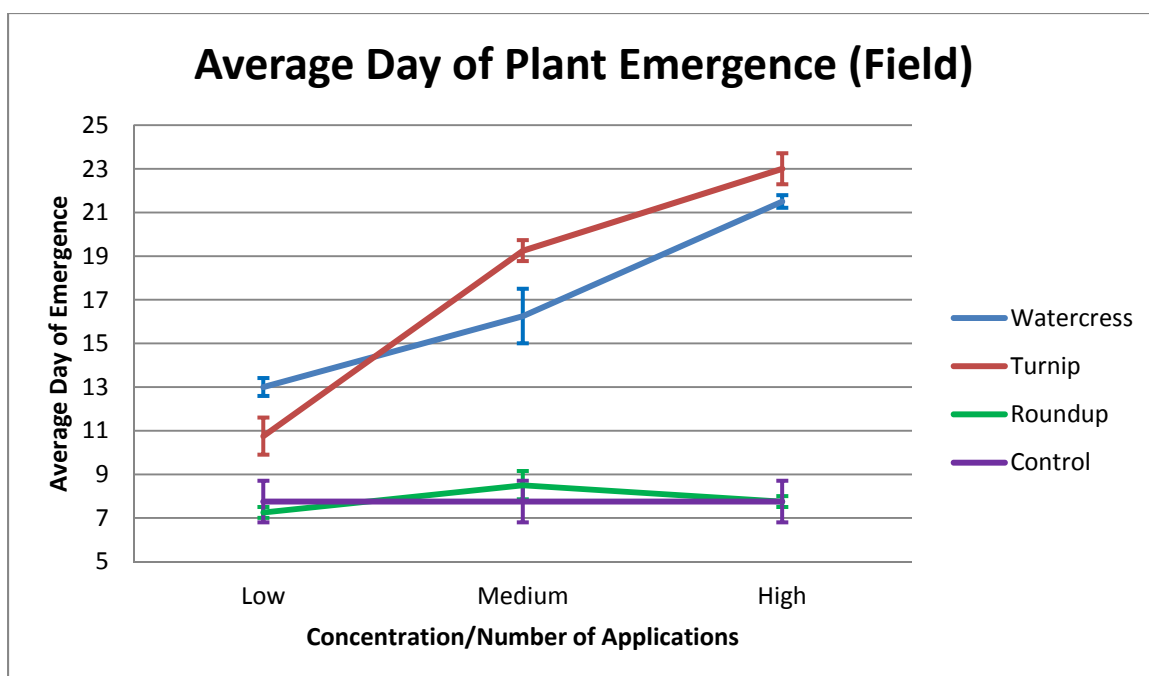


Figure 7. Graph depicting the average day of purple nutsedge emergence in the field following treatment. Allelopathic amendments were applied at low (100 g/plot), medium (150 g/plot), and high (200 g/plot) concentrations. Roundup was applied once (low), twice (medium), and thrice (high). The control is represented as a line by a single value, the mean day of emergence for the group.

4.1.2 Potted Trial

A one-way ANOVA run on data for average day of purple nutsedge emergence in the potted experiment showed a highly significant between-group difference at the $p < 0.05$ level [$F(9,30) = 5.399$, $p < 0.001$]. Mustard seed powder at the highest concentration (150 g/pot) delayed initial plant emergence to the greatest extent, at an average of 4 d later than corresponding controls. Purple nutsedge grown in pots treated with low and medium concentrations (50 g/pot, 100 g/pot) of mustard seed powder broke ground at an average of 1 d later than the controls. Weeds in pots treated with any concentration of the sunflower amendment and pots to be treated with Roundup displayed almost identical temporal emergence compared with controls (Figure 8). Independent-samples T tests performed between treatment and control data revealed that the highest concentration of

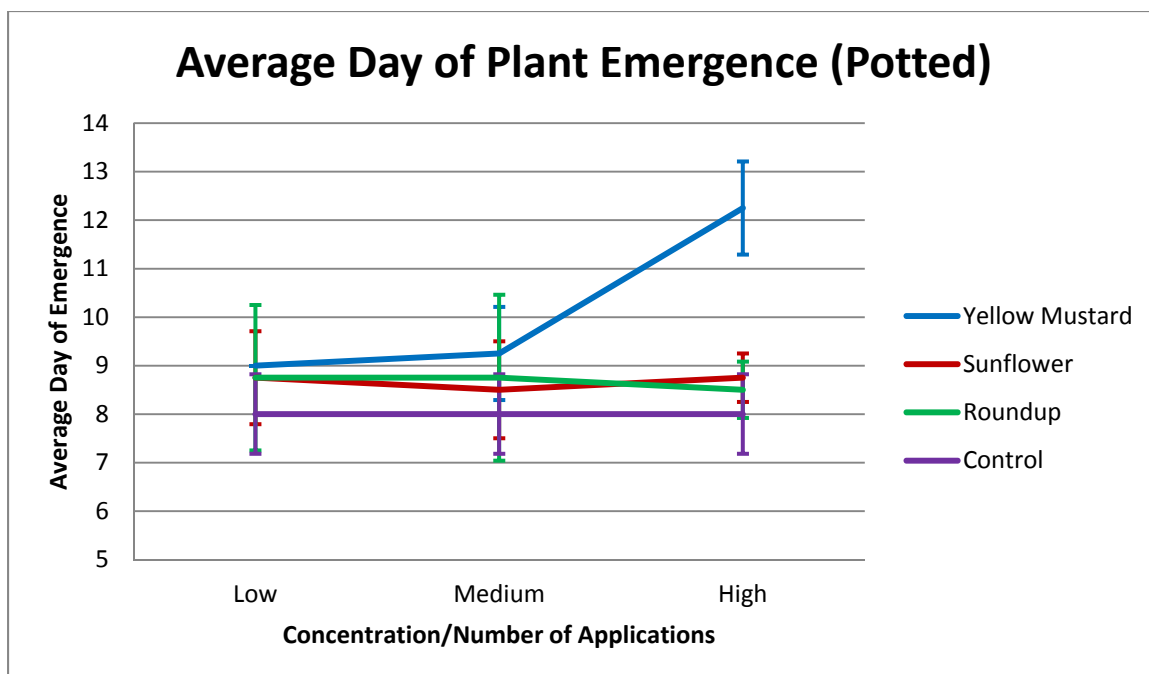


Figure 8. Graph depicting the average day of purple nutsedge emergence in pots following treatment. Allelopathic amendments were applied at low (100 g/plot), medium (150 g/plot), and high (200 g/plot) concentrations. Roundup was applied once (low), twice (medium), and thrice (high). The control is represented as a line by a single value, the mean day of emergence for the group.

mustard seed powder caused significant delay of weed emergence ($p < 0.002$) while low and medium concentrations of mustard seed powder contributed to marginally significant delays ($p < 0.051$, $p < 0.095$ respectively).

4.2 Plant Number

4.2.1 Field Trial

After 90 d, plots treated with three applications of Roundup contained the lowest amounts of living purple nutsedge averaging 11 plants less than the control groups. Plots treated with one or two applications of the herbicide also averaged lower weed counts than the controls. Although all of the allelopathic treatments averaged higher weed counts than the controls, the means plot clearly shows downward trends in the numbers of purple nutsedge per plot with increasing concentrations of either turnip or watercress seed

powder (Figure 9). A one-way ANOVA test showed a significant difference between subjects at the $p < 0.05$ level [$F(9,30) = 3.331$, $p < 0.007$]. However, independent-samples T tests found that only plots treated with medium and high concentrations of watercress seed powder (150 g/plot, 200 g/plot) or three applications of Roundup showed marginally significant differences in weed numbers compared to corresponding control data ($p < 0.094$, $p < 0.068$, $p < 0.068$ respectively).

4.2.2 Potted Trial

At the conclusion of the experiment, pots treated with one or three applications of Roundup completely controlled purple nutsedge while those treated with two applications proved almost as effective. All seed powder treatments sustained thicker stands of purple nutsedge than the controls. The means plot reveals that increasing concentrations of

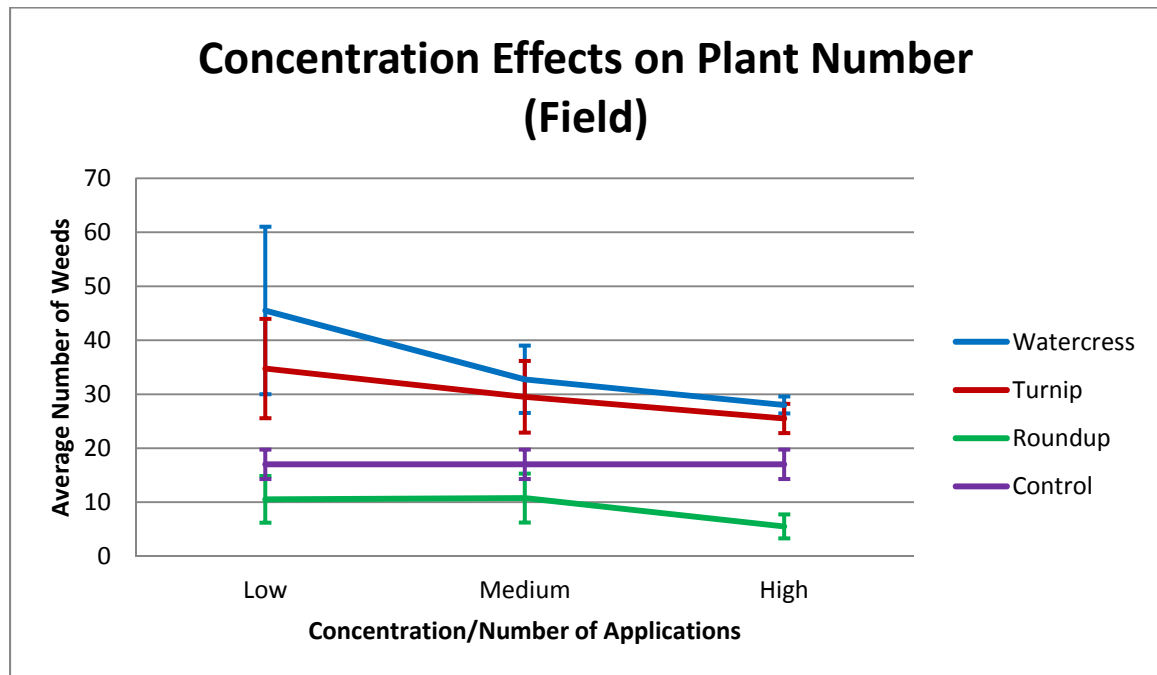


Figure 9. Graph depicting the average number of weeds in the field at the conclusion of the experiment. Allelopathic amendments were applied at low (100 g/plot), medium (150 g/plot), and high (200 g/plot) concentrations. Roundup was applied once (low), twice (medium), and thrice (high). The control is represented as a line by a single value, the mean number of weeds for the group.

mustard seed powder reduced the number of weeds per plot while no distinct patterns were evident for pots treated with sunflower seed powder (Figure 10). A one-way ANOVA showed a highly significant between-group difference in the number of living weeds per pot at the $p < 0.05$ level [$F(9,30)=19.173$, $p < 0.001$]. Independent-samples T tests found significant differences between control data and every treatment except for pots receiving one or three applications of Roundup which produced marginally significant results (Table 2).

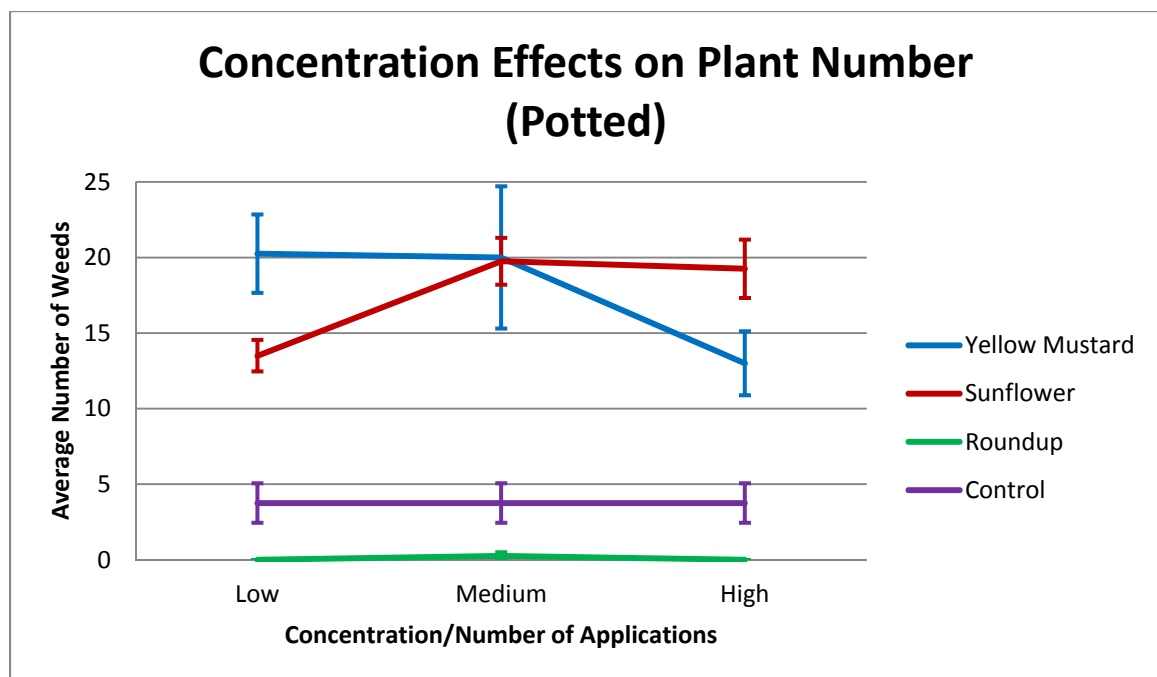


Figure 10. Graph depicting the average number of weeds in pots at the conclusion of the experiment. Allelopathic amendments were applied at low (100 g/plot), medium (150 g/plot), and high (200 g/plot) concentrations. Roundup was applied once (low), twice (medium), and thrice (high). The control is represented as a line by a single value, the mean number of weeds for the group.

Table 2. Independent-samples T test results comparing potted trial treatment data for number of plants to control data.

Potted Trial Plant Number Independent Samples T Test Results	
Treatment	p-Value
Yellow Mustard 50 g/pot	<0.002
Yellow Mustard 100 g/pot	<0.017
Yellow Mustard 150 g/pot	<0.011
Sunflower 50 g/pot	<0.002
Sunflower 100 g/pot	<0.001
Sunflower 150 g/pot	<0.002
Roundup – 1 Application	<0.066
Roundup – 2 Applications	<0.041
Roundup – 3 Applications	<0.066

4.3 Shoot Length

4.3.1 Field Trial

Measurements showed that weeds enduring in plots treated with medium and high concentrations (150 g/plot, 200 g/plot) of turnip or watercress seed powders averaged slightly longer shoot lengths than those in control plots. Plots treated with low concentrations of either seed powder sustained plants with slightly shorter shoot lengths than controls while Roundup treated plots averaged the shortest shoot lengths (Figure 11). A one-way ANOVA run on the data revealed no significant difference between the groups at the $p < 0.05$ level [$F(9,30) = 1.469$, $p < 0.206$]. Independent-samples T tests also found no significant difference in shoot lengths when treatments were compared with controls.

4.3.2 Potted Trial

Weeds surviving in pots receiving any mustard seed powder treatment averaged longer shoot lengths than controls with the data displaying an upward trend correlating with increased concentration (Figure 12). All concentrations of sunflower seed powder resulted in longer shoot lengths compared to controls as well. The shortest shoot lengths

were again recorded from plants in pots treated with Roundup. A one-way ANOVA found highly significant differences between group data at the $p < 0.05$ level [$F(9,30)=17.559$, $p < 0.001$]. Independent-samples T tests run between treatment and control data showed that medium and high concentrations of mustard seed powder (100 g/pot, 150 g/pot) contributed to significantly longer shoot lengths ($p < 0.044$, $p < 0.04$ respectively). No significance was detected for shoot length data from pots treated with any concentration of sunflower seed powder. The T tests also reported marginally significant differences for one or three applications of Roundup ($p < 0.066$, $p < 0.066$, respectively) as these treatments eliminated all purple nutsedge plants.

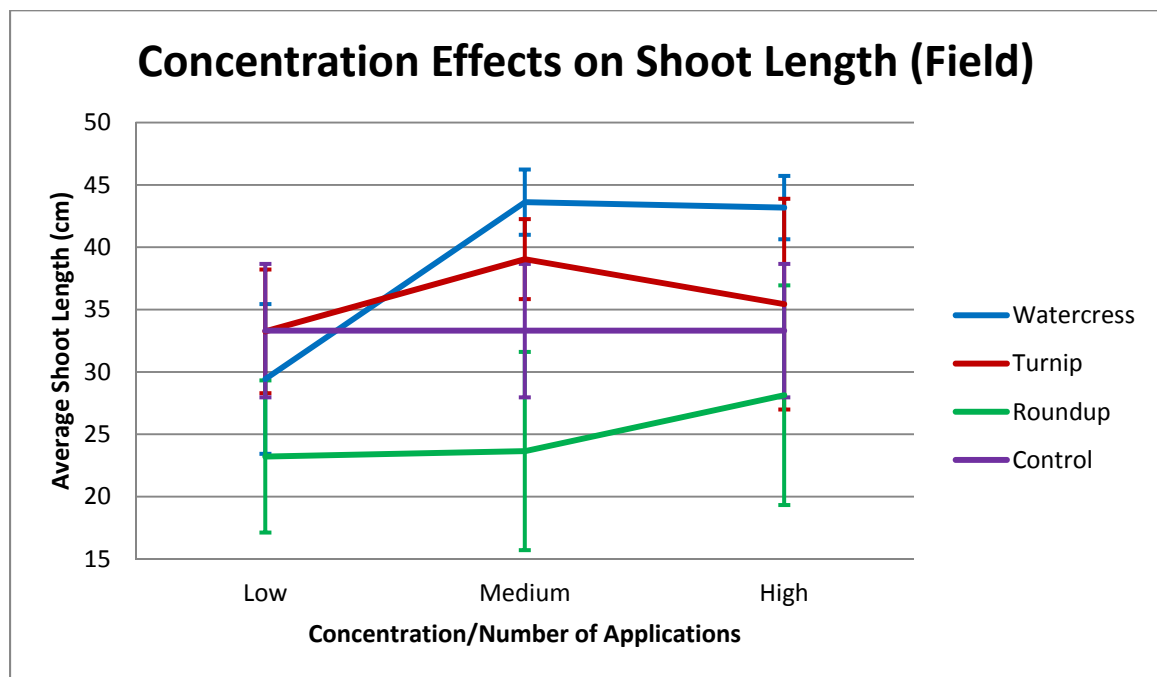


Figure 11. Graph depicting average shoot length of weeds in the field at the conclusion of the experiment. Allelopathic amendments were applied at low (100 g/plot), medium (150 g/plot), and high (200 g/plot) concentrations. Roundup was applied once (low), twice (medium), and thrice (high). The control is represented as a line by a single value, the mean shoot length for the group.

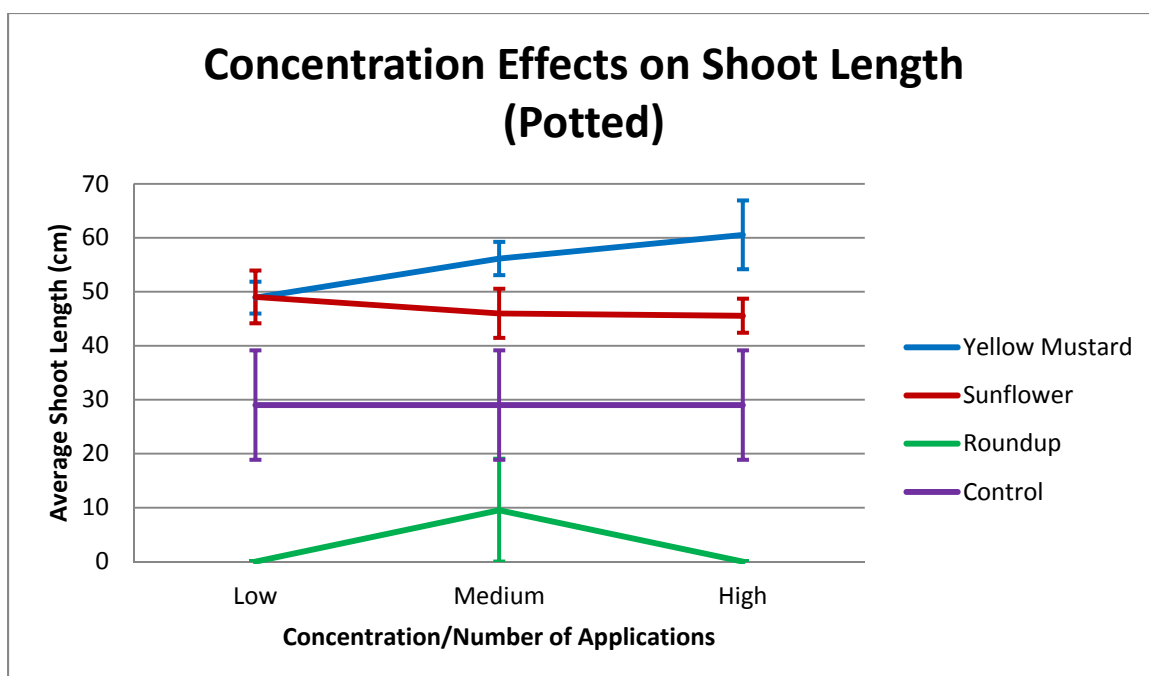


Figure 12. Graph depicting the average shoot length of weeds in pots at the conclusion of the experiment. Allelopathic amendments were applied at low (100 g/plot), medium (150 g/plot), and high (200 g/plot) concentrations. Roundup was applied once (low), twice (medium), and thrice (high). The control is represented as a line by a single value, the mean shoot length for the group.

4.4 Tuber Number

4.4.1 Field Trial

Plots treated with Roundup recorded the lowest averages of remaining viable tubers among treatments and controls. All allelopathic treatments caused higher averages of viable tubers than were found in control plots. The means plot shows distinct patterns of diminishing tuber counts with increasing concentrations or applications of any treatment type (Figure 13). A one-way ANOVA revealed significant differences at the $p < 0.05$ level between-groups [$F(9,30) = 3.628$, $p < 0.005$]. Independent-samples T tests showed that the highest concentration of watercress seed powder (200 g/plot) maintained a significantly greater amount of viable tubers ($p < 0.011$) than controls while plots receiving three applications of Roundup ended up with significantly less viable tubers

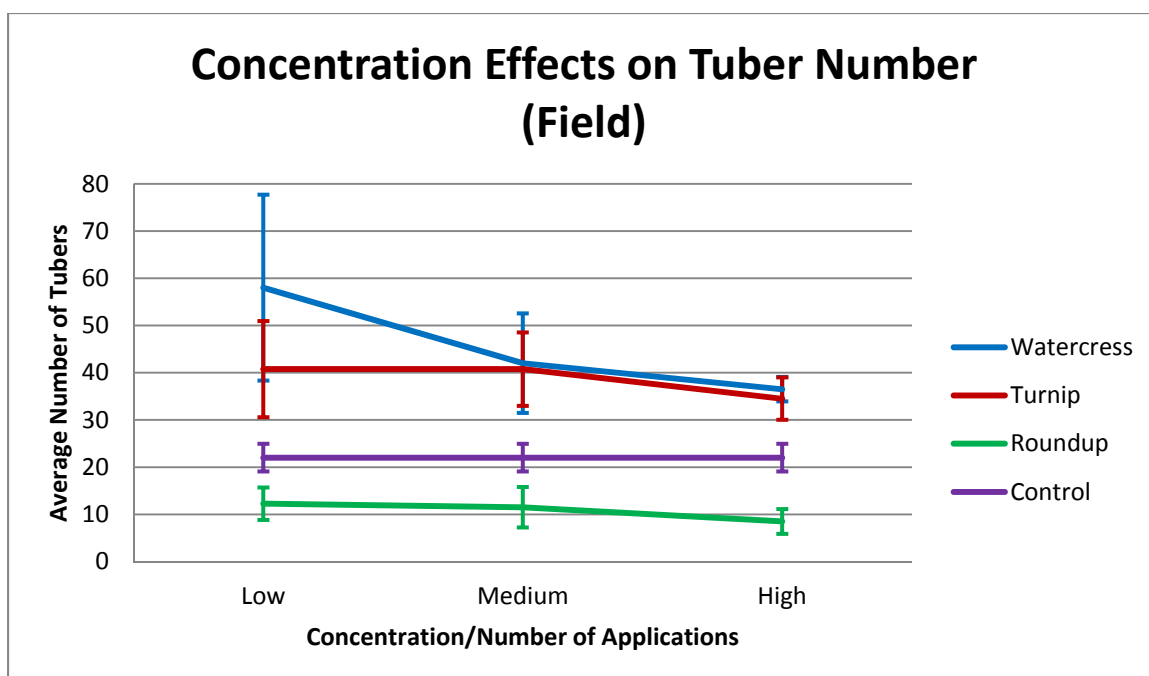


Figure 13. Graph depicting average number of tubers in the field at the conclusion of the experiment. Allelopathic amendments were applied at low (100 g/plot), medium (150 g/plot), and high (200 g/plot) concentrations. Roundup was applied once (low), twice (medium), and thrice (high). The control is represented as a line by a single value, the mean number of tubers for the group.

($p < 0.015$) than controls. Furthermore, the T tests revealed marginal significance for a higher number of tubers recorded in plots treated with medium and high concentrations of turnip seed powder (150 g/plot, 200 g/plot) ($p < 0.066$, $p < 0.06$, respectively) as well as the smaller tuber counts reported for plots receiving one or two applications of Roundup ($p < 0.076$, $p < 0.091$, respectively).

4.4.2 Potted Trial

As was the case in the field trial, Roundup treated plots exhibited the fewest remaining tubers among the groups while all allelopathic treatments averaged much higher numbers of viable tubers than controls. Examination of the means plot reveals a downward trend in the number of viable tubers with increasing concentrations of mustard seed powder while no obvious patterns can be observed for corresponding sunflower seed

powder treatments (Figure 14). A one-way ANOVA testing between-group differences presents high significance at the $p < 0.05$ level [$F(9,30) = 21.26$, $p < 0.001$]. Independent samples T tests revealed all treatment data to be significantly different from control data with the exception of pots treated with one or two applications of Roundup which offered marginal significance (Table 3).

4.5 Average Tuber Weight

4.5.1 Field Trial

Laboratory measurements revealed that tubers recovered from all allelopathic treatment plots averaged slightly lower weights than those recovered from control plots with the exception of plots treated with the lowest turnip seed powder concentration (100 g/plot). Contrastingly, plots treated with any number of Roundup applications averaged

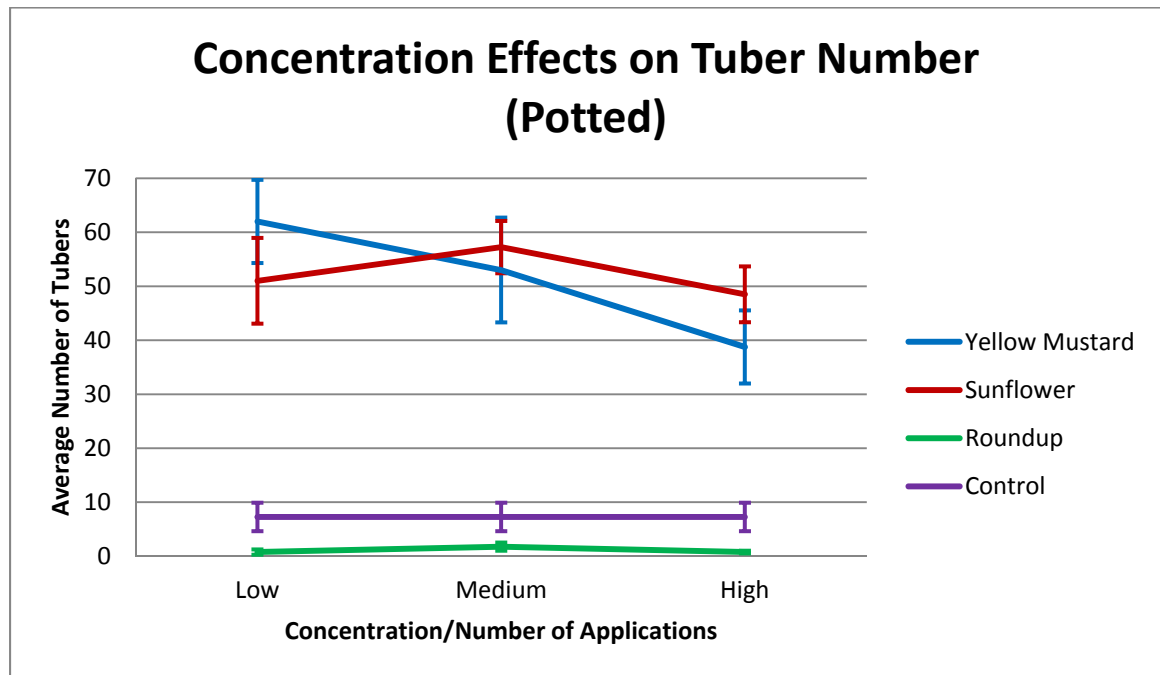


Figure 14. Graph depicting the average number of tubers in pots at the conclusion of the experiment. Allelopathic amendments were applied at low (100 g/plot), medium (150 g/plot), and high (200 g/plot) concentrations. Roundup was applied once (low), twice (medium), and thrice (high). The control is represented as a line by a single value, the mean number of tubers for the group.

Table 3. Independent-samples T test results comparing potted trial treatment data for number of tubers to control data.

Potted Trial Tuber Number Independent Samples T Test Results	
Treatment	p-Value
Yellow Mustard 50 g/pot	<0.002
Yellow Mustard 100 g/pot	<0.005
Yellow Mustard 150 g/pot	<0.014
Sunflower 50 g/pot	<0.003
Sunflower 100 g/pot	<0.001
Sunflower 150 g/pot	<0.001
Roundup – 1 Application	<0.052
Roundup – 2 Applications	<0.092
Roundup – 3 Applications	<0.050

higher tuber weights than corresponding controls (Figure 15). A one-way ANOVA found no significance between group differences at the $p < 0.05$ level [$F(9,30)=1.288$, $p < 0.285$]. Likewise, independent-samples T tests run between treatment and control data showed no significant differences.

4.5.2 Potted Trial

Tubers recovered from all treated pots demonstrated higher average weights than control pots with the exception of those receiving two Roundup applications. Analysis of the means plot uncovers no evident patterns in the data among treatment types (Figure 16). A one-way ANOVA run on the data discloses no significant differences between the groups at the $p < 0.05$ level [$F(9,30)=0.917$, $p < 0.525$]. Independent T tests, however, detect significantly higher average tuber weights for every allelopathic soil powder treatment as compared to the controls (Table 4). The T tests reported no significance for differences between data from Roundup treated pots and control data.

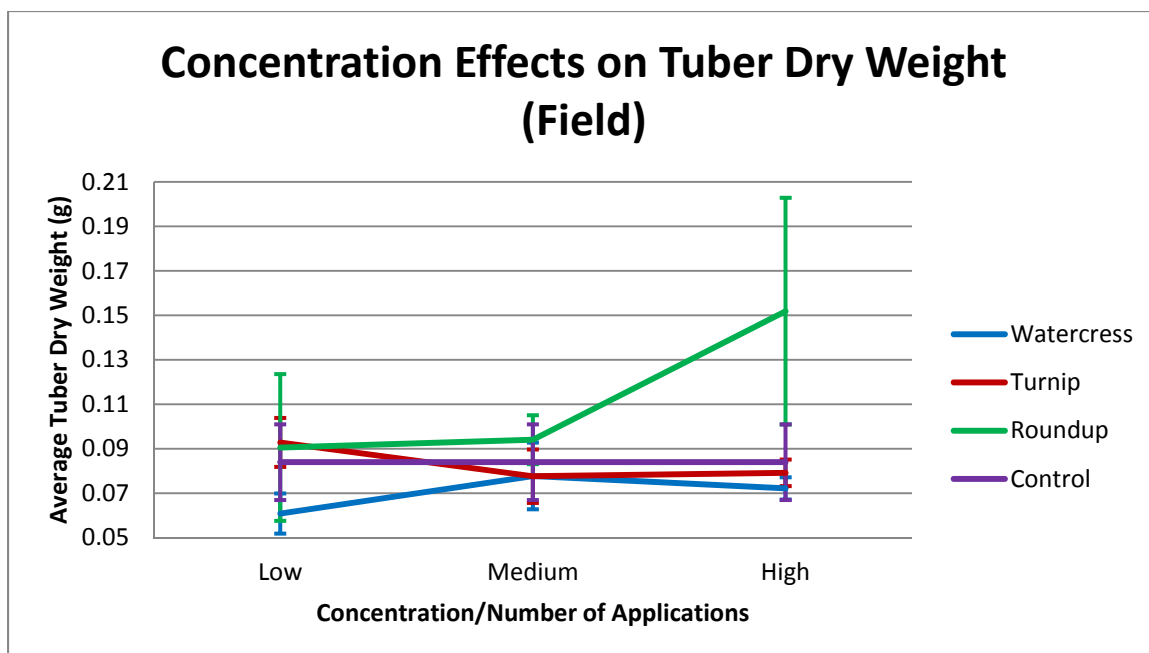


Figure 15. Graph depicting average dry weight of tubers in the field at the conclusion of the experiment. Allelopathic amendments were applied at low (100 g/plot), medium (150 g/plot), and high (200 g/plot) concentrations. Roundup was applied once (low), twice (medium), and thrice (high). The control is represented as a line by a single value, the mean dry weight of tubers for the group.

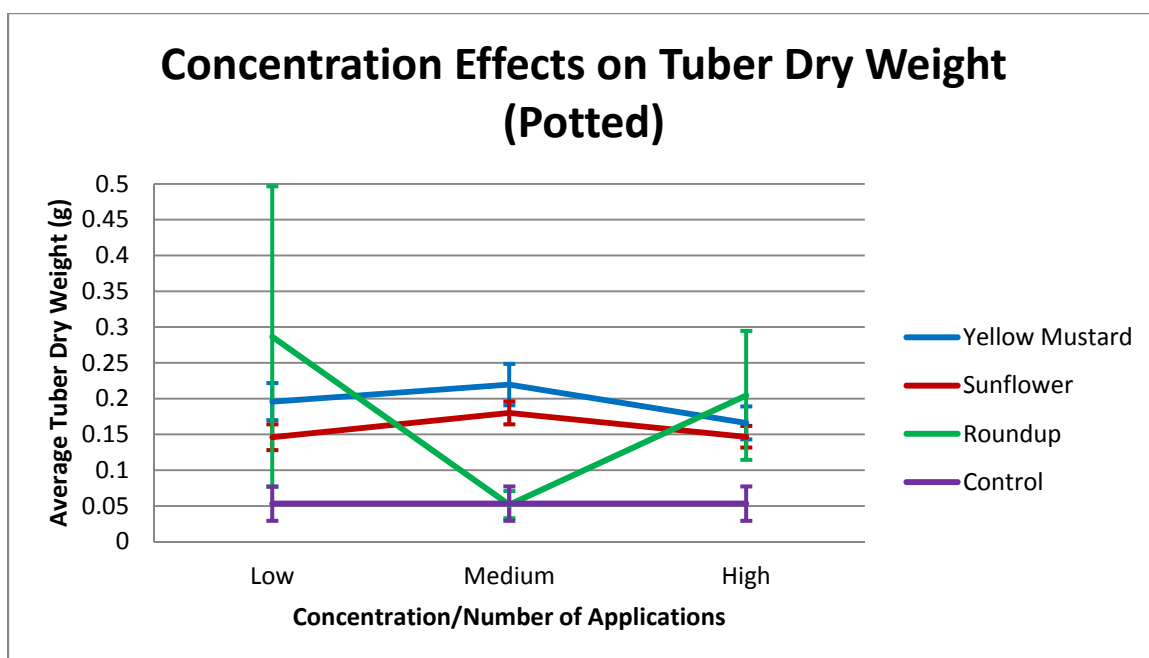


Figure 16. Graph depicting the average dry weight of tubers in pots at the conclusion of the experiment. Allelopathic amendments were applied at low (100 g/plot), medium (150 g/plot), and high (200 g/plot) concentrations. Roundup was applied once (low), twice (medium), and thrice (high). The control is represented as a line by a single value, the mean dry weight of tubers for the group.

Table 4. Independent-samples T test results comparing potted trial allelopathic treatment data for average dry weight of tubers to control data.

Potted Trial Tuber Average Dry Weight Independent Samples T Test Results	
Treatment	p-Value
Yellow Mustard 50 g/pot	<0.008
Yellow Mustard 100 g/pot	<0.005
Yellow Mustard 150 g/pot	<0.016
Sunflower 50 g/pot	<0.022
Sunflower 100 g/pot	<0.006
Sunflower 150 g/pot	<0.017

4.6 Emergence Percentage

4.6.1 Field Trial

In the field trial, emergence percentages for all concentrations of watercress seed powder treatment were extremely similar to controls. The plots treated with the lowest concentration of turnip seed powder (100 g/plot) experienced a high rate of emergence while emergence percentage was reduced compared to controls for medium to high concentrations (150 g/plot, 200 g/plot) of the same treatment type. Emergence percentage was also very high for plots treated with one or two applications of Roundup but much lower for those receiving three applications (Figure 17).

4.6.2 Potted Trial

In the potted trial, all treatments maintained a lower emergence percentage than corresponding controls. All yellow mustard seed powder treatments sustained similar rates of emergence while sunflower seed powder promoted greater emergence percentages with increasing treatment concentration (Figure 18). Roundup applied one or three times completely controlled purple nutsedge while pots receiving two applications collectively only allowed one plant to survive to the end of the experiment.

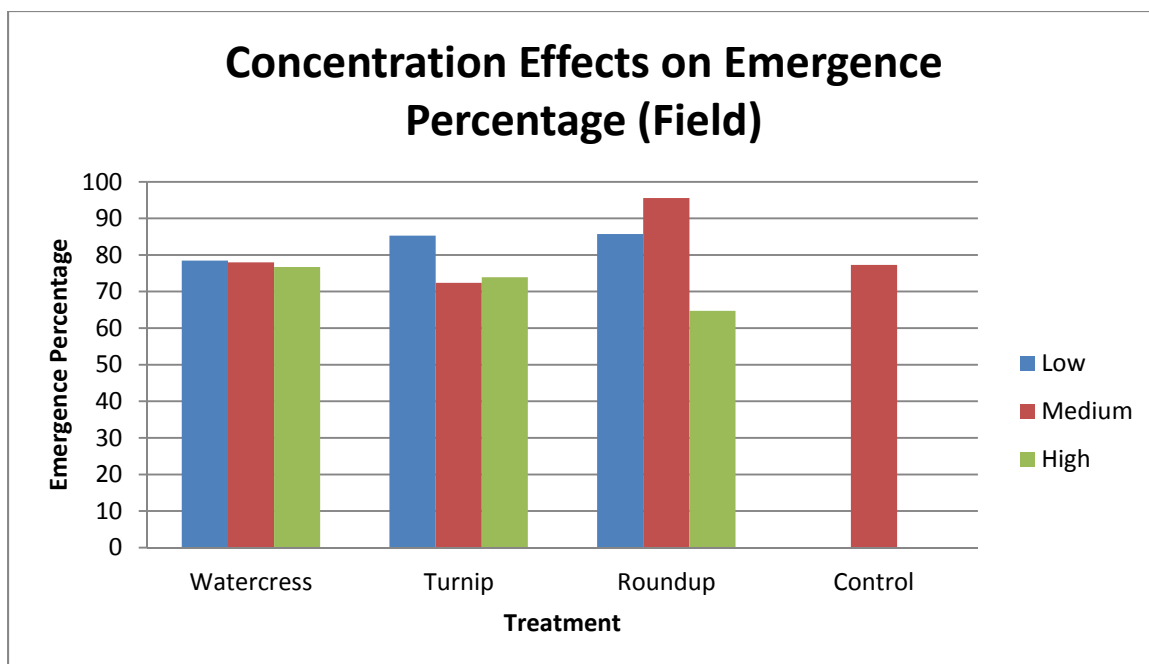


Figure 17. Graph depicting the field emergence percentage of purple nutsedge at the conclusion of the experiment. Allelopathic amendments were applied at low (100 g/plot), medium (150 g/plot), and high (200 g/plot) concentrations. Roundup was applied once (low), twice (medium), and thrice (high). The control is represented as a bar by a single value, the mean emergence percentage for the group.

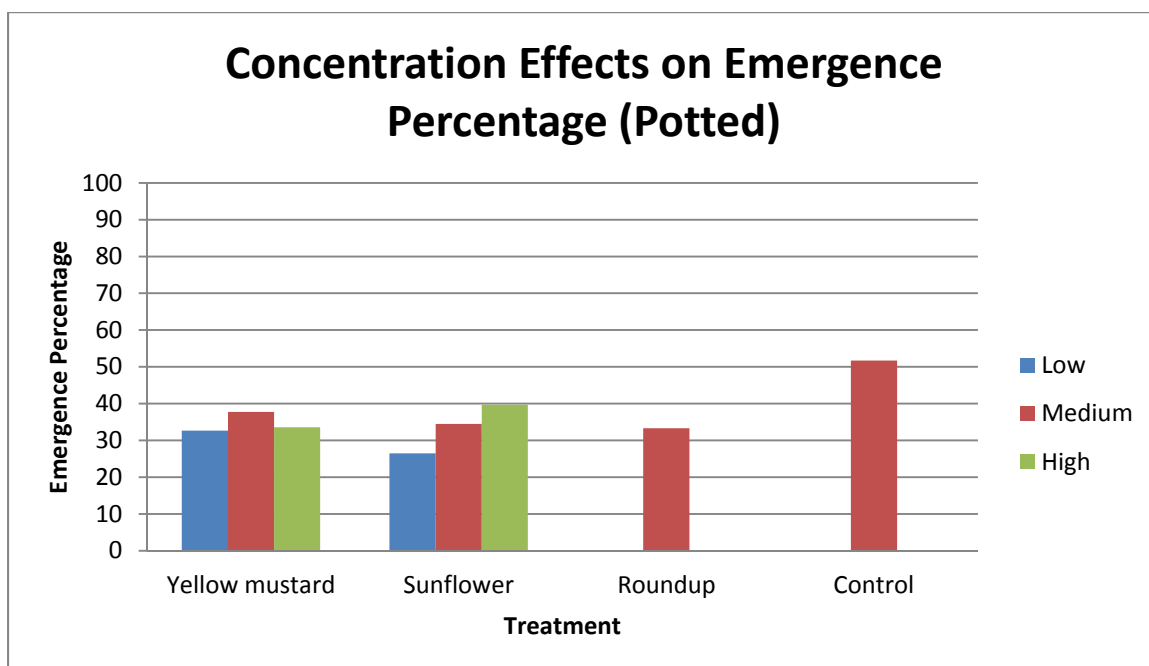


Figure 18. Graph depicting the emergence percentage of purple nutsedge in pots at the conclusion of the experiment. Allelopathic amendments were applied at low (100 g/plot), medium (150 g/plot), and high (200 g/plot) concentrations. Roundup was applied once (low), twice (medium), and thrice (high). The control is represented as a bar by a single value, the mean emergence percentage for the group.

5. DISCUSSION

5.1 Hypothesis A

Results from the field trial partially support my first hypothesis in that Roundup treatments caused the greatest purple nutsedge mortality. Three applications of Roundup provided the best control among treatments with the least remaining individual weeds, underground organs, and shoot lengths (although high variance kept shoot length data from being significant). As expected, Roundup, a post-emergence treatment, held no influence over the average day of weed emergence. Interestingly enough, Roundup treated plots averaged among the highest tuber weights and tended to support high emergence percentages. The outcome can be explained by naturally reduced diffusion of the herbicide to primary tubers upon weed maturation resulting in survival of only the largest, hardiest tubers. Smaller tubers were likely killed outright before entering dormancy within the soil resulting in higher emergence percentages. The amount of Roundup administered per application was lower than the amount that is typically used by the average gardener, landscaper, or agriculturalist. Application of Roundup at slightly higher volumes should easily provide complete control of purple nutsedge stands.

The remainder of my first hypothesis did not prove correct as neither turnip nor watercress seed powder treatments provided satisfactory control over purple nutsedge. Although every allelopathic amendment impressively postponed initial weed emergence in the field, they all bolstered rapid late-season resurgences in weed populations. All concentrations of either seed powder amendment promoted higher averages for numbers of viable tubers and individual purple nutsedge plants by the conclusion of the experiment. Several factors likely played a part in influencing the confounding results

including high temperature and precipitation, nutrient effects, microbial activity, low phytotoxin residence time, and lack of interspecific competition. Shoot length data were inconsistent resulting in inconclusive analysis of this parameter. One positive takeaway from the datasets is that every plant residue treatment produced lower average tuber weights with the exception of the lowest turnip seed powder concentration (100 g/plot). Unfortunately, the most feasible interpretation of the tuber weight findings is that the higher number of tubers within these EUs induced intense intraspecific competition for nutrients leading to stunted growth overall. Finally, the medium and high concentrations (150 g/plot, 200 g/plot) of turnip seed powder demonstrated lower emergence percentages compared to the control group suggesting that specific phytotoxins released from turnip residues may be capable of either encouraging purple nutsedge tuber dormancy or permanently preventing germination.

5.2 Hypothesis B

Results collected from the potted trial partially support my second hypothesis in that Roundup provided the best control of purple nutsedge populations. One or three applications of Roundup were sufficient to completely suppress the weed while maintaining the fewest viable tubers. Two applications of the herbicide provided the next most efficient treatment. Lack of plants resulted in no shoot length data for Roundup treatments applied one or three times as well as 0% emergence. Two applications of Roundup produced the next shortest shoot length data among treatments and exhibited one of the lowest emergence percentages. As in the field trial, tubers recovered from plots treated with one or three herbicide applications averaged much higher dry weights than

control plots. Tubers exhumed from EUs treated with two Roundup applications returned anomalously low weight data and are considered outliers.

Predictions made concerning the efficacy of yellow mustard seed powder treatments as purple nutsedge controls did not reflect actual outcomes. As a Brassicaceae plant high in ionic thiocyanates, yellow mustard residues were expected to have a pronounced negative effect on weed germination and persistence. All concentrations of yellow mustard amendment did in fact delay weed emergence but fostered more rapid weed growth later in the season. At the conclusion of the experiment, pots amended with yellow mustard contained higher numbers of weeds and tubers as well as plants with longer average shoot lengths and lower rates of overall emergence. Downward trends in the number of weeds and tubers per pot corresponding to increasing treatment concentration reveals the lasting allelopathic effect yellow mustard plant residues have on purple nutsedge despite late-season growth. Contrastingly, average shoot lengths were found to increase with higher concentrations of treatment likely as a result of greater amounts of plant available phosphorus added to the soil by the seed powder. The low emergence percentages can be attributed to tuber dormancy or death caused by yellow mustard allelochemicals similar to the effects seen with turnip treatments. Average tuber dry weights for pots treated with yellow mustard seed powder were found to be higher than controls. The unexpected tuber weight findings contradict results obtained in the field trial from plots receiving allelopathic treatments. One reason for this outcome may be that the weeds more efficiently absorbed nutrients added via treatment within the smaller soil profile of the pots.

None of the predictions made for sunflower seed powder performance as a bioherbicide for purple nutsedge were accurate. Experimental results observed for sunflower plant residue amendments were often inconsistent and counterintuitive. Despite containing relatively high levels of allelochemicals, no amount of sunflower seed powder had any discernable effect on delaying weed emergence contrary to several previously reported findings (Narwal, 1999; Batish *et al.*, 2002; Matloob *et al.*, 2010). Furthermore, pots treated with sunflower seed powder finished with higher numbers of weeds and tubers, longer shoot lengths, and higher average tuber dry weights than controls. Emergence rates were found to be lower than corresponding controls but increased coinciding with higher seed powder concentrations. Nutrient availability may have drastically undermined the phytotoxicity of sunflower phenolic acids. Hall *et al.* (1983) showed that phenolic acids and N added to the soil by sunflower plant residues were negatively correlated with pigweed dry weight while levels of P and K were positively correlated. He also reported that amendment with Hoagland's nutrient solution nullified the allelopathic effects of sunflower plant residues. Nutrient stress seems to play a significant role in enhancing the effectiveness of phenolic acids for pest control. Lack of nutrient stress at least partially explains the emphatically positive purple nutsedge response to sunflower seed powder treatments. Carbohydrates, amino acids, and other organic compounds are also known to influence the phytotoxicity of phenolics acids. Research shows that morning glory seedling biomass production is suppressed to a greater degree by *p*-coumaric acid when in the presence of either non-inhibitory levels of glucose or inhibitory levels of methionine (Blum *et al.*, 1993; Pue *et al.*, 1995). The composition of topsoil used in the experiment might also have had a hand in negating

some sunflower seed powder allelopathy. Lastly, the possibility must also be considered that sunflowers concentrate the bulk of their allelochemicals within shoots or other tissues outside of the seed as opposed to Brassicaceae plants.

5.3 Precipitation and Temperature Effects

The increased weed growth observed within treatment plots in the later stages of the experiments was certainly unexpected considering the excellent season-long control recently demonstrated by watercress and turnip residues (Messiha *et al.*, 2013). Efficacy of the allelopathic treatments in the experiments at hand may have been hampered by optimal growing conditions maintained for the weeds throughout the experiment. Excessive waterlogging of the systems was likely a major contributing factor to the poor results. The experiments were carefully planned to begin during the heart of the wet season in order to stimulate immediate allelopathic activity and to accurately gauge treatment viability under climatic conditions often experienced in Florida where purple nutsedge is a major pest for agriculturalists. Starting the experiments in the wet season was deemed reasonable considering the findings of a Morra and Kirkegaard (2002) study showing glucosinolate-to-ITC conversion to be 1.9 times greater in waterlogged soils treated with brown mustard leaf tissues compared to soils with a water content of -32 kPa. Unfortunately, abnormally high levels of precipitation encountered at the very start of the experiments at hand would have leached or washed away large portions of the water soluble glucosinolate degradation products, thereby hindering long-term treatment effectiveness. Heavy rains inundated the trial sites during the first three days of the experiments while the highest amount of precipitation during the growing season occurred over the first ten days. Overall, Miami during August saw 15 cm greater

precipitation than the typical average for the month (Figure 19). Later stages of the experiments saw below average levels of precipitation which were countered with consistent irrigation to ensure that plants had access to sufficient moisture. In addition to the high levels of early precipitation, the trial sites endured temperatures higher than average throughout much of the experiments' duration. In fact, temperatures during August were 1.25 °C higher than the monthly average (Figure 20). The high temperatures and constantly water-saturated soils provided optimal growing conditions for the weeds and contributed to some of the counterintuitive data recorded.

5.4 Nutrient and Competition Effects

The late-season recovery of purple nutsedge in seed powder amended EUs may also have been the result of growth facilitation from supplemental nutrients and organic matter.

Initially, the watercress, turnip, and yellow mustard treatments caused delays in

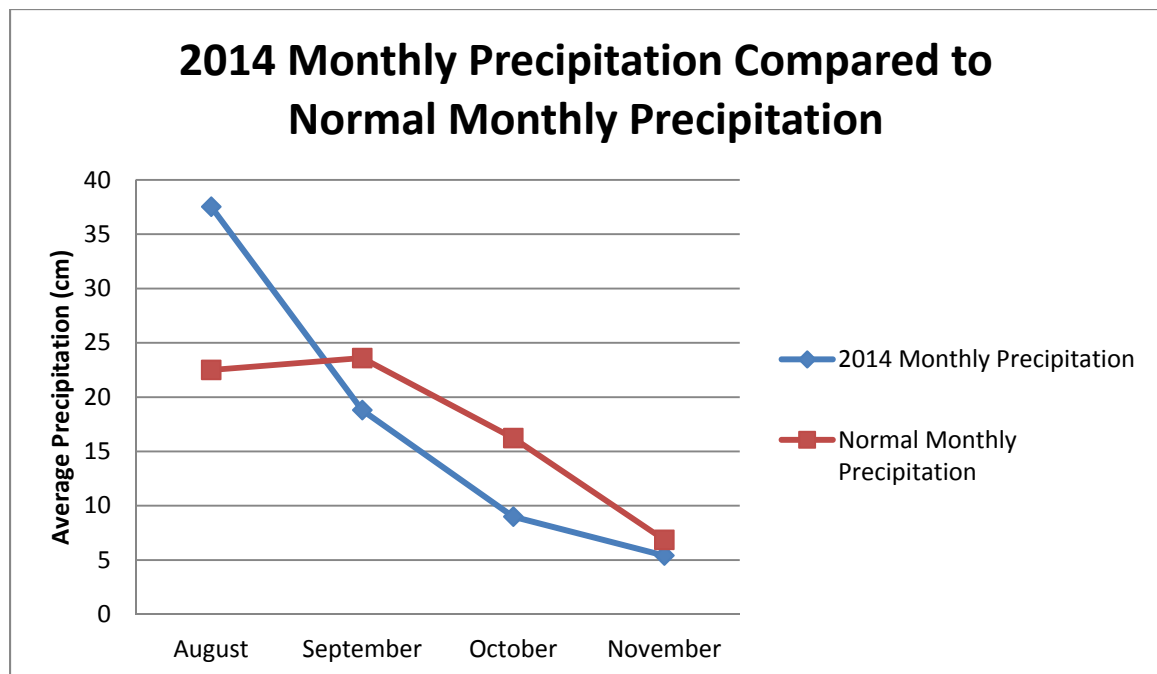


Figure 19. Graph depicting the total monthly precipitation for 2014 compared to monthly precipitation averages.

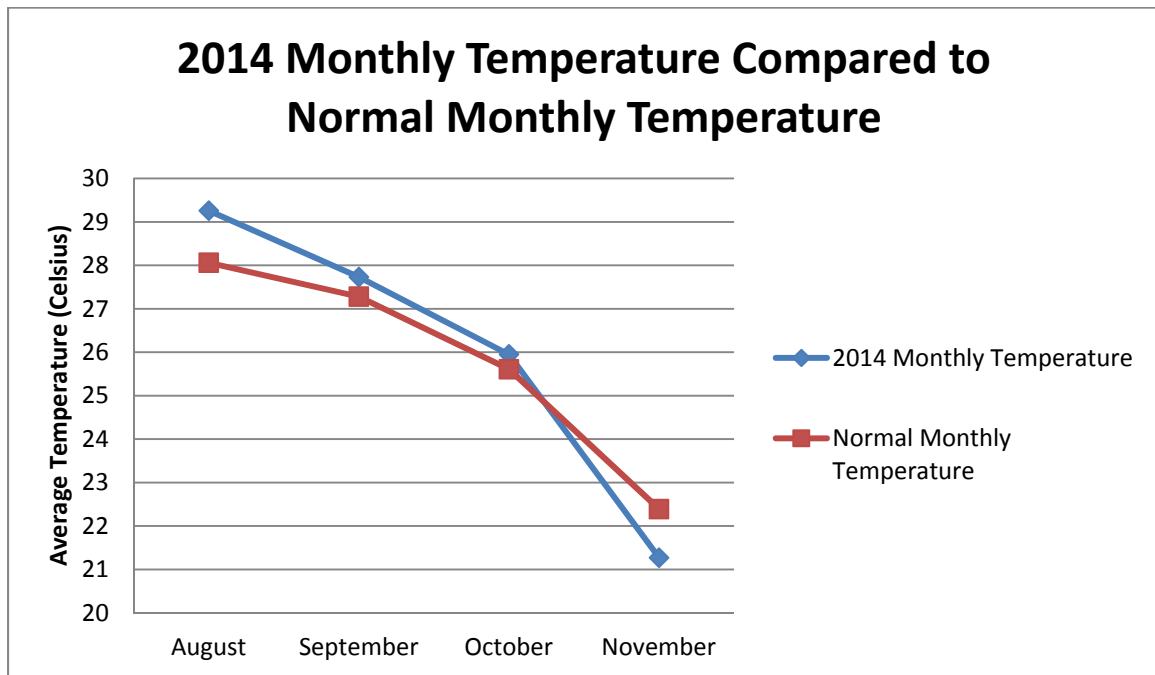


Figure 20. Graph depicting average monthly temperatures for 2014 compared to normal monthly temperature averages.

purple nutsedge emergence. However, following microbial degradation and the washing away of allelochemicals there was nothing to stop the weed stands from rebounding. To make matters worse, the seed powder treatments seem to have actually benefitted the remaining dormant tubers by enriching the soil with nutrients and organic matter otherwise unavailable to the untreated EUs and EUs treated with Roundup. It was not obvious at the outset of the experiments that the purple nutsedge stands would make late-season resurgences since the seed powders of watercress, turnip, and yellow mustard have never previously been investigated as exclusive factors for purple nutsedge control. The techniques for this thesis were adapted from a prior study in which turnip and watercress seed powders were used as soil amendments for purple nutsedge control in pots where corn was also being grown (Messiha *et al.*, 2013). Improved control over

purple nutsedge foliage and tuber growth characters in the study was accomplished by increasing concentrations of either turnip or watercress seed powders with corn growth characters being enhanced at low to medium concentrations (25 g/kg soil, 50 g/kg soil). The large discrepancy between the findings of Messiha *et al.* (2013) and the results of the two experiments at hand can be explained by a lack of interspecies interaction, a factor that was not formerly considered to be an important element to purple nutsedge control. When purple nutsedge is treated with satisfactory concentrations of certain allelopathic plant residues, tuber germination is impeded as a consequence of ITC interference with enzymes important for glycolysis and respiration. Purple nutsedge emergence can also be delayed by the induction of secondary tuber dormancy caused by ITCs applied at lower concentrations (Drobinca *et al.*, 1977; Peterson *et al.*, 2001). In the absence of purple nutsedge, plants exhibiting resistance to the allelochemicals act as nutrient sinks for the supplemental plant available nutrients added to the soil via treatment. The small window of impaired weed growth provided by allelopathic treatment allows crop plants to become quickly established making them powerful late-season resource competitors that can successfully hold off the surviving weeds. For the experiments at hand, intruding foreign plants were consistently removed from the EUs every 3-4 d resulting in absolutely no natural interspecies competition for purple nutsedge.

5.5 Allelochemical Residence Time and Conversion Efficiency

A significant hurdle to overcome with the use of allelochemicals as purple nutsedge bioherbicides is their brief residence time within the soil. The vast majority of ITCs are volatile and highly unstable in soil which leads to relatively rapid dissipation from the environment. According to one laboratory study, allyl ITC produced from

brown mustard seed meal is known to have a short half-life of about 20-60 h within the soil while the half-life for allylnitrile ranges between 80 and 120 h (Borek *et al.*, 1995). Some ITCs can have even shorter half-lives; 2-phenylethyl and n-butyl ITCs produced from chopped turnip-rape mulch displayed half-lives of 16 hours and less than 1 hour, respectively (Peterson *et al.*, 2001). Brown *et al.* (1991) found that ITCs released from rapeseed seed meal can be eliminated from the environment at rates greater than 90% only 24 h after application. Other allelochemicals such as ionic thiocyanates have half-lives ranging from 60 to 120 h as they are quickly removed from the soil profile through processes such as microbial degradation and leaching (Brown and Morra, 1993).

There is evidence to show that Brassicaceae plants generally have low glucosinolate-to-ITC release efficiencies, somewhat hampering their usefulness. Gimsing and Kirkegaard (2006) showed that high glucosinolate containing varieties of rapeseed and brown mustard exhibited ITC conversion rates of 26 and 56%, respectively, 30 minutes after being added to the soil. Of the total glucosinolates incorporated, 7% rapeseed and 13% brown mustard glucosinolates remained unhydrolyzed. Bangarwa *et al.* (2011) took measurements 3 hours after incorporation of seven different Brassicaceae cover crops into plots of soil and discovered extremely low ITC conversion rates of 3-39% for 2-propenyl glucosinolate, 1-11% for benzyl glucosinolate, and 1-10% for 2-phenylethyl glucosinolate. Complete cell disruption is crucial in order to maximize ITC conversion efficiency therefore allelopathic plant tissues should be thoroughly chopped or grinded prior to soil incorporation. Cellular disturbance caused by the freezing and thawing of allelopathic tissues has also been shown to improve ITC conversion efficiency. One study found that freezing and thawing brown mustard leaf tissues

increased ITC conversion rates from 0.03 to 13.7% (Morra and Kirkegaard, 2002).

Freezing and thawing the seeds used in the study at hand prior to grinding could possibly have improved the results obtained.

5.6 Microbial Degradation

Microorganisms will readily metabolize many allelochemicals as they serve as valuable sources of available carbon. Microbial degradation of phenolic acids results in conversion to different phenolic compounds, recalcitrant organic matter, or other organic substances which are typically less phytotoxic than the original phenolic acids (Gerig and Blum, 1991; Blum, 1998). Furthermore, organic molecules exuded from roots into the surrounding rhizosphere provide the microbial community with an additional source of carbon. The stimulation of microbial growth and reproduction in the rhizosphere creates a formidable barrier for allelochemicals to overcome in order to reach root surfaces (Curl and Truelove, 1986). Blum, Shafer, and Lehman (1999) showed that under optimal conditions, microorganisms utilized *p*-coumaric acid at rates 880 times that of soil fixation via non-biological means. Microbial degradation of allelochemicals likely played a major role in the lack of treatment effectiveness for the two experiments at hand as the utilized soils were left unsterilized. In addition to volatilization, microbial degradation, and leaching, soil sorption can play a role in undermining the effectiveness of ITCs (Price *et al.*, 2005; Primo *et al.*, 2003).

6. RECOMMENDATIONS

6.1 Concentration Effects and Multiple Applications

Although complete season-long weed suppression using only glucosinolate-producing plant residues seems unattainable, future studies should continue to test the effects of utilizing higher concentrations of seed powder treatment, especially under field conditions (Haramoto and Gallandt, 2005; Norsworthy *et al.*, 2005). Increased treatment concentrations should improve the longevity of allelopathic compounds in the face of heavy precipitation. Potential downsides would be harm inflicted by the same allelochemicals to surrounding crop plants and delayed sowing dates for crop seeds. Perhaps an even better alternative might be planning for multiple low concentration seed powder applications during the growing season in a fashion which would avoid soil manipulation. Multiple applications can conceivably be accomplished with surface amendments as long as the soil is immediately irrigated to minimize treatment losses to wind erosion.

6.2 Specific Toxicity

Much more information should be gleaned about the specific composition, toxicity, and persistence of the allelochemicals produced by turnip, watercress, and yellow mustard. A better comprehension must also be built regarding precisely which crop and pest species may be negatively affected by their allelopathic properties and to what extent. Scientists have known for quite some time that allelochemicals are selective of certain species causing specific sensitivity for some while having no effect on others. For example, one study testing the weed control capabilities of four Brassicaceae plant residues for soybean found that while the emergence of kochia (*Kochia scoparia*), green

foxtail (*Setaria viridis*), and shepherd's purse (*Capsella bursa-pastoris*) were reduced by all green manures, redroot pigweed was unaffected by brown mustard and velvetleaf was unaffected by brown mustard or rapeseed (Krishnan *et al.*, 1998).

6.3 Allelopathic Plant Residue Mixtures

Broader application of allelopathic plant residues as weed control agents will necessitate incorporation of multiple species into the soil profile simultaneously in order to satisfactorily control a variety of problematic weed species. At the moment, the issue with this approach is that it is unknown whether the new mixture of phytotoxins would affect plant processes in an antagonistic, synergistic, or additive fashion when compared to the effects wrought by the compounds individually (Blum, 1996). An experiment performed to discover the weed control potential of sunflower, leafy turnip, and sorghum (*Sorghum bicolor*) for purple nutsedge found that while each individual treatment reduced final sprouting percentage by 41-45%, sorghum and leafy turnip used in conjunction provided complete control and the combination of sorghum and sunflower provided only 27% inhibition (Matloob *et al.*, 2010). It would be highly advantageous to identify allelopathic plant residues that can provide effective pest control when mixed together at concentrations below their known individual inhibitory levels (Blum, 1996).

6.4 Polyethylene Mulches

Another interesting direction for future research would be the control of purple nutsedge stands using allelopathic seed powders in a plasticulture system. The spreading of low-permeability polyethylene mulches or tarps over treated soils greatly increases the amount of heat retained while minimizing the escape of volatile ITCs during the glucosinolate break down process (Earlywine *et al.*, 2010). Polyethylene mulches allow

allelochemicals to be absorbed more efficiently by purple nutsedge tubers occurring within treated soils. Superb pest control results have been achieved with the combined use of allelopathic plant residues and plasticulture. For example, reduced weed emergence and biomass were reported for barnyardgrass, redroot pigweed, and hairy vetch (*Vicia villosa*) grown in tarped EUs treated with 8,533 kg/ha of yellow mustard seed meal (Hoagland *et al.*, 2008). Greenhouse experiments conducted by Earlywine *et al.* (2010) found that tarping containers treated with brown mustard seed meal decreased emergence of several weed and turfgrass species by up to 50% while reducing biomass up to 57%.

Of course, some polyethylene mulches are more suitable for purple nutsedge control than others. It has been calculated that the half-life of phenyl ITC under low-density polyethylene mulch ranges from 5.82 to 6.37 d while its half-life under virtually impermeable film mulch extends to between 8.34 and 9.42 d (Bangarwa *et al.*, 2010). According to laboratory results, virtually impermeable film mulch also retains methyl ITC 40% better than low-density polyethylene mulch (Austerweil *et al.*, 2006). It has been postulated that virtually impermeable film mulch provides better purple nutsedge control than low-density polyethylene mulch because it is thicker and tougher for weeds and fumigants to penetrate through and because it contains a polamide barrier permitting lower permeability for volatile compounds (Yates *et al.*, 2002; Santos *et al.*, 2007). Clear or translucent polyethylene film mulches present another control measure which may work favorably with allelopathic plant residue treatments. Light exposure through clear polyethylene prompts purple nutsedge to open its leaves before penetrating the film, thus trapping the weed and hindering its growth (Patterson, 1998; Chase *et al.*, 1998).

Impressive purple nutsedge control has also been demonstrated in northern Florida using thermal-infrared retentive plastic film which heats the soil more intensely than clear polyethylene causing tubers to lose viability (Chase *et al.*, 1999).

6.5 Best Management Practices

Before plant residues of turnip, watercress, yellow mustard, or sunflower can be recommended for general use as purple nutsedge bioherbicides best management practices for their use must be established. Further investigations should be carried out to discover the proper amount of time before crop seeds may be sown into areas that have been treated with allelopathic plant residues so that the harvest is minimally affected. For instance, one study showed that 3% yellow mustard seed meal mixed into soil suppressed total lettuce emergence to 3-17% if seeds were sown within 4 weeks of treatment application (Rice *et al.*, 2006). The effectiveness of allelopathic plant residues may drastically differ in higher latitudinal geographic locations where growing seasons are naturally curtailed. The influence of soil type, pH, and salinity on the weed suppression effectiveness of allelopathic plant residues is currently unknown. Finally, considerations must also be made for local soil moisture, climate, and type of irrigation employed in planning for optimal planting dates.

7. CONCLUSIONS

The results of this study indicate that soil amendment with allelopathic seed powders of turnip, watercress, yellow mustard, or sunflower does not provide sufficient full season control over purple nutsedge. Utilization of phytotoxic plant residues cannot be considered as a primary weed management strategy for commercial crop production systems in lieu of methyl bromide, especially when inexpensive, proven herbicides such as Roundup remain on the market. In fact, Bangarwa *et al.* (2010) calculated that an application rate as high as 4,561 kg/ha of phenyl ITC may be necessary to provide control over purple nutsedge infestations equivalent to 390 kg/ha of methyl bromide. The effective use of allelopathic plant residues for purple nutsedge control is further complicated by several factors including local weather conditions, available nutrients, interspecies competition, low allelochemical residence time, inefficient glucosinolate-to-ITC conversion, and microbial degradation. Despite lackluster capability for late-season weed control, soil treatment with Brassicaceae plant tissues can be a valuable tool for agronomic systems and turf installations where traditional synthetic herbicides are not an option. Organically-certified agricultural systems can incorporate the use of allelopathic plant residues into carefully planned integrated pest management systems. Precisely timed applications of allelopathic plant residues would benefit organic farmers by providing early season purple nutsedge control and allowing for the rapid establishment of crop plants. The competitive advantage in nutrient acquisition gained by the crops would further inhibit purple nutsedge encroachment. The process may be expedited if fully mature plants are transplanted into treated areas and multiple lower concentration

surface plant residue applications are achieved during the growing season to minimize crop injury.

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