PURPLE NUTSEDGE BIOLOGY AND VEGETABLE TOLERANCE TO EPTC

by

REBEKAH DANIELLE WALLACE

(Under the Direction of Timothy L. Grey)

ABSTRACT

Studies were conducted from 2007 to 2009 to establish the tolerance of vegetable crops to *S*-ethyl dipropylthiocarbamate (EPTC) and to evaluate temperature variation effect on purple nutsedge tubers. For the vegetable experiments, data evaluated included crop tolerance and weed control. EPTC was applied at three rates with two mulches; vegetables evaluated were pepper, tomato, eggplant, and watermelon. Vegetable crops had varying levels of sensitivity to EPTC. Experiments were conducted to determine the effect of diurnal temperature fluctuation on sprouting of purple nutsedge tubers. No significant differences were noted in cumulative sprouting between any of the temperature variations. Additional experiments were conducted to evaluate for genetically modified cotton resistance to glyphosate and glufosinate. Cotton cultivars were screened with varying schedules of glyphosate and glufosinate. Cotton was tolerant to all application schedules and weed control was dependant on schedule and herbicide resistance.

INDEX WORDS: EPTC, glyphosate, glufosinate, cotton, purple nutsedge, pepper, tomato, eggplant, watermelon

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DEDICATION

To my family and friends, thank you for supporting me for the past two and a half years as I have completed this adventure. Thanks for listening to me, even when you had no clue what I was talking about. Remember, just nod and smile.

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Chapter 1. Introduction

Early accounts of weed management describe many hours spent hand pulling weeds from cropped areas. As technology advanced, tools were introduced to aid humans, first the hoe then animals to pull weed control contraptions (Bryson et al. 1999). During the 1930s and 40s advancements, such as the steel plow and the ability to mount cultivators to tractors, there was a shift from animal labor to mechanical (Abernathy and McWhorter 1992; Ridgeway et al. 1984). Following World War II, the development of the first synthetic herbicides combined with mechanical tillage replaced hand-hoeing in many crops (Bryson et al. 1999). The top three troublesome weeds for fruiting vegetables are nutsedge spp., pigweed spp. and morningglory spp. (Webster 2006).

Herbicides are a vital component for many vegetable crops. For vegetable production, herbicide use varies with crop: fresh snap beans (*Phaseolus vulgaris* L.) 69%, fresh cabbage (*Brassica oleracea* L.) 28%, fresh sweet corn (*Zea mays* L.) 99%, onion (*Allium cepa* L.) 81%, squash (*Cucurbita moschata* L.) 39%, tomato (*Lycopersicon esculentum* L.) 85%, and watermelon (*Citrullus lanatus* L.) 46% (USDA-NASS 2007).

Weed populations are affected by many factors including cropping sequences, herbicide programs, herbicide-resistances, and environmental situations (Bryson 1996; Buchanan et al. 1975; Hoveland et al. 1976; Shaner 1995; Weber et al. 1974). In 1993, a methyl bromide phase out program was implemented by the Montreal Protocol and as of 2005 methyl bromide may only be applied with a Critical Use Exemption (EPA 2008). This has changed the face of vegetable production in many areas and alternative fumigants are being researched for efficacy.

Chapter 2. Literature Review

Vegetable

For over 60 years methyl bromide (CH_3Br) was a common agriculture fumigant (Subbarao 2002). Methyl bromide's (MBr) insecticidal properties were first described in 1932 and it was an important part of land preparation for many crops (Le Goupil 1932). It was mainly used as a soil fumigant, though it also functions well as a fumigant for perishable commodities.

This versatile chemical acts as an acaricide, fungicide, herbicide, insecticide, nematicide, and rodenticide (NPTN 2000). MBr is formulated as a pressurized liquid at 1,420 mm Hg at 20 C (ATSDR 1997). The MBr gas seeps into the soil pores and binds to deoxyribonucleic acid (DNA), lipids and proteins; it is believed to be directly toxic to cells due to observations of damage to multiple cellular sites. As MBr is a gas, it is covered by a mulch barrier for retention and to provide maximum efficacy. MBr is produced naturally, but for commercial uses, it is produced synthetically. The major sources of emission for MBr are oceans, biomass burning, and commercial fumigation (NPTN 2000). Though it is both acutely and chronically toxic, controlled application measures have increased user safety. It is utilized mainly in high-input, high-value crops in both agronomic and horticultural growing systems, primarily in California and Florida.

Methyl bromide was determined by the US Environmental Protection Agency to be a Class I ozone depleting substance. In 1993, a phase out program was implemented by the Montreal Protocol. By 2005, MBr use was limited to Critical Use Exemptions (CUE) only (EPA 2008).

Some current alternatives under examination include chloropicrin, 1,3-dichloropropene (1,3-D), methyl isothiocyanate (MITC) generators (metam sodium and dazomet), and methyl iodide.

Chloropicrin was first used as an insecticide in 1917, and then as a soil fumigant since 1920. It primarily controls soil borne fungi, diseases and nematodes as a preplant soil fumigant (Anonymous 2007). It can be used with MBr as a warning agent, as it has an irritating odor (Extension Toxicology Network 2008). MBr used under CUEs generally include chloropicrin to control a broad spectrum of pests due to synergistic effects (Duniway 2002). There is potential for this chemical to be a stand-alone fumigant as yields of strawberry are 94-96% of that with MBr (Duniway et al. 1997). However, as chloropicrin lacks weed control, herbicides may need to be included in the management program.

1,3-dichloropropene (1,3-D), more commonly known as Telone, is a pesticide used to control nematodes and diseases. Telone was originally marketed as C-17, containing 78% 1,3-D and 17% chloropicrin, and as C-35, containing 63% 1,3-D and 35% chloropicrin (Anonymous 2005). Telone II is also available now with 1,3-D as the only active ingredient (Anonymous 2006c). 1,3-D is a fumigant alternative that has a narrow weed control spectrum and could be utilized more effectively in mixtures with other fumigants or pesticides.

Metham sodium converts to MITC after soil incorporation (Ajwa et al. 2003). It has activity against weeds, nematodes, symphylids, and some soil-borne diseases (Anonymous 2008c). To achieve maximum effectiveness with metham sodium, soil moisture must range from 50 to 80% field capacity with temperatures of 4 to 32 C. California strawberry production has utilized metham sodium, but yields have been significantly lower as compared to MBr and chloropicrin (Duniway 2002). Combining metham sodium with other fumigants has not been successful but a careful sequential program may supplement other pesticide programs (Trout and Ajwa 1999).

Dazomet is another MITC generator and, as with metham sodium, will not be used as a stand-alone commercial fumigant (Duniway 2002). It has similar soil requirements to metham sodium and could be beneficial to other fumigant programs (Duniway 2002).

Methyl iodide is registered for control of weed seeds, nematodes, insects and diseases (Anonymous 2008b). Methyl iodide as a fumigant has equal or greater efficacy against fungi, nematodes, and weeds as compared to MBr (Ohr et al. 1996). However, in a study comparing pest populations in bell pepper-squash rotation, methyl iodide had a significantly greater population of purple nutsedge, as well as a significantly lower pepper yield as compared to MBr (Webster et al. 2001). Further testing needs to be conducted to accurately assess the efficacy and economic feasibility of methyl iodide as a soil fumigant.

In addition to chemical control options, research is also being conducted to determine the effectiveness of various mulches made of polyethylene and other materials for pest control in vegetables. The standard mulch in vegetable production for the southeast US for weed and disease control is low-density polyethylene (LDPE) (Webster 2005a; Patterson 1998). LDPE has many positive characteristics such as trapping soil heat promoting rapid plant growth, restricting light which can reduce weed growth, and restricting fumigant escape from soil after application for maximum pest control. Many other mulches are available to producers for use in vegetables: metalized, high-density polyethylene, and virtually impermeable film, though these have varying fumigant retention qualities (Santos et al. 2006b; Santos et al. 2007). Using a volatile chemical with a mulch with low permeability may allow for lower rates to achieve a similar control as a high rate with a more permeable film (Gilreath et al. 2005a). Low-volatility fumigants could be used with black paper mulches, but this mulch does not retain heat as effectively as LDPE, and since paper is placed loosely over the soil beds, piercing by nutsedge is less likely to occur (Gilreath et al. 2004a).

With the elimination of MBr as a soil fumigant, alternatives are being evaluated for economic feasibility, ease of application, and range of pest control. One alternative fumigant is a combination of 59.4% chloropicrin and 39% 1,3-dichloropropene (1,3-D) (Pic-Clor 60). This fumigant effectively controls soil-borne fungi, diseases, and nematodes, but not weeds (Anonymous 2002; Anonymous 2005; Anonymous 2006c; Extension Toxicology Network 2008). Lack of weed control limits chloropicrin plus 1,3-D applicability in purple nutsedge infested areas. Incorporating herbicides effective against purple nutsedge into alternative fumigant systems would be beneficial.

EPTC (*S*-ethyl dipropylthiocarbamate) is a pre-plant incorporated (PPI) thiocarbamate herbicide that has a moderate vapor pressure and the most important factor involved in dissipation is soil moisture (Gray and Weterich 1965; Senseman et al. 2007). It has activity on annual grasses and some broadleaf weeds, causing failure to emerge, malformed and twisted seedlings, cupping and crinkling of leaves, and death of seedling (Vencill 2002).

Nutsedge

Nutsedges are troublesome weeds for many crops in Georgia (Webster 2006). In Florida purple nutsedge (*Cyperus rotundus* L.) can reach densities of over 100 plants/m^2 (Morales-Payan et al. 1997). Purple nutsedge is a perennial that can reproduce sexually from seed and asexually from roots. As seeds have low viability (Justice and Whitehead 1946; Bryson 1990), tubers are the primary method of reproduction (Stoller and Sweet 1987; Wills 1987). Purple nutsedge tubers are spread out along the rhizomes that grow from the primary tuber after it sprouts aboveground and a single tuber can produce thirty-four tubers in ten weeks of growth (Webster et al. 2008). Many of the tubers in the rhizome chain are suppressed through apical dominance; when the chain is broken through mechanical means (i. e. tillage) the dominance is no longer expressed and the tubers will sprout (Musik and Cruzado 1953; Smith and Fick 1937).

Tubers begin to form from four to six weeks after seedling emergence, with greater than 80% of tubers located in the top 25 cm of soil and greater than 95% located within the top 45 cm (Andrews 1940; Bell et al. 1962; Loustalot et al. 1954; Smith and Fick 1937; Stoller and Sweet 1987; and Tripathi 1969). Tubers contain phenolic compounds that have allelopathic properties (Jangaard et al. 1971; Sanchez-Tames et al. 1973). Purple nutsedge tubers have buds all along the length from where shoots will sprout (Loustalot et al.1954; Ranade and Burns 1925). When rhizomes emerge from tubers, the first will grow vertically to the soil surface after exposure to sunlight, plants will begin to form a basal bulb underground (Stoller and Sweet 1987). After the primary shoot emerges, secondary rhizomes will grow underground parallel to the soil surface forming secondary basal bulbs and onward to form a complex system of underground vegetative growth (Stoller and Sweet 1987).

Purple nutsedge shoot morphology allows it to pierce polyethylene mulch used in vegetable production. Webster (2005a) reported that under black-opaque low-density polyethylene mulch a single tuber can multiply to a patch of 3,440 shoots over an area of 22.1 m^2 in 60 weeks. Nutsedge growth through the polyethylene barrier competes with the crop for nutrients and water that is supplied under the mulch. Increasing purple nutsedge densities cause a linear decrease in shoot dry weight during flowering and fruiting in pepper (*Capsicum annum* L.) and tomato (*Lycoperscion esculentum* L.) (Morales-Payan et al. 1997). According to William and Warren

(1975), competition season-long with a field infested with purple nutsedge (160 plants/0.1 m^2) reduced yield of garlic (*Allium sativum* L.) (89%), okra (*Abelmoschus esculentus* L.) (62%), cucumber (*Cucumis sativus* L.) (43%), tomato (*Lycipersicon esculentum* L.) (53%), with similar reductions in several other crops. *Cyperus* has also reduced tomato yield by 51% with season-long interference of 105 plants/m^2 when compared to weed free plots (Gilreath and Santos 2004c). The critical weed free time for tomato and other vegetable crops is prior to the crop reproductive development (Kasasian and Seeyave 1969). Purple nutsedge is among the top ten most troublesome weeds in vegetables for Georgia, Alabama, Louisiana, North Carolina, and South Carolina (Webster 2006). It was also reported as a top ten most troublesome weed species for cotton and peanut in Virginia (Webster 2009).

Efforts to control purple nutsedge in vegetable have mainly involved research into film mulches and herbicides. Whereas most weeds are controlled by opaque polyethylene mulch, nutsedges are able to pierce through the film (William 1976). Purple nutsedge has been reported to pierce black polyethylene film 127 µm thick, though it was unable to pierce 254 µm film (Henson and Little 1969). Research shows that color of polyethylene mulch can affect the growth of yellow nutsedge, black and clear polyethylene mulch reduced shoot production 46 and 72%, respectively, compared to nonmulched (Webster 2005a). Patterson (1998) observed purple nutsedge tuber production was reduced when using translucent film. However, Webster (2005a) reported that purple nutsedge growth had few detectable differences comparing nonmulched, clear polyehtlyene, and black polyethylene mulch. This could prompt a weed shift from yellow nutsedge to purple, as purple is relatively insensitive to these polyethylene barriers in comparison.

With the inability of mulch to completely control research has also reviewed herbicides for efficacy on purple nutsedge. Parker et al. (1969) tested 98 herbicides with a variety of modes of actions for control of purple nutsedge, only thirty were found to cause greater than 60% reduction of vigor and only five had the potential to kill the plant. As this test was conducted in a glasshouse, the efficacy of the herbicides in a field setting is likely to be less than was reported (Parker et al. 1969). Some of these findings, such as pebulate efficacy, were supported by Gilreath and Santos (2004b) when evaluating several herbicide combinations effect on purple nutsedge density. A combination of 1,3-d (82%) plus chloropicrin (17%) (410 kg ai/ha) and pebulate (4.5 kg ai/ha) provided the best control of purple nutsedge (Gilreath and Santos 2004b).

Research into weed biology began in the 1930s with Pavlychenko (1937) studying root characteristics of weeds and crops in competitive and noncompetitive associations. In the 1970s a series of literature reviews were published documenting the importance of weed biology, including a review of yellow nutsedge (Mulligan and Junkins 1976). Current research is focused on understanding basic weed biology to predict how weed species, populations, and biotypes respond to agricultural practices in areas of seedbank dynamics, tuber dormancy, and modeling seedling emergence (Bhowmik 1997).

Previous work has indicated that some species requires a certain amount of temperature fluctuation to achieve optimum germination, or sprouting, or to achieve optimum growth. Sun and Nishimoto (1999) reported that greater shoot elongation of purple nutsedge occurred when diurnally alternating temperature. Germination, or sprouting, is sometimes initiated or enhanced by diurnal fluctuations (Benech Arnold et al. 1988; Harrington 1923; Morinaga 1926; Nishimoto and McCarty 1997; Thompson and Grime 1983; Thompson et al. 1977; Totterdel and Roberts

1980). However, growth under a diurnal temperature flux does not have an effect on all species (Dale 1964; Friend and Helson 1976).

Objectives

Objectives of this research revolve around determining the crop tolerance and weed control with herbicides applied in vegetable production, and to evaluate effect of temperature on purple nutsedge sprouting. Knowledge of these factors will assist researchers and extension agents in maintaining and developing management strategies for weeds, and help to educate growers on the efficacy of herbicides in vegetables.

CHAPTER 3

VEGETABLE RESPONSE TO EPTC APPLIED PREEMERGENCE UNDER LOW BARRIER AND HIGH BARRIER PLASTIC MULCH[1]

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[1] Wallace, R. D., A. S. Culpepper, A. W. MacRae, L. M. Sosnoskie and T. L. Grey. To be submitted to Weed Technology.

Chapter 3. Vegetable Response to EPTC Applied Under Plastic Mulch

Abstract: The continued phase out of methyl bromide challenges a vegetable grower's ability to control weeds in plasticulture production systems. Herbicides will be needed as part of a methyl bromide (MBr) alternative system. An experiment was conducted during the spring of 2008 and 2009 in Ty Ty, GA to determine tomato (*Lycopersicon esculentum* L.), pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), and watermelon (*Citrullus lanatus* L.) tolerance to EPTC applied under mulch. Treatments consisted of a factorial arrangement with four rates of EPTC including 0, 2, 3, or 4 kg ai/ha and two plastic mulch types including a low density polyethylene (LDPE) mulch or a high barrier mulch (HBM). Each crop was planted 28 days after applying herbicides and laying mulch. EPTC, regardless of rate, applied under LDPE mulch did not impact plant development or yields of any crop. In contrast to LDPE mulch, pepper, tomato, and eggplant heights were reduced 65 to 72%, 30 to 75%, and 9 to 32%, respectively, by EPTC at 2 to 4 kg/ha when applied under HBM. Yields followed similar trends with fruit number or weights harvested being reduced for pepper (71 to 84%), tomato (36 to 76%), and eggplant (7 to 15%) by EPTC at 2 to 4 kg/ha when compared to the no EPTC HBM control. Watermelon noted 12% or less early season stunting, and no impact of EPTC rate on runner lengths or yield was noted. It appears plausible that high barrier mulches reduce the loss of EPTC through volatilization thereby increasing the dose present at time of planting. EPTC could be included as part of a MBr alternative system for tomato, pepper, eggplant and watermelon when applied under LDPE mulch but only for watermelon when applied under high barrier mulches.

Introduction

Many fruit and vegetable crops applied Methyl bromide (MBr) to sterilize the soil prior to planting (USDA-NASS 2009). In 1992, prior to the classification of MBr as a ozone depleting substance, MBr was applied to 28% of bell pepper ha (USDA-NASS 2009). After the reduction program by the Montreal protocol, this decreased to 17% as of 2006 (USDA-NASS 2009).

Methyl bromide was determined by the Montreal Protocol to be a Class I ozone depleting substance (Anbar et al. 1996). In 1993, a MBr reduction use program was implemented by the Montreal Protocol but it was not until 2005 before growers felt the impact from this reduction program (UNEP 1995). By 2005, MBr used for vegetable production was limited to Critical Use Exemptions (Anonymous 2008a) which allowed use in six GA crops including pepper, tomato, eggplant, cantaloupe, cucumber, and squash (Anonymous 2009a). As the amount of MBr became more limiting the cost of MBr increased significantly (Gilreath et al. 2005a; Noling 2002). Alternative fumigant research has yielded promising results, but alternatives to methyl bromide continue to be less effective in controlling some weeds, especially nutsedge, when compared to MBr (Yates et al. 2002).

Purple nutsedge (*Cyperus rotundus* L.) and yellow nutsedge (*Cyperus esculentus* L.) are two most troublesome weeds in cucurbits, peaches, and fruiting vegetable crops in Georgia (Webster 2006). Both nustedges are perennial with erect growth and triangular stems. This shoot morphology allows nutsedges the ability to pierce polyethylene mulch (William 1976). In addition to penetrating the mulch, the plants reproduce primarily by tubers (Wills 1987) allowing nutsedge levels to increase rapidly under the mulch with continual emergence during the season (Webster 2005b). Webster (2005b) reported that under black-opaque LDPE mulch, a single purple nutsedge tuber can multiply to 3,440 shoots covering an area of 22.1 m^2 in 60 weeks.

However, the study reported that yellow nutsedge was slightly suppressed by the black-opaque LDPE mulch, the nonmulched patch expanding out to twice the area of the LDPE (Webster 2005b). Vegetable production is sensitive to heavy infestations of nutsedge as William and Warren (1975) noted season-long purple nutsedge competition (160 plants/0.1 m^2) reduced yields of cabbage 35%, cucumber 43%, and tomato 53%. Season long purple nutsedge interference reduced eggplant yield 22% (Morales-Payan and Stall 1997). Season long yellow nutsedge interference has reduced watermelon yield 98% (Buker et al. 1998). Gilreath et al. (2005) reported that nutsedge densities of approximately 0.5 plant/ft^2 during crop fruit set reduced bell pepper yield by 31%. Motis et al. (2003) reported that a yellow nutsedge density of approximately 9 plants/ft^2 may reduce bell pepper yield by 70%.

With the eventual elimination of MBr as a soil fumigant and alternatives to MBr providing inadequate weed control, the need for a fumigant plus herbicide system may be in order (Noling and Gilreath 2002; Noling et al. 2006; Santos et al. 2006b). EPTC (*S*-ethyl dipropylthiocarbamate) is a thiocarbamate herbicide having a moderate vapor pressure, 3.4×10^{-2} mm Hg at 25 C, with activity on grasses, nutsedges, and other broadleaf weeds (Parker et al. 1969; Santos 2009; Wallace et al. 2007; Vencill 2002). A herbicide that offers control of problematic weeds and has a moderate vapor pressure with the potential to disperse more uniformly under various mulches may improve weed control in a MBr alternative system.

Numerous mulch types are now available for plasticulture production systems. These mulches have been shown to vary in their ability to reduce the movement of fumigants from the soil into the atmosphere (Austerweil et al. 2006; Minuto et al. 1999; Ou et al. 2008; Qin et al. 2008; Santos et al. 2006a; Santos et al. 2007; Yates et al. 2002). It may be possible that these mulches also impact the movement of EPTC when applied under mulches since the herbicide has moderate vapor pressure. The standard mulch in vegetable plasticulture production for the southeast US has been LDPE mulch (Webster 2005b; Patterson 1998); however, regulations placed on fumigants by the EPA are forcing growers to consider mulches with greater barrier characteristics.

Before EPTC could be integrated into a MBr alternative system, vegetable tolerances in the field must be determined. Thus, an experiment was developed to determine the response of tomato, pepper, eggplant and watermelon to EPTC applied under two mulch types. Information of this nature will be essential in developing sustainable systems for the replacement of MBr.

Materials and Methods

Experiments were conducted in the spring of 2008 and 2009 in Ty Ty, Georgia. Soil type, typical for the vegetable production region, was a Tifton loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults) with 90% sand, 6% silt, 4% clay, 1.1% organic matter, and pH 6.4. The randomized complete block experiment included a factorial arrangement of treatments replicated three times. The factorial arrangement included an herbicide option of EPTC[5] at 0, 2, 3, or 4 kg ai/ha and a mulch option of LDPE mulch[6] and a HBM[7].

Soil within the trial area was disk harrowed twice and moldboard plowed 25-30 cm deep in January of each year. On February 27, 2008 and February 17, 2009, raised beds (0.9 m wide, 30.5 m long and 15 cm high) were established with a super bedder. While preparing beds, chloropicrin at 176 kg ai/ha plus 1,3-D at 115 kg ai/ha[4] was injected 20 cm below the bed top using three shanks spread evenly across the bed. Immediately following the bed shaper and fumigant application, the plastic layer laid a single drip tape 2.5 cm below the bed surface in the center of the bed, applied EPTC at 0, 2, 3,or 4 kg ai/ha at 165 kPa with a spray volume of 37 L/ha to the soil surface of the final formed bed, and then covered the bed with mulch.

Herbicide and mulch treatment plot size was 30.5 m but plots were then split into four even sections to determine crop response for bell pepper (‘Heritage’), tomato (‘Bella Rosa’), eggplant (‘Santana’), and watermelon (‘Delight Seedless’). Transplant holes were mechanically punched through the mulched beds followed immediately by hand transplanting crops on March 27, 2008 and March 12, 2009. Two rows of bell pepper were planted 5 cm deep and 30 cm between plants. Eggplant and tomato were planted in single rows 5 cm deep and 51 cm between plants. Watermelon were planted in single rows 5 cm deep and 61 cm between plants.

Visual injury estimates of crop stunting and plant loss were taken at least every three weeks after (WAP) planting using a scale of 0 (no injury) to 100% (plant death). Ten tomato, pepper, and eggplant heights and watermelon runner lengths were taken three times during the season at 2 (early season), 4 to 6, and 6 to 8 (late season) WAP. Pepper harvested included Jumbo fruit picked once a week for three to four weeks; tomato harvests consisted of picking fruit 6 to 7 times as soon as the fruit initiated reddening followed by fruit size grading; eggplant were harvested 7 to 9 times by picking fruit approximately 13 cm in diameter at the widest point having a length of 20 cm; and watermelon when mature were harvested 1 to 3 times. The experimental area was chosen for its light weed and nematode pressure. Additionally, the entire area was fumigated in order to eliminate other pests in an effort to focus specifically on crop response to EPTC under various mulches. All other aspects of plasticulture production followed recommendations by the University of Georgia Extension Service and the Alabama Cooperative Extension System (Anonymous 1999; Anonymous 2006a; Anonymous 2009b; Kemble et al. 1998)

Vegetable injury estimates, heights, and total yield for the season, were subjected to a SAS mixed model analysis of variance (MMANOVA) to evaluate the main effects and their interaction. A MMNOVA was used due to the factorial design and the desire to partition out the influence of the variables. Mulch and rate were the main effects of the model, and replication and year were random effects. Regression analysis were used to evaluate the affect of rate and the rate by mulch interaction when MMANOVA indicated a significant influence ($P \leq 0.05$) Data were combined across years with the lack of a year interaction. The pepper late rating was regressed using an exponential rise to maximum

$$y = a^*(1\text{-}e^{(-b^*x)}) \qquad [1]$$

where *y* is crop injury, *a* is the measure of the no EPTC control, and *b* is the influence of herbicide on increasing injury. Pepper late season height, yield in number/ha and kg/ha were regressed using an exponential decay:

$$y = a^*e^{(-b^*x)}) \qquad [2]$$

where *y* is crop injury, *a* is the measure of the no EPTC control, and *b* is the influence of herbicide on decreasing height and yield. Pepper early season injury, early season height, and all variables for tomato, eggplant and watermelon were regressed using a linear regression.

Results and Discussion

Bell pepper, tomato, eggplant, and watermelon were tolerant to EPTC applied under LDPE mulch, regardless of EPTC rate applied (Figures 4.1 A, 4.1 B, 4.4 A, 4.4 B, 4.7 A, 4.7 B, 4.10 A, 4.10 B). The greatest level of injury was noted with pepper but injury was at most 5% during the season. Plant heights followed similar trends with EPTC rate having no impact on plant heights (Figures 4.2, 4.5, 4.8, 4.11). Bell pepper, tomato, eggplant and watermelon produced 4,690, 133,400, 344,800, and 235,300 kg/ha of marketable fruit when no EPTC was applied under LDPE mulch; applying EPTC at 2, 3, or 4 kg/ha under LDPE mulch did not impact yield of these crops (Figures 4.3 B, 4.6 B, 4.9B, 4.12 B).

In contrast to LDPE mulch, significant and often severe injury was noted with all crops planted into EPTC applied under HBM. Bell pepper was injured 29, 33, and 34% with EPTC at 2, 3, and 4 kg/ha at 2 WAP, respectively (Figure 4.1A). Pepper injury increased rapidly through the season with 66 to 75% injury noted 6 to 8 WAP (Figure 4.1B). Pepper heights just prior to harvest noted a reduction of plant growth for 2, 3, and 4 kg/ha of 65 to 72% when compared to the no EPTC HBM treatment (Figure 4.2). When accumulating jumbo pepper yield over the 3-4 harvests, fruit numbers and weights were reduced 71 to 84% with all rates of EPTC (Figure 4.3 A, 4.3 B).

Similar to pepper, tomato was very sensitive to EPTC applied under HBM mulch. Visual evaluations during early season noted 18, 24, and 31% injury for 2, 3, and 4 kg/ha, respectively (Figure 4.4 A). Injury was greater during late season with 30, 43, and 76% injury noted at the aforementioned rates, respectively (Figure 4.4 B). Similar to late season visual estimates, a linear decrease in tomato plant height was noted with a reduction of 30, 48, and 75% with 2, 3, and 4 kg/ha of EPTC, respectively (Figure 4.5). Tomato fruit numbers and weights were reduced for each harvest (data not shown) and when cumulating yields over the 6 to 7 harvest a linear decrease in yield was noted. Total number of tomato fruit harvested from the control was 678,000 and declined as the rate of EPTC increased from 435,000 (2 kg/ha EPTC), 268,000 (3 kg/ha EPTC), to 156,000 (4 kg/ha EPTC (Figure 4.6 B). This linear decrease with increasing rate was also noted in tomato number harvested (Figure 4.6 A).

Eggplant was more tolerant than tomato or pepper to EPTC when applied under HBM, but crop response was still not commercially acceptable. Visual eggplant injury of 9 to 32% was noted with 2 to 4 kg/ha of EPTC throughout the entire season (Figure 4.7A, 4.7B). Eggplant heights 6 to 7 WAP were reduced 17, 29, and 35% at 2, 3, and 4 kg/ha, respectively, when compared to the no EPTC HBM control (Figure 4.8). With increasing rate of EPTC eggplant yield decreased linearly (Figure 4.9 A, 4.9 B).

Watermelon was the most tolerant to EPTC applied under HBM mulch when compared to the fruiting vegetables of tomato, pepper, and eggplant. EPTC at 2, 3,and 4 kg/ha caused only 6 to 12% stunting at 6 to 8 WAP with no injury noted during late season (Figures 4.10 A, 4.10 B). Watermelon runner lengths were not impacted by EPTC rate (Figure 4.11) and no differences in yield were noted when comparing the number or weight of watermelon harvested during individual harvests or for cumulative harvests (Figures 4.12 A, 4.12 B).

Results from these efforts show that crop tolerance of vegetables to EPTC in plasticulture productions systems is directly influenced by the mulch type used. It appears plausible that high barrier mulches reduce the loss of EPTC through volatilization thereby increasing the dose present at time of planting as noted with numerous fumigants (Austerweil et al. 2006; Minuto et al. 1999; Ou et al. 2008; Qin et al. 2008; Santos et al. 2006a; Santos et al. 2007; Yates et al. 2002). EPTC could be included as part of a MBr alternative system for tomato, pepper, eggplant and watermelon when applied under LDPE mulch, but only for watermelon under high barrier mulches. If regulatory agencies continue to force growers to adopt high barrier mulches when fumigating in an effort to reduce or eliminate fumigant gas emissions, the use of EPTC is not feasible for fruiting vegetables production.

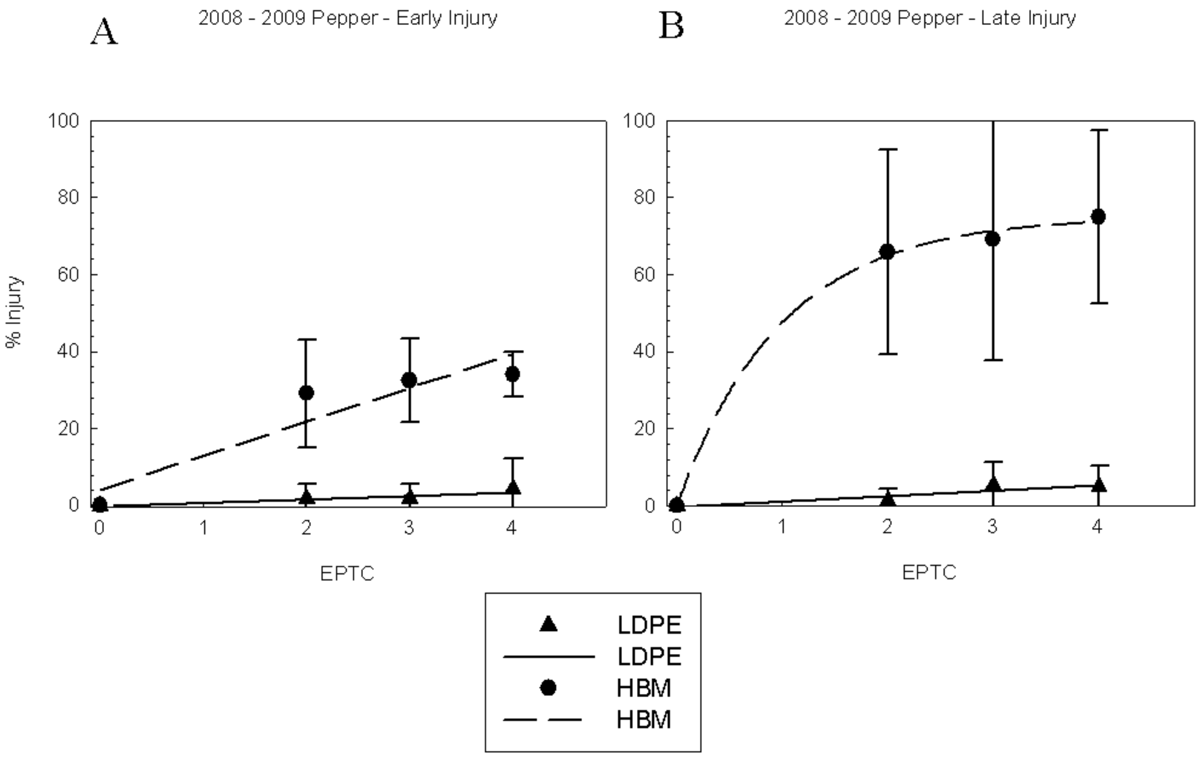

Figure 4.1A. Early season (14 DAP) pepper injury from EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals.

Figure 4.1 B. Late season (42-56 DAP) pepper injury from EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. LDPE data HBM data was non-linearly regressed using an exponential rise to maximum equation.

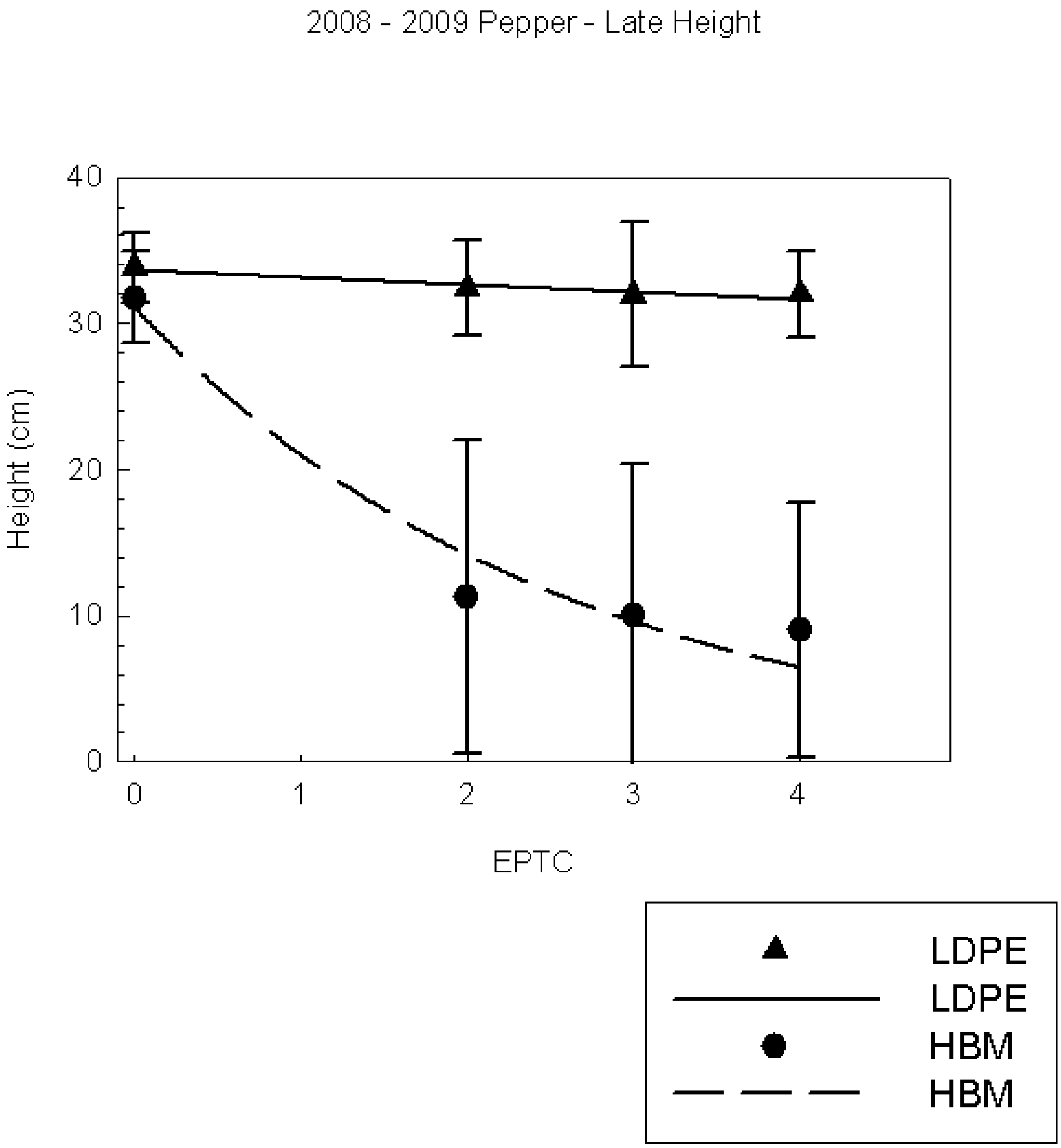

Figure 4.2. Late season (39-49 DAP) pepper heights with EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. LDPE data was linearly regressed with mean separation using 95% asymptotic confidence intervals. HBM data was non-linearly regressed using an exponential decay equation.

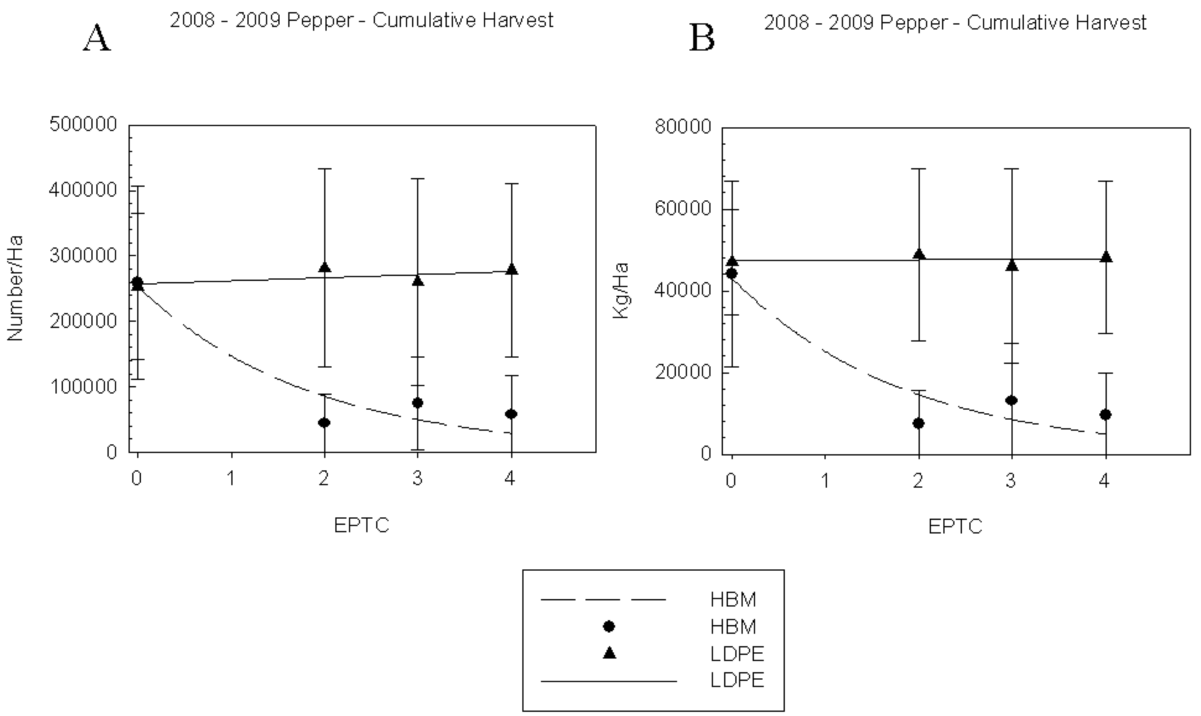

Figure 4.3 A. Cumulative pepper harvest as number of fruit/ha with EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. LDPE data was linearly regressed with mean separation using 95% asymptotic confidence intervals. HBM data was non-linearly regressed using an exponential decay equation.

Figure 4.3 B. Cumulative pepper harvest as kg/ha with EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. LDPE data was linearly regressed with mean separation using 95% asymptotic confidence intervals. HBM data was non-linearly regressed using an exponential decay equation.

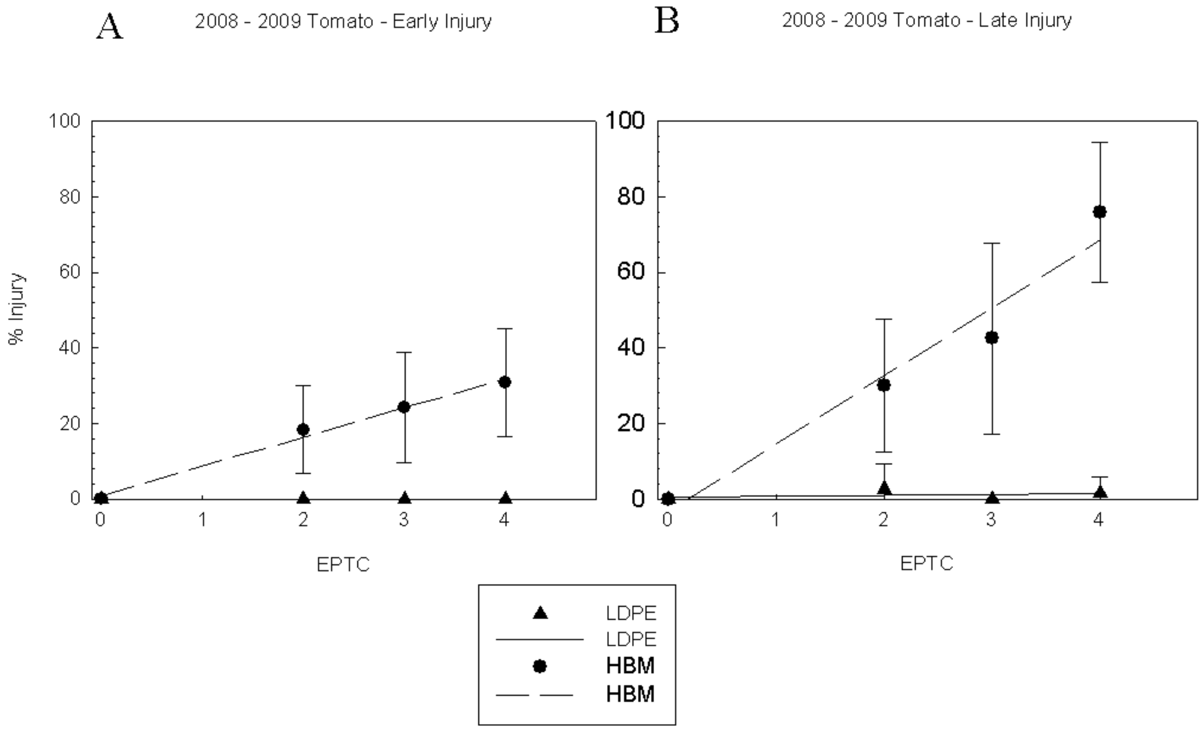

Figure 4.4 A. Early season tomato injury from EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals.

Figure 4.4 B. Late season (42-56 DAP) tomato injury from EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals.

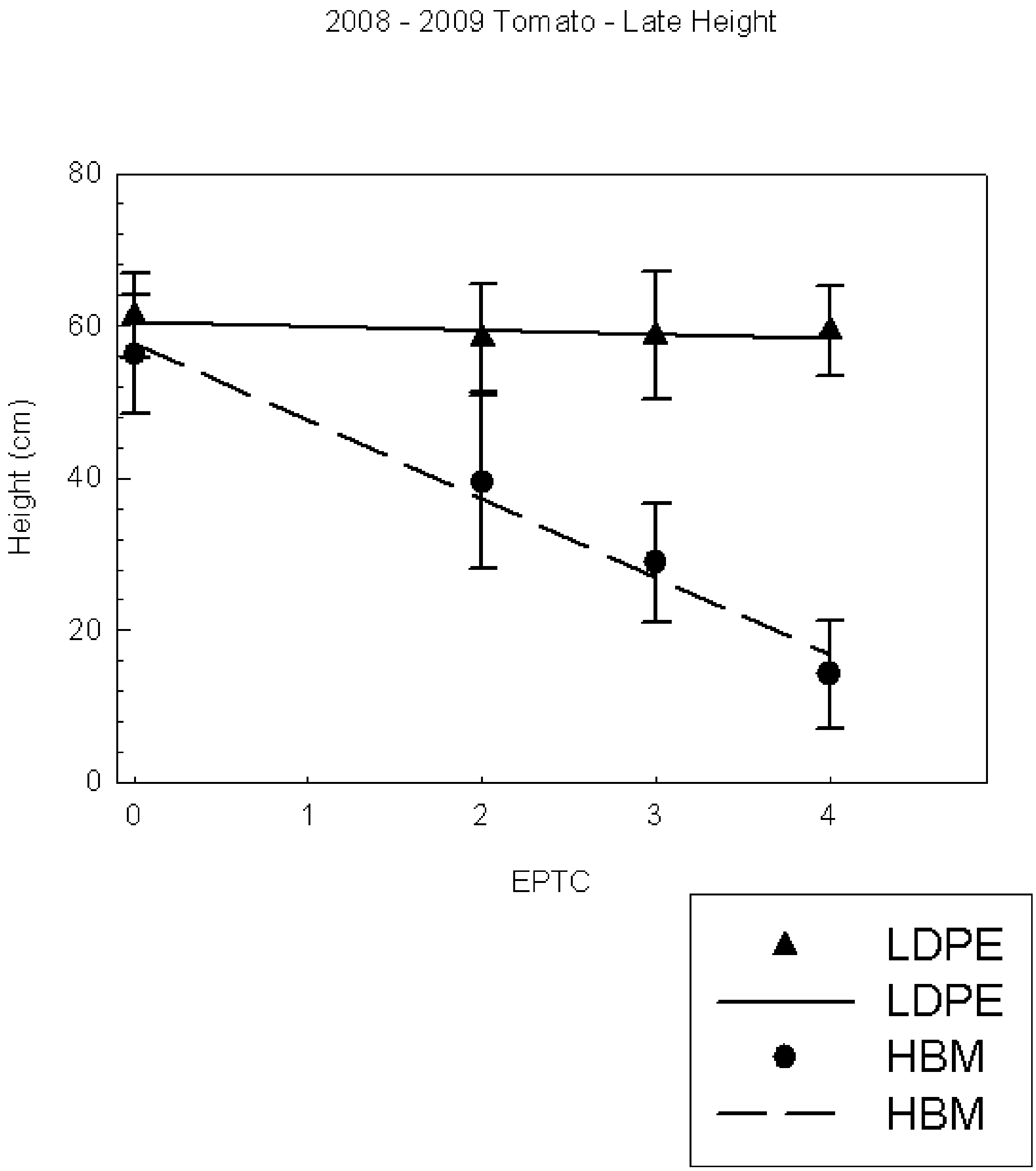

Figure 4.5. Late season (39-49 DAP) tomato height with EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals.

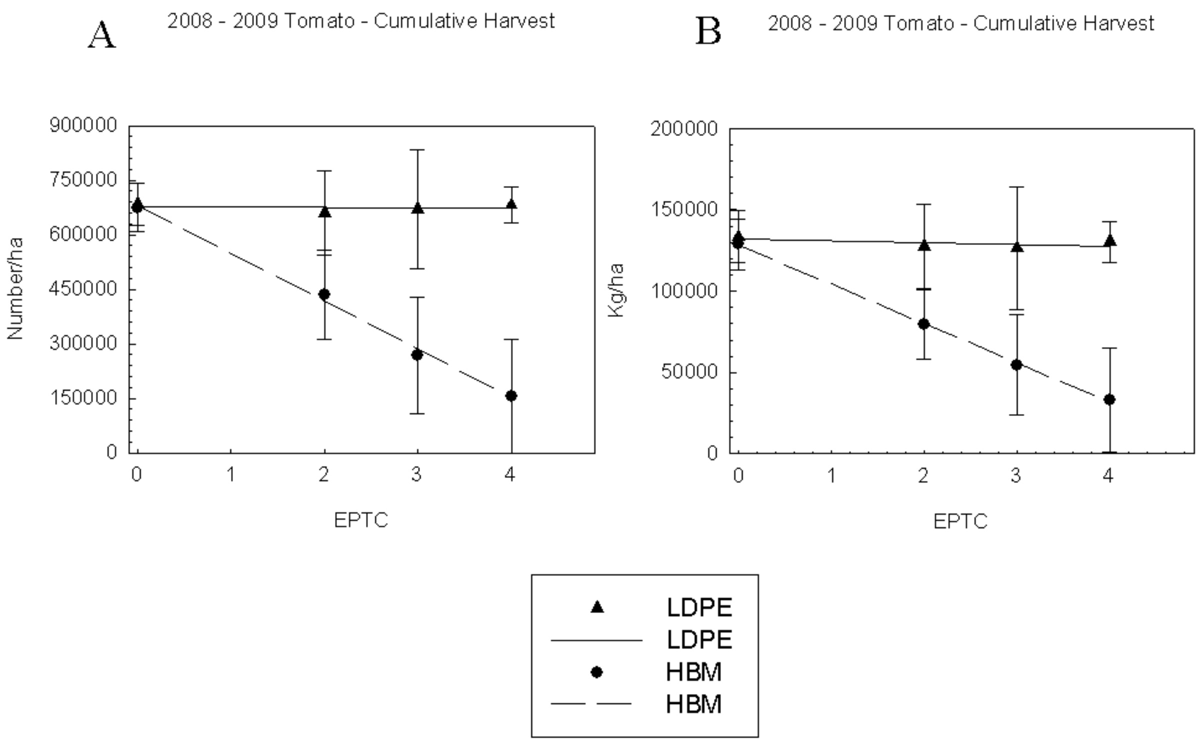

Figure 4.6 A. Cumulative tomato harvest as number of fruit/ha with EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals.

Figure 4.6 B. Cumulative tomato harvest as kg/ha with EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals

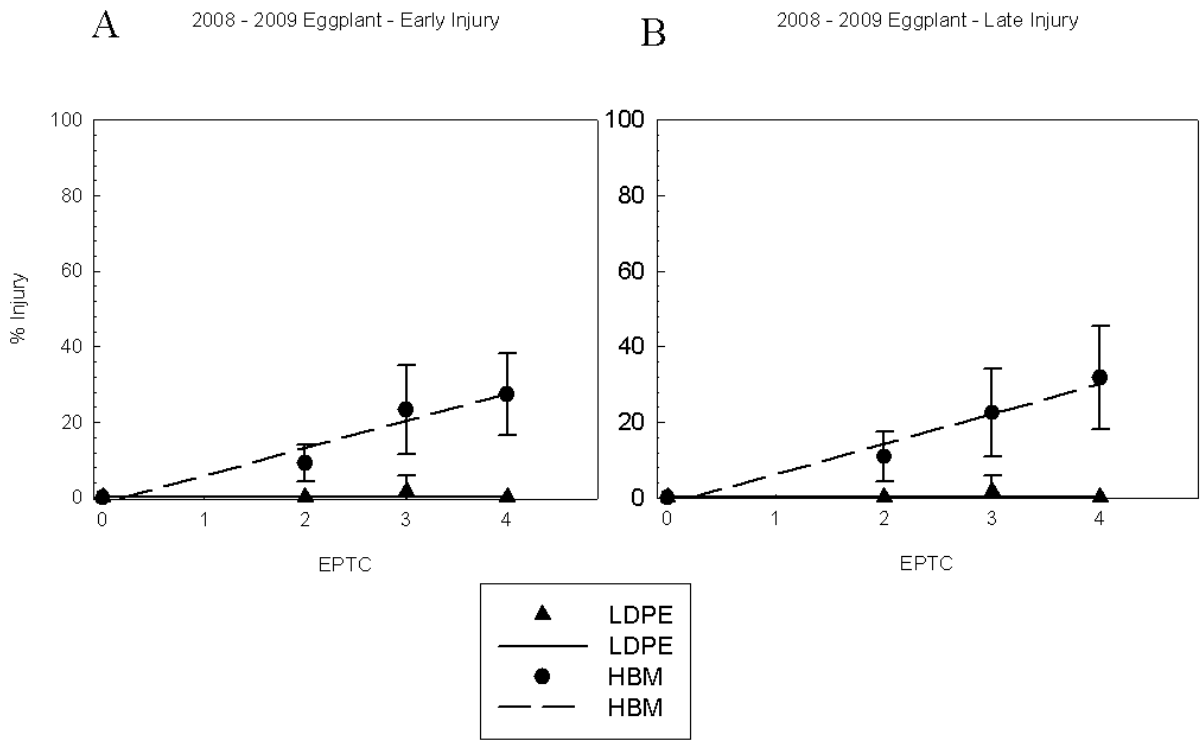

Figure 4.7 A. Early season eggplant injury from EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals.

Figure 4.7 B. Late season (42-56 DAP) eggplant injury from EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals.

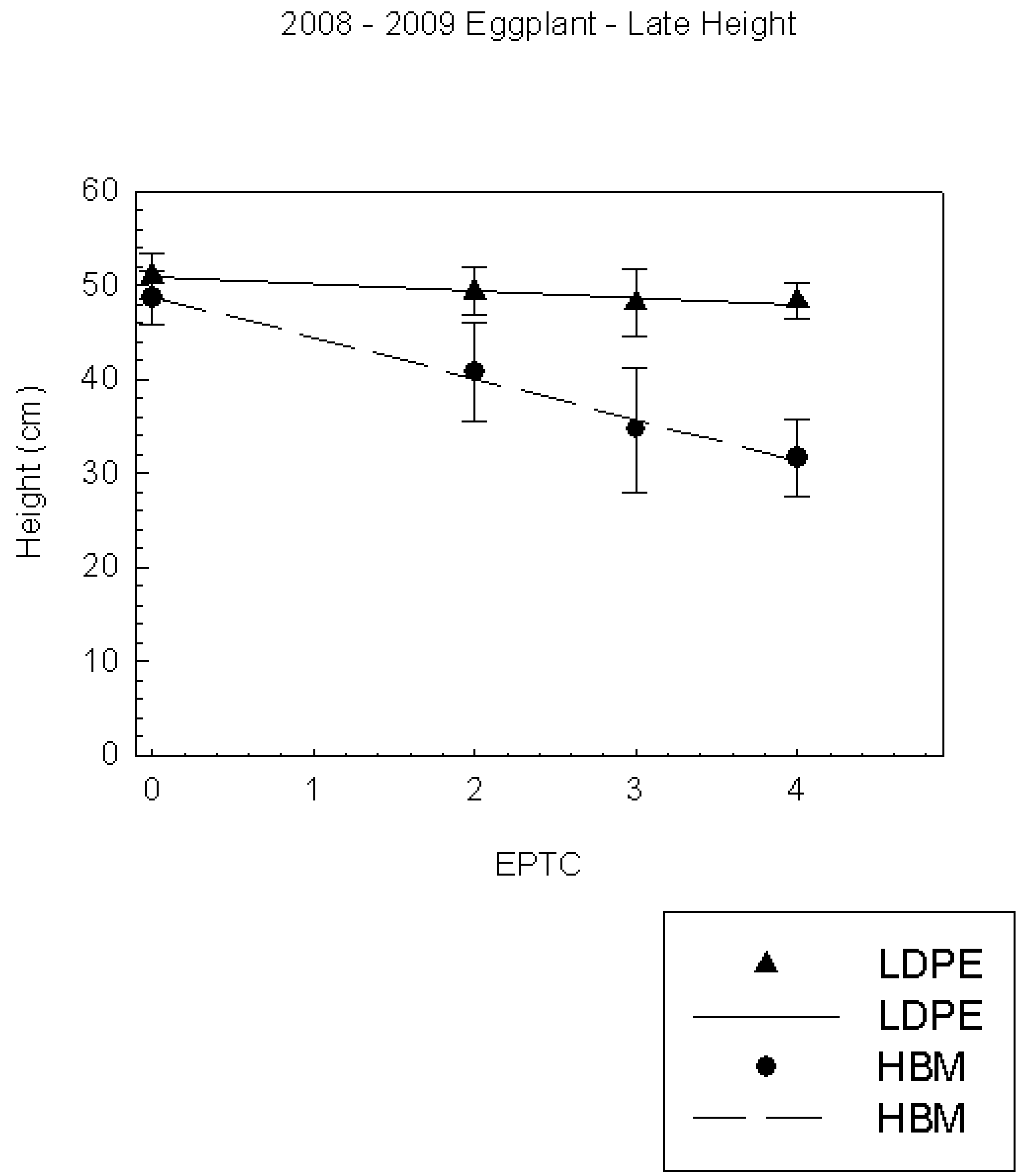

Figure 4.8. Late season (39-49 DAP) eggplant height with EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals.

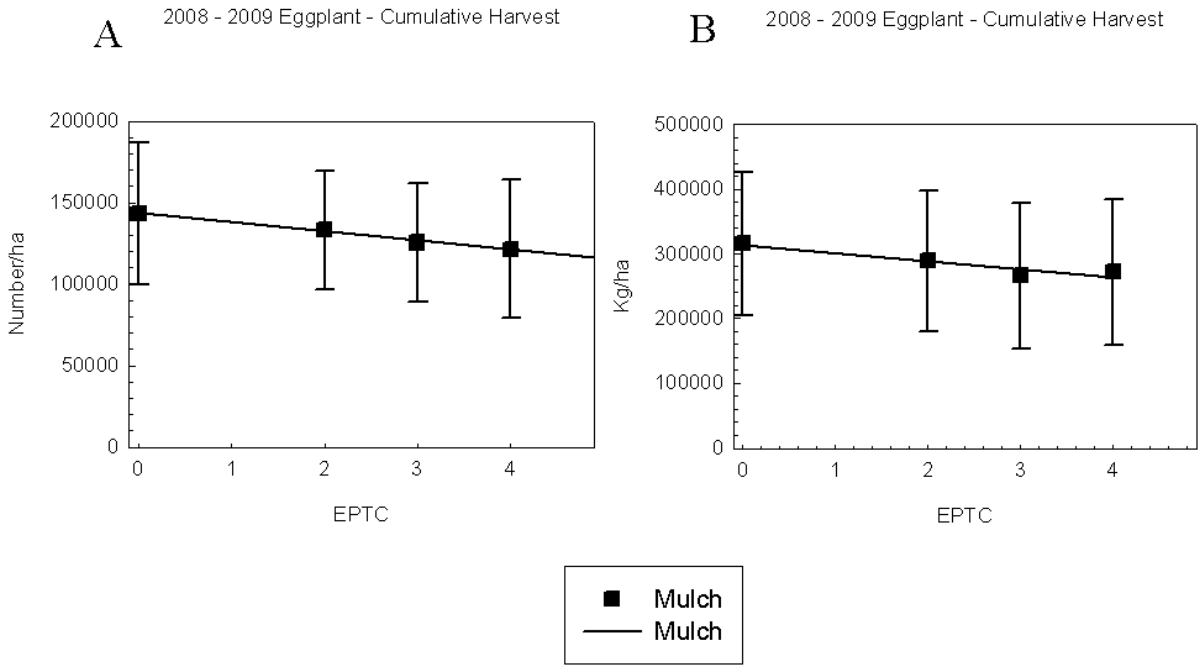

Figure 4.9 A. Cumulative eggplant harvest as number of fruit/ha with EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Rate by mulch interaction was not significant, data was combined across mulches. Data points are the means of three replications with bars indicating the standard error of the mean.

Figure 4.9 B. Cumulative eggplant harvest as kg/ha with EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Rate by mulch interaction was not significant, data was combined across mulches. Data points are the means of three replications with bars indicating the standard error of the mean.

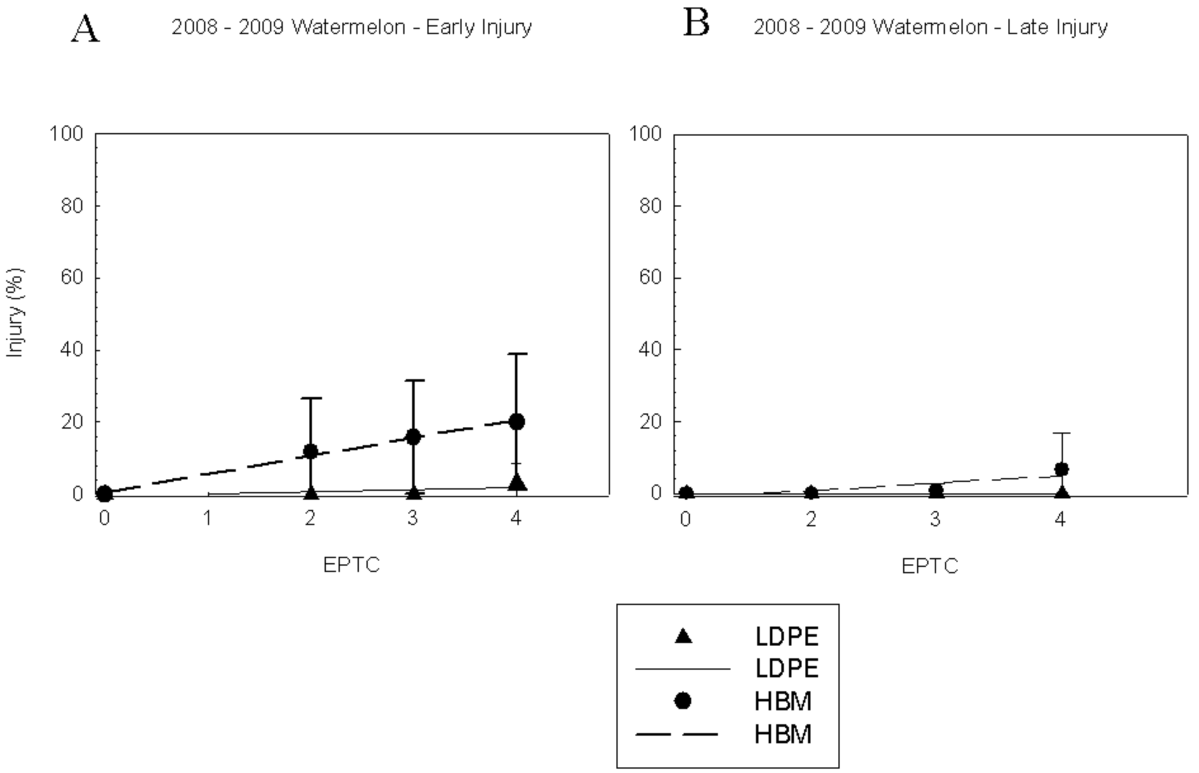

Figure 4.10 A. Early season watermelon injury from EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals.

Figure 4.10 B. Late season (42-56 DAP) watermelon injury from EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals.

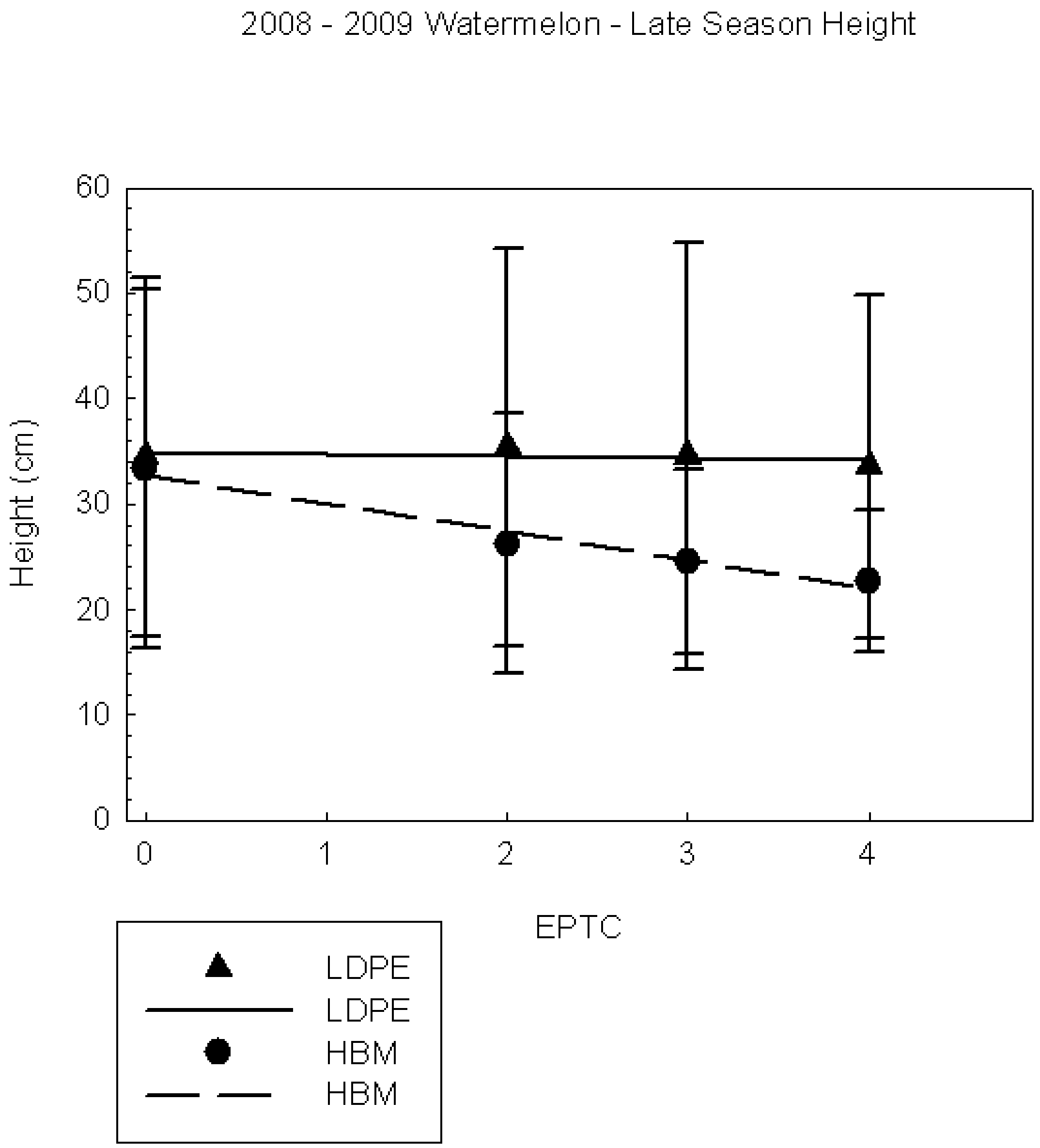

Figure 4.11. Late season (27-28 DAP) watermelon height with EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals.

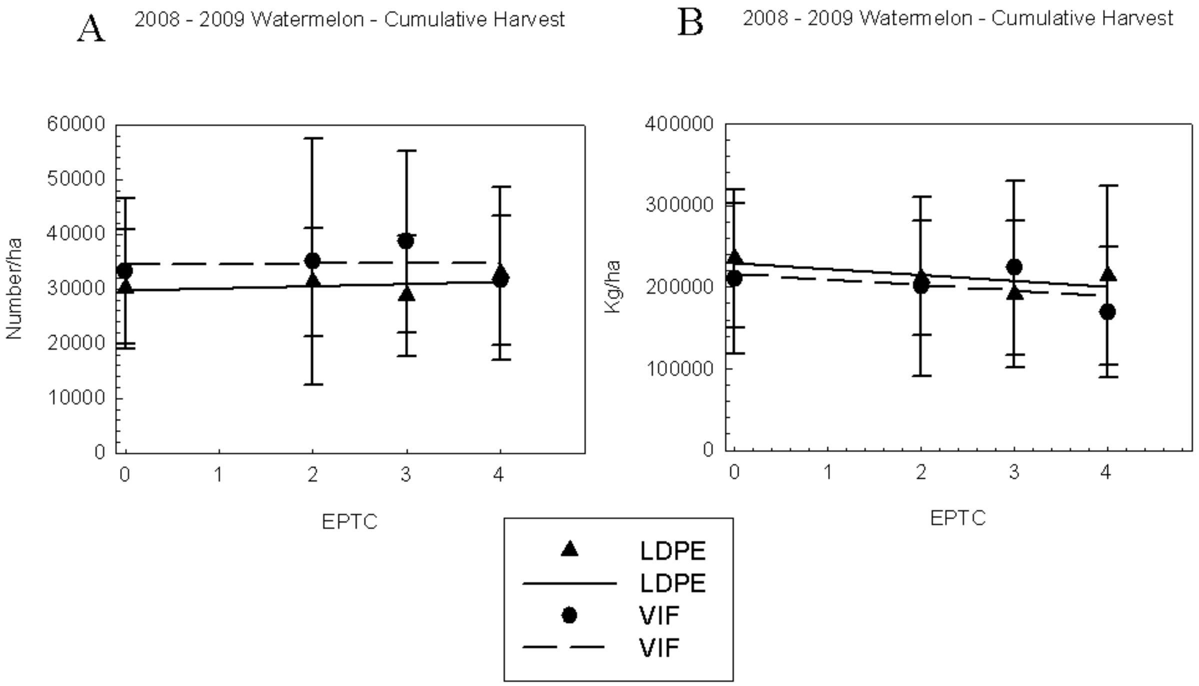

Figure 4.12 A. Cumulative watermelon harvest as number of fruit/ha with EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals.

Figure 4.12 B. Cumulative watermelon harvest as kg/ha with EPTC rates of 0, 2, 3, and 4 kg ai/ha with two mulches: low density polyethylene (LDPE) or high barrier mulch (HBM). Data points are the means of three replications with bars indicating the standard error of the mean. Data was linearly regressed with mean separation using 95% asymptotic confidence intervals.

CHAPTER 4

TEMPERATURE VARIATION EFFECT ON PURPLE NUTSEDGE (*CYPERUS ROTUNDUS*) TUBER SPROUTING.[2]

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[3] Wallace, R. D., T. L. Grey,T. M. Webster, and W. K. Vencill. To be submitted to Weed Science.

Chapter 4. Temperature Variation Effect on Purple Nutsedge (*Cyperus rotundus*) Tuber Sprouting.

Abstract: Experiments were conducted to determine the effect of simulated diurnal temperature variation on cumulative sprouting of purple nutsedge tubers. Tubers were placed on a thermogradient table in petri dishes. Variation in temperature was accomplished by alternating the petri dishes on the table from one zone to another. Diurnal temperatures varied by 0, 4, 9, 15, and 23 C, though all tubers accumulated the same amount of growing degree days. Purple nutsedge tubers were alternated between cool (8 hrs) and warm (16 hrs) everyday and sprouted tubers were counted once per day. Cumulative sprouting over eight days was recorded for each amplitude of variation. Cumulative sprouting was: 88, 92, 92, 90, and 90% for diurnal variations of 0, 4, 9, 15, and 23 C, respectively. No significant differences were noted in cumulative sprouting between any of the temperature variations.

Introduction

Nutsedge species are troublesome weeds for many crops in Georgia (Webster 2006). Purple nutsedge (*Cyperus rotundus* L.) is a perennial that can reproduce sexually from seed and asexually from the roots. As the seeds have low viability (Justice and Whitehead 1946; Bryson 1990), tubers are the primary method of reproduction (Wills 1987). Purple nutsedge tubers are spread out along the rhizomes that grow from the primary tuber after it sprouts aboveground and a single tuber can produce thirty-four tubers in ten weeks of growth (Webster et al. 2008). Many of the tubers in the rhizome chain are suppressed through apical dominance; when the chain is broken through mechanical means (i. e. tillage) the dominance is no longer expressed and the tubers will sprout (Musik and Cruzado 1953; Smith and Fick 1937).

Until recently, high-value vegetable production in the Southeast U.S. used methyl bromide to control a multitude of pests, including plant pathogens, soil borne insects, nematodes, and weeds (Motis and Locascio 2004; Gilreath et al. 2005b; Gilreath and Santos 2004a; Dowler 1999). However, use of methyl bromide has been eliminated as a fumigant for vegetable production due to concerns that it is an ozone-depleting chemical, with exception to critical use exemptions that are granted by the Methyl Bromide Technical Options Committee of the United Nations. As growers transition from methyl bromide to alternative fumigants, purple nutsedge has become a more common weed in Georgia vegetables, from the tenth most common weed in 1999 (Dowler 1999) to the fifth in 2006 (Webster 2006). This weed shift necessitates more understanding of the biology and physiology of purple nutsedge to improve management tactics.

Purple nutsedge shoot morphology allows it to pierce polyethylene mulch used in vegetable production. Webster (2005b) reported that under black-opaque low-density polyethylene mulch a single tuber can multiply to a patch of 3,440 shoots over an area of 22 m^2 in 60 weeks.

Nutsedge growth through the polyethylene barrier competes with the crop for nutrients and water that is supplied under the mulch. According to William and Warren (1975), season-long competition with a field infested with purple nutsedge (160 plants/0.1 m^2) in bareground production reduced yield of garlic (*Allium sativum* L.) (89%), okra (*Abelmoschus esculentus* L.) (62%), cucumber (*Cucumis sativus* L.) (43%), and tomato (*Lycipersicon esculentum* L.) (53%).

Previous work has indicated that some weed species requires a certain amount of temperature fluctuation to achieve optimum germination, or sprouting, or to achieve optimum growth. Sun and Nishimoto (1999) reported that greater shoot elongation of purple nutsedge occurred when tubers were exposed to diurnally alternating temperature. Germination, or sprouting, is sometimes initiated or enhanced by diurnal fluctuations (Benech Arnold et al. 1988; Harrington 1923; Morinaga 1926; Nishimoto and McCarty 1997; Thompson and Grime 1983; Thompson et al. 1977; Totterdel and Roberts 1980). The minimum, maximum and constant temperatures for optimum purple nutsedge sprouting are 10 to 13 C, 44 to 45 C, and 30 to 35 C (Holt and Orcutt 1996; Miles 1991; Tripathi 1967; and Ueki 1969). However, growth under a diurnal temperature flux does not have an effect on all species (Dale 1964; Friend and Helson 1976).

In order to identify the effects of varying temperature, five different ranges of temperature variance were chosen and nutsedge tuber sprouting was recorded. Experiments were conducted to evaluate the optimum temperature for purple nutsedge sprouting to better understand the biology and physiology of this important weed.

Materials and Methods

Purple nutsedge tubers were collected from the USDA-ARS Jones farm near Chula, GA on September 14, September 23, and October 27 in 2008, and in 2009 on January 7. Experiment was conducted four times. Tubers were harvested using a field cultivator to move chains of tubers to the field edge, after which tubers were separated from chains by hand. Purple nutsedge tubers were collected and used in the study within three days; tubers were kept at 4 C until used in the study. Each experimental unit consisted of twenty-five similarly sized tubers on a blotter disk[9] in a Petri dish[10] with 10 ml of deionized water and kept moist for the duration of the experiment. The dishes were placed on a thermogradient table with nine subsamples of the Jones farm population for each diurnal temperature variation.

The thermogradient table was constructed as a solid aluminum block 2.4 m long by 0.9 m wide by 7.6 cm thick. It is divided into 9.5 cm by 9.5 cm squares and the table is 9 rows by 24 columns for a total of 216 cells. The thermogradient was regulated by water from a cold water bath on one side and a hot water bath on the other side that was pumped into opposite ends of the table through a 1.0 cm hole at 3.8 L per minute. The gradient ranged from 10.5 to 35.9 C, with the temperature variation in each cell less than 1 C. Thermocouples were inserted into holes drilled into the bottom of the table at the center of each cell. The holes were 8 mm wide by 7 cm deep and thermocouples were inserted into the hole with thermocouple metal coming within 0.5 cm of the table surface. The temperature of each cell used was recorded every 30 minutes using a datalogger[11].

The experiment was conducted by placing purple nutsedge tubers in petri dishes remaining for 16 hours on the warm side of the table and 8 hours on the cool side of the table to create a diurnal effect. All dishes accumulated the same growing degree days (GDD) (Figure 1), though there were five distinct levels of variation in temperature between warm and cool: 0 C (constant 28.5 C), 4 C (26 C for 8 h and 30 C for 16 h), 9 C (23 C for 8 h and 32 C for 16 h), 15 C (19 C for 8 h and 33 C for 16 h), and 23 C (13 C for 8 h and 36 C for 16 h). Dishes were evaluated every 24 h and tubers with a root or shoot emerged at least 5 mm were counted and removed from the dish. The experiment ran for eight days and non-germinated tubers were counted on the last day. The GDD were calculated using the equation:

$$y = [(T_{max} + T_{min})/2] - T_{base}. \quad [1]$$

Purple nutsedge has a T_{base} of 12 C based on the work by Holt and Orcutt (1996). T_{max} and T_{min} are the maximum and minimum temperature recorded during the time the tubers were on the warm or cool temperatures. As the exact temperatures were recorded and used, there were slight variations in temperature when tubers were on the warm or cool sides of the thermogradient table. The equation had to be modified to reflect these fluctuations in temperature as well as to account for the exact amount of time the tubers were on the warm and cool sides of the table. During the 8 hours that the tubers were on the cool temperature, the GDD was multiplied by 0.33, and the 16 hours the tubers were on the warm temperature, the GDD was multiplied by 0.66. To reflect the amount of GDD actually accumulated over a twenty-four hour period, the resulting equation is:

$$y = [(((T_{max} + T_{min})/2) - 12)*0.33] + [(((T_{max} + T_{min})/2) - 12)*0.66] \quad [2]$$

Parameter estimates were calculated by nonlinear regression equation:

$$y = a/[(1 + ((a-b_1)/b_1)*e^{(-b2x)})] \quad [3]$$

using tuber sprouting with respect to time based on growing degree days. For the equation; a is the height of horizontal asymptote at a very large X, b_1 is the expected value of Y at time X = 0, b_2 is the measure of growth rate. This is a modified version of the equation described by Chen and Nelson (2006). Data were combined across the four replications for analysis. A mixed model analysis was used where tests were considered a random effect. General linear models procedures were used with mean separation using 95% asymptotic confidence intervals.

Results and Discussion

Data analysis indicated that there was no significant difference for any of the parameter estimates (Table 1). Comparing the cumulative sprouting of purple nutsedge tubers for all temperature variations, there was no significant difference between constant and alternated (Figure 1). Purple nutsedge tubers held at a constant temperature (29 C) had a maximum cumulative sprouting of 88%. The diurnal variations of 4, 9, 15, and 23 C had maximum cumulative sprouting rates of 92, 92, 90, and 90%, respectively (Table 1, Figure 1). Miles et al. (1996) experimented with alternating temperature and evaluating its effects on dormant purple nutsedge tuber sprouting. Their experiment utilized an incubator and had fluxes of 0 (constant), 2, 4, and 6 C for 12 hours and counted tubers to 36 days after planting. An increase in nutsedge sprouting with increasing temperature variation was reported.

Miles et al. (1996) reported that 100% sprouting occurred with a fluctuation of 25 to 35 C (12:12 h) as compared to 66% sprouting with a constant temperature of 30 C. Sprouting also occurred earlier under the alternating temperature as compared to a constant temperature. Due to the difference in reporting about the effect of temperature on purple nutsedge tuber sprouting, there is a lack of understanding how this weed breaks dormancy with respect to temperature fluctuation.

Kawabata and Nishimoto (2003) experimented on single tubers and tubers in chains of various numbers (2, 4 and 6 tubers long) with applying a high-temperature pulse (35 C) to dormant tubers for varying lengths of time to evaluate sprouting. The experiment included tubers from Osaka, Australia, Florida, Palau, and Singapore to record the differences in ecotype. Single tubers held at a constant temperature of 20 C had 11 to 85% budbreak for all ecotypes. Tubers that experienced a high-temperature pulse of any length exhibited increased budbreak as compared to the constant temperature for each specific ecotype. The current experiment used single tubers, removed from their chains and the influence of apical dominance, and there was no significant difference between tubers held at a constant temperature and those exposed to the diurnal simulated temperatures. Kawabata and Nishimoto (2003) included a single temperature amplitude (20 to 35 C), plus a constant, and varied the time spent on the high-temperature pulse. The current experiment included four temperature variations, plus a constant, and had one set amount of time on the cool and warm temperatures.

Travlos et al. (2009) experiment focused on alternating temperature affect on tubers from different depths. Four alternating temperatures of 18 to 22, 26 to 30, 34 to 38 and 42 to 46 C were evaluated for sprouting. Tubers showed a similar response to other experiments that alternating temperature around the established optimum sprouting temperature, 30 to 35C (Ueki 1969), stimulated faster and more tuber sprouting (Miles et al. 1996 and Kawabata and Nishimoto 2003).

As demonstrated by Kawabata and Nishimoto (2003), Miles et al. (1996) and the current experiment, there are many factors that can influence sprouting of nutsedge tubers. Apical dominance, ecotype and environmental factors (i. e. temperature and moisture) are all important variables to consider when predicting sprouting of tubers. As demonstrated by previous work, in certain instances, temperature variation can increase tuber sprouting on dormant tubers. This experiment focused on simulating a diurnal flux of temperature and evaluating tuber sprouting. Evaluating for those factors indicated that no significant difference was noted comparing any variation of diurnal flux (4, 9, 15, or 23 C) to a constant temperature.

The most probable explanation regarding the difference comparing the current experiment and those who have done similar experiments and reported a difference in sprouting and elongation in purple nutsedge tubers when comparing a constant temperature and alternating temperatures is in the handling of the tubers prior to the beginning of the experiment. Miles et al. (1996), Kawabata and Nishimoto (2003) and Travlos et al. (2009) tubers were set on a lab bench overnight at 20 to 22 C before the experiment. The tubers for the current experiment were stored at 4 C overnight before the experiment. Shamsi et al. (1978) reported earlier sprouting and an overall increase in sprouting of tubers chilled to 0 C over tubers not exposed to chilling temperatures both for mature (dormant) and immature tubers. As the current experiment is the only one to indicate chilling tubers overnight this may have stimulated all tubers, including tubers at the constant temperature, to sprout.

Table 5.1. Parameter estimates and 95% confidence interval (CI)[a] for sprouting of purple nutsedge when varying temperature using a thermogradient

	Parameter *a*[b]		Parameter *b1*[b]		Parameter *b2*[b]	
Temperature Variation[c]	Maximum Rate	95% CI	Maximum Rate	95% CI	Maximum Rate	95% CI
0	87.8	±11.8 NS	4.7	±7.4 NS	0.1	±0.04 NS
4	92.0	±8.8 NS	4.1	±5.9 NS	0.08	±0.04 NS
9	92.1	±5.7 NS	2.1	±2.6 NS	0.1	±0.03 NS
15	90.0	±5.7 NS	1.5	±2.2 NS	0.1	±0.03 NS
23	90.1	±6.1 NS	1.3	±2.2 NS	0.1	±0.04 NS

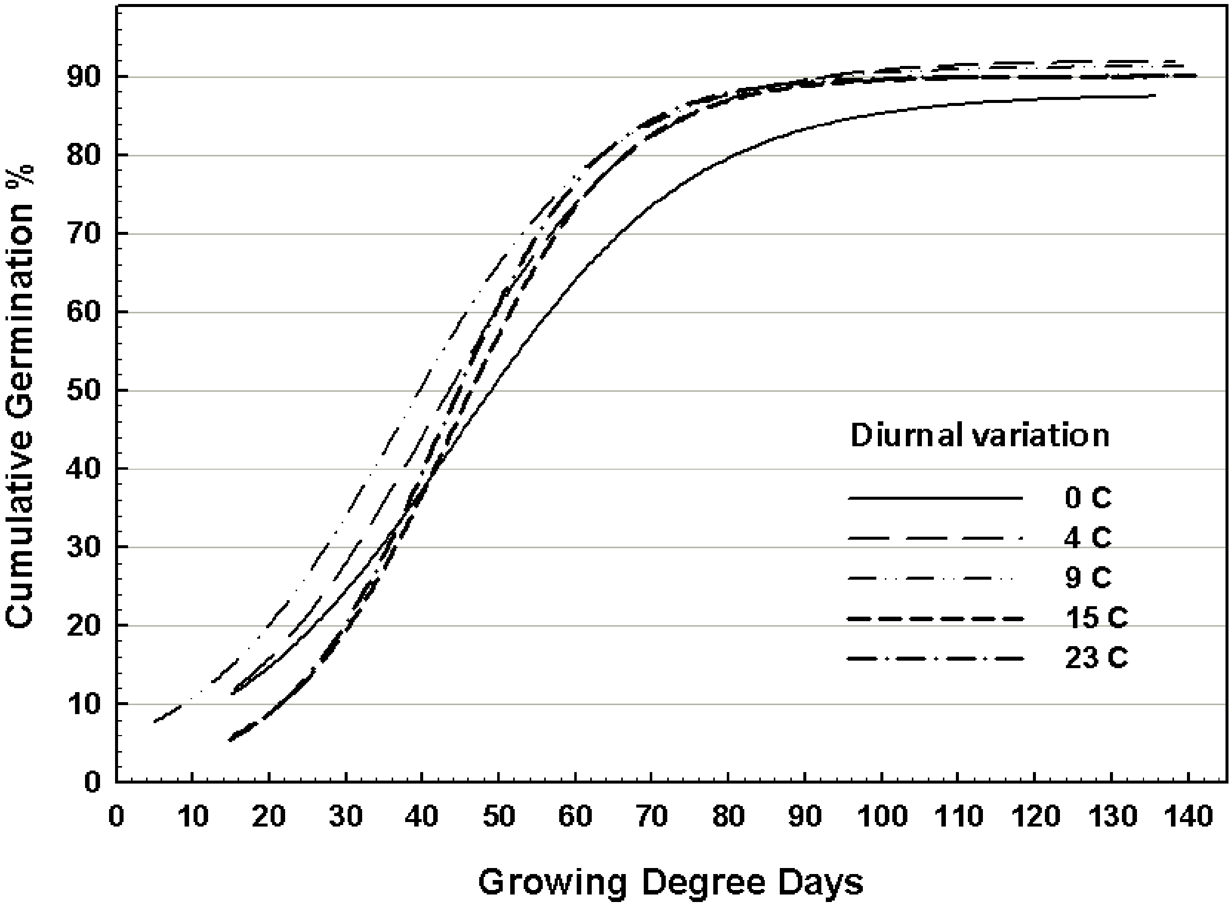
[a] Abbreviation: CI, Confidence Interval

[b] Parameter estimates calculated by nonlinear regression equation, $Y = a/[(1 + ((a-b_1)/b_1)*e^{(-b2x)})]$ using tuber sprouting with respect to time based on growing degree days.

[c] Temperature varied to simulate a diurnal period of 8 hours of cool temperature and 16 hours of warm temperature. 0 - Constant temperature of approximately 29 C for 24 hours a day. Temperature variation was approximately 26 to 30 C, 23 to 32 C, 19 to 33 C, and 13 to 36 C for fluctuations of 4, 9, 15 and 23 C, respectively.

[d] Values followed by NS indicate that the values do not significantly contribute to the respective equation at the 5% probability level. General linear models procedures were used with mean separation using 95% asymptotic confidence intervals.

Figure 5.1. Temperature varied to simulate a diurnal period of 8 hours of cool temperature and 16 hours of warm temperature. 0 - Constant temperature of approximately 28.5 C for 24 hours a day. Temperature variation was approximately 26 to 30 C, 23 to 32 C, 19 to 33 C, and 13 to 36 C for fluctuations of 4, 9, 15 and 23 C, respectively. See Table 1 for parameter estimates and 95% Confidence Intervals.



CHAPTER 6. SUMMARY AND CONCLUSIONS

Studies indicated that vegetable tolerance to EPTC is dependent on the crop sensitivity, EPTC rate, and mulch chosen. Pepper was the most sensitive crop evaluated and was not injured with 4.0 kg ai/ha on LDPE, but was significantly injured by the same rate on HBM mulch. Watermelon was the least sensitive crop evaluated and was not significantly injured by 4.0 kg ai/ha on HBM mulch.

Experiments with purple nutsedge tubers were conducted to evaluate the effect of diurnal temperature fluctuation on tuber sprouting. No significant difference was noted comparing any variation of diurnal flux (4, 9, 14, or 19 C) and the constant temperature (0 C). As tubers were chilled prior to planting, the effect of vernalization may have stimulated sprouting of tubers.

SOURCE OF MATERIALS

[1] Bayer CropScience. D - 40789 Monheim am Rhein, Germany.

[2] Glyfos X-tra. Cheminova. DK-7673 Harboøre, Denmark.

[3] TeeJet nozzles. Spraying Systems Co.; Wheaton, IL

[4] Pic-Chlor 60. Cardinal Professional Products, Mt. Zion, IL 62549

[5] EPTAM 7E, Gowan Company, Yuma, AZ 85364

[6] Low density polyethylene agricultural film, Pliant Corporation, Schaumburg, IL

[7] Blockade agricultural film, Pliant Corporation, Schaumburg, IL

[8] SigmaPlot 10th for Windows. SPSS Inc. 444 N Michigan Ave., Chicago, IL. 60611.

[9] Extra-deep, disposable polystyrene dishes (diameter, 10 cm; height 2 cm), Fischer Scientific, 200 Park Lane, Pittsburgh, PA 15275

[10] Anchor Steel Blue Germination Blotter Paper, SDB3.5, Anchor Paper Company 480 Broadway, St. Paul, Minnesota 55101

[11] Graphtec Midi Logger, GRAPHTEC Corporation, 503-10 Shinano-cho, Totsuka-ku Yokohama 244-8503, Japan

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APPENDIX 1

Cotton Literature Review

Cotton (*Gossypium hirsutum* L.) is an important crop for the southeastern US. In 2007 766,000 ha was planted in Virginia, North Carolina, South Carolina, Georgia, Florida and Alabama (USDA-NASS 2008). Pesticide use is needed to protect this economically important crop from insects, diseases, and weeds. Due to the warm weather and humid conditions, weed control is particularly difficult in the mid-south and southeastern United States (Snipes and Mueller 1992; Snipes and Mueller 1994). Prior to herbicide-resistant cotton, weed management was labor intensive and required numerous herbicide applications to achieve control at many different stages of cotton growth (Byrd and York 1987; Culpepper and York 1997; Culpepper and York 1999; Guthrie and York 1989; Jordan et al. 1993; Wilcut et al. 1995; Wilcut et al. 1997). While many of the conventional herbicide programs can also be used with herbicide-resistant cotton production systems, resistant cultivars have the added benefit of an additional herbicide that can be applied broadcast during the growing season.

Transgenic cotton has increased the production efficiency and decreased pesticide cost of managing pests, especially weeds (Young 2006). Herbicide-resistant crops help conserve environmental resources such as soil by encouraging farmers to increase their ha devoted to no-till and conservation tillage. In 1996 there were 206,000 ha practicing no-till in the US; as of 2002, it had risen to 822,000 ha (Sankula and Blumenthal 2004). Herbicide-resistant crop production can be more efficient and cost-effective than traditional weed management by lowering multiple herbicide use, reduced tillage, and simplify weed management (Young 2006). Sankula and Blumenthal (2004) reported an overall net reduction of production costs by $221 million and a reduction in pesticide application of 4.1 million kg for the United States. For the southeast (SE) alone, this is a savings of $68.1 million (Sankula and Blumenthal 2004). As of 2009, the top planted varieties in the SE were: DP 555 BG/RR (58%), PHY 375 WRF (6%), DP 0935 B2RF (5%), ST 4427 B2RF (3%), and ST 4554 B2RF (2%), all of which are transgenic varieties (USDA-AMS 2009). The percent of transgenic cotton planted in the SE ranged from 96.2% in Alabama to 100% in South Carolina (USDA-AMS 2009).

At least three methods exist for engineering herbicide resistance in plants: initiating overproduction of the herbicide-sensitive biochemical target, alteration of the target structure causing reduced sensitivity, or detoxification or degradation of the chemical prior to the herbicide reaching the target within a plant cell (Stalker et al. 1988).

Glufosinate is a foliar-applied, broad-spectrum, nonselective herbicide that controls annual and perennial grasses and broadleaf weeds. It causes a build-up of toxic levels of ammonia that destroys plant cells (Devine et al. 1993). Glufosinate-resistance was developed using a *bar* gene encoding an enzyme, phosphinothricin acetyl transferase (PAT) , that detoxifies glufosinate (Murakami et al. 1986; Thompson et al. 1987). This method is the above described “detoxification or degradation” method. The resistance trait patent is owned by Bayer CropScience and is known as *LibertyLink*® and cotton has been commercially available for production since 2004. Glufosinate-resistant cotton is produced mainly in Texas (USDA-AMS 2009).

Glyphosate resistance, known by the brand name *Roundup Ready®*, is the most widely adopted form of engineered herbicide-resistance in crops. Glyphosate is foliar applied, has a broad weed control spectrum, is nonselective, and controls annual and perennial grasses and broadleaf weeds. In susceptible plants, glyphosate inhibits growth followed by chlorosis and necrosis (Vencill 2002). Glyphosate causes deregulation of the shikimate pathway by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme in the formation of three aromatic amino acids: tyrosine, tryptophan, and phenylalanine (Devine et al. 1993; Franz et al. 1997). Glyphosate tolerance in cotton was achieved by alteration of the target site, introducing an insensitive EPSPS. A gene was inserted through *Agrobacterium tumefaciens*-mediated transformation and caused the production of insensitive EPSPS, known as CP4, in the cell chloroplasts (Dill 2005).

Grower acceptance and planting Roundup Ready crops has vastly increased glyphosate use. Since the introduction of glyphosate-resistant soybean in 1996, the annual volume of glyphosate increased from 2.5 million to 30 million kg per year, and the average number of applications rose slightly from 1 to 1.4 annually from 1995 to 2002 (Young 2006). Annual glyphosate use in glyphosate-resistant cotton increased sharply from 0.7 to 3.9 million kg per year from 1997 to 2002, and applications nearly doubled from 1 to 1.8 annually from 1996 to 2001 (Young 2006). With increased postemergence glyphosate applications and decreased use of other herbicide modes of action, it was predicted that there could be a shift of weeds to plants with some natural tolerance to glyphosate may become problematic (Shaner 2000).

Changes in weed management tactics (i.e. applying different herbicides or altering land management) can prompt a shift in weed species composition (Aldrich and Kremer 1997; Culpepper et al. 2004; Tuesca et al. 2001). With the rapid adoption of glyphosate-resistant crops and the subsequent reliance on glyphosate, Benghal dayflower (*Commelina benghalensis* L.), which is only suppressed by glyphosate, has become a problem for south Georgia farmers (Culpepper et al. 2004; Prostko et al. 2005). The use of a single mode of action for season long control of weeds has been a factor in promoting weed shifts in grower's fields.

In Florida, the most troublesome weed in cotton is Benghal dayflower (Webster 2009). As of 2009 Palmer amaranth and its resistant biotypes are three of the top five most troublesome weeds for Georgia cotton; most troublesome – glyphosate and ALS resistant Palmer amaranth, second – glyphosate-resistant Palmer amaranth, and fourth – Palmer amaranth (Webster 2009). Previously, Palmer amaranth was the second most common and troublesome weed with no resistant biotypes listed (Webster 2005c). Palmer amaranth is an annual broadleaf weed that can grow to 2 m in height, producing unbranched terminal thyresis that can be up to 0.5 m long (Elmore 1990). Palmer amaranth is dioecious, having male and female flowers on separate plants (Elmore 1990). A Texas study indicated cotton lint yield was linearly reduced 13 to 54% as Palmer amaranth density increased from 1 to 10 plants in 9.1 m of row (Morgan et al. 2001). In Oklahoma, a similar study reported cotton lint yield was reduced 5.9 to 11.5% with increased Palmer amaranth population in 10 m of row (Rowland et al. 1999).

There are several documented Palmer amaranth populations in the United States that have developed herbicide resistance to several herbicide modes of action including EPSP, ALS, photosystem II, and mitotic inhibitors (Culpepper et al. 2006, Sprague et al. 1997, Horak et al. 1995, Heap 2008, Wise et al. 2009; and Gossett et al. 1992). In Georgia triazine, ALS and EPSP resistance have been documented (Culpepper et al. 2006, Vencill et al. 2009, Wise 2009). With increased herbicide resistance in weeds, there is a need to develop methods of control through new herbicides and new management techniques. Currently there are efforts to not only combine existing resistances into crops, but also incorporate genes containing new herbicide resistant traits to allow for use of existing herbicides on crops that were previously sensitive.

Novel genes have the potential to provide growers with effective weed management alternatives. However, it can take many years for researchers to produce a commercially viable cultivar. Utilizing existing herbicide traits to create crops with multiple herbicide-resistances may prove to take less time and be available to growers who have herbicide-resistant weeds before they need to adopt costly weed management programs.

CROP TOLERANCE AND PALMER AMARANTH CONTROL IN GLYTOL AND GLYTOL PLUS LIBERTY LINK COTTON (*GOSSYPIUM HIRSUTUM* L.) IN THE SOUTHEASTERN U. S.[3]

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[3] Wallace, R. D., L. M. Sosnoskie, A. S. Culpepper, A. C. York, K. Edmisten, M. Patterson, M. A. Jones, G. L. Cloud, J. Pierson, and M. Rinehardt. To be submitted to Cotton Science.

Chapter 3. Crop Tolerance and Palmer amaranth Control in GlyTol and GlyTol plus LibertlyLink Cotton (*Gossypium hirsutum* L.) in the Southeastern U. S.

Abstract: Cotton (*Gossypium hirsutum* L.) tolerant to topical applications of both glyphosate and glufosinate will aid growers in the control of weeds, including glyphosate-resistant (GR) Palmer amaranth (*Amaranthus palmeri* S. Wats). An experiment was conducted at eight locations in five southeastern states to determine the tolerance of GlyTol plus LibertyLink 'FiberMax 958' cotton to sequential applications of 1) glyphosate (1.3 kg ae ha^{-1}) applied four times, 2) glufosinate (0.68 kg ae ha^{-1}) applied four times, 3) altering applications of glufosinate, glyphosate, glufosinate, and glyphosate, 4) altering application of glyphosate, glufosinate, glyphosate, and glufosinate, and 5) glufosinate plus glyphosate applied four times. GlyTol 'Coker 312' cotton was included in the study for comparison and was treated with the four glyphosate applications. Applications were made topically to cotton in the 1- to 3-, 6- to 8-, 14- to 16-leaf stages and again at 50% boll crack. A non-herbicidal control was included within each technology. Compared to the controls, no herbicide application visually injured cotton throughout the experiment at any location. Similar to cotton injury, herbicide applications did not impact cotton stand, height, boll morphology, boll opening, lint yield, or fiber qualities within cultivar. GlyTol plus LibertyLink 'FiberMax 958' cotton had 19% greater seedling emergence, 9% higher cotton yields, 11.8% lower micronaire, 2.4% lower fiber length, 3.3% lower fiber strength, and 1.7% lower fiber uniformity when compared to GlyTol 'Coker 312'. An additional study near Rocky Mount, NC and Garden Valley, GA evaluated Palmer amaranth response to the aforementioned herbicide systems, excluding the boll crack application. In NC, glyphosate-sensitive Palmer amaranth control was 99% or greater throughout the season for all herbicide systems. In GA, GR Palmer amaranth was controlled only 15% late in the season with

the four glyphosate applications. Applying glyphosate first in the system of rotating glyphosate and glufosinate only provided 48% late season control. In contrast, any program applying glufosinate first to cotton in the 1- to 3-leaf stage when Palmer was 7 cm in height or less provided complete GR Palmer amaranth control.

Introduction

Cotton production is a critical component of farm sustainability in the Southeast with plantings on 766,000 ha in VA, NC, SC, GA, FL, and AL during 2009 (USDA-NASS, 2009). Weed control, more specifically glyphosate- and glyphosate plus ALS-resistant Palmer amaranth control, has become the greatest pest management challenge for these cotton producers (Sosnoskie et al., 2009, Webster, 2009). In fields infested with glyphosate- and glyphosate plus ALS-resistant Palmer amaranth, residual herbicides applied throughout the crop do offer control (Marshall, 2009; Whitaker et al., 2007). However, control is unpredictable in production areas without irrigation to ensure timely herbicide activation. When biotypes of Palmer amaranth with resistance to both glyphosate and ALS herbicides escape residual herbicides applied at planting, only two topical herbicide options exist for cotton producers. Fluometuron plus MSMA can be applied over-the-top of cotton but this option provides ineffective control of emerged Palmer amaranth and can adversely affect yield and maturity of cotton (Byrd and York, 1987; Guthrie and York 1989). Glufosinate applied very timely over-the-top of glufosinate-tolerant crops can control *Amaranthus* species (Beyers et al., 2002; Gardner et al., 2006; Marshall, 2009; Steckel et al. 1997) and glufosinate-based systems are more effective than glyphosate-based systems in controlling GR Palmer amaranth (Marshall, 2009). Although glufosinate is more effective than glyphosate in the control of GR Palmer amaranth, glufosinate often does not effectively control other common weeds infesting southeastern cotton fields such as large annual grasses, and larger glyphosate-sensitive *Amaranthus*, species (Coetzer et al., 2002; Corbett et al., 2004; Dodds et al., 2005). Many of the weeds tolerant to glufosinate can be controlled effectively and easily with glyphosate (Tharp et al., 1999). Thus, growers with cotton cultivars allowing them the option to spray glyphosate and/or glufosinate over-the-top of cotton would improve weed control options.

Current cotton cultivars are available that provide tolerance to topical applications of glyphosate (ie Roundup Ready Flex®) or glufosinate (LibertyLink®). One current cotton technology, Phytogen Widestrike™ cultivars, can tolerate applications of both glyphosate and glufosinate (Culpepper et al., 2009). Phytogen'sWidestrike™ cotton such as 'PHY 485 WRF' expresses remarkable tolerance to glyphosate and was developed by jointly introducing two CP4 *epsps* gene expression cassettes into cotton along with a meristem-active promoter (Chen et al., 2006). However, Phytogen's Widestrike™ cotton tolerance to glufosinate is less acceptable with injury often ranging from 20 to 50% (Culpepper et al., 2009). In Phytogen's Widestrike™ cotton, the phosphinothricin acetyltransferase (*pat*) gene was inserted for use as a selectable marker during a plant transformation conferring resistance to lepidopteran pests. This *pat* gene of *Streptomcyces viridochromogenes* codes for phophinothricin-acetyltransferase, an enzyme which catalyzes the conversion of lethal L-phosphinothricin to non-lethal N-acetyl-L-phosphinothricin (Devine et al., 1993; Hinchee et al., 1993). The Widestrike™ cotton detoxification process using the *pat* gene often does not provide the level of tolerance desired by most growers.

A cotton that can withstand normal use rates of both glyphosate and glufosinate without injury would be more readably accepted by growers when compared to the Phytogen's Widestrike™ cotton technology. GlyTol plus LibertyLink cotton may offer growers just such a technology. GlyTol plus LibertyLink cotton confers tolerance to glyphosate in response to an insertion of the *2mepsps* gene, using *Agrobacterium*-mediated gene transfer (Bayer CropScience 2006). The presence of the gene produces a modified protein that has a decreased binding affinity for glyphosate thereby conferring tolerance. GlyTol plus LibertyLink cotton also confers tolerance to glufosinate in response to the insertion of a *bar* gene which, similar to the *pat* gene in Phytogen's Widestrike^{TM} cotton, converts lethal L-phosphinothricin to non-lethal N-acetyl-L-phosphinothricin (Devine, 1993; Hinchee et al., 1993). Although the glufosinate applied to both Widestrike^{TM} and GlyTol plus LibertyLink is being detoxified through similar processes, the GlyTol plus LibertyLink technology may be more effective in the detoxification process thereby eliminating crop injury and yield concerns. Research was conducted to determine the response of GlyTol plus LibertyLink cotton and Palmer amaranth to topical applications of glufosinate and glyphosate. Information of this nature will be essential in developing sustainable management systems for cotton production in the southeastern U.S.

Materials and Methods

A crop tolerance experiment was conducted at eight locations across five southeastern U.S. states during 2008. Locations were selected for there light weed pressure and were located near Attapulgus, GA a Lucy loamy sand (Loamy, kaolinitic, thermic Arenic Kandiudults); Brooks county, GA a Tifton loamy sand (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults); Elko, SC a Varina loamy sand (Fine, kaolinitic, thermic Plinthic Paleudults); Laurell Hill, NC a Noboco loamy sand (Fine-loamy, siliceous, subactive, thermic Oxyaquic Paleudults); Lewiston, NC a Goldsboro loamy sand (Fine-loamy, siliceous, subactive, thermic Aquic Paleudults); Sellers, SC a Clarendon loamy sand (Fine-loamy, siliceous, semiactive, thermic Plinthaquic Paleudults); Tallahassee, FL a Notcher fine sandy loam (Fine-loamy, siliceous, subactive, thermic Plinthic Paleudults); and Tuscaloosa, AL a Compass fine sandy loam (Coarse-loamy, siliceous, subactive, thermic Plinthic Paleudults) (USDA-NRCS 2009). An experiment focusing on Palmer amaranth control was conducted near Rocky Mount, NC, a Norfolk loamy sand (fine-loamy, siliceous, thermic Typic Paleudults) (USDA-NRCS 2009) with 70 glyphosate-sensitive Palmer amaranth plants m^{-2} and near Garden Valley, GA, a Dothan loamy sand (fine-loamy, siliceous, thermic, Plinthic Paleudults) (USDA-NRCS 2009) with 60 GR Palmer amaranth plants m^{-2}. Production practices, other than weed control, were according to local standards for both experiments.

Cultivars planted included Coker 312 with tolerance to glyphosate (GlyTol), and FiberMax 958 with tolerance to glyphosate and glufosinate (GlyTol plus LibertyLink). Cotton at 4 seed m^{-1} was planted into conventionally prepared seedbeds at all locations between April 29 and May 30, 2008. In the tolerance experiment, treatments consisted of six herbicide options in the GlyTol plus LibertyLink cotton and two herbicide options in the GlyTol cotton. In GlyTol cotton, no herbicides or glyphosate at 1.3 kg ai ha^{-1} (Glyfos X-tra; Cheminova Durham; NC) was applied four times. GlyTol plus LibertyLink cotton was treated with 1) no herbicide, 2) four applications of glyphosate at 1.3 kg ha, 3) four applications of glufosinate at 0.68 kg ha^{-1} (Ignite 280 S; Bayer CropScience; Monheim am Rhein; Germany), 4) altering applications of glufosinate, glyphosate, glufosinate, and glyphosate, 5) altering applications of glyphosate, glufosinate, glyphosate, and glufosinate, and 6)four applications of glyphosate plus glufosinate. Herbicide applications were made topically to cotton in the 1- to 3-, 6- to 8-, 14- to 16-leaf stages and again at 50% boll crack. For the weed control experiment, treatments were identical to those in the tolerance experiment minus the boll crack application. Palmer amaranth ranged from 5 to 7 cm during the initial application and no other weeds were present. Both experiments were randomized complete block designs with treatments replicated four times. Herbicides were applied using a CO_2-pressureized backpack sprayer equipped with flat-fan nozzles (TeeJet XR 11002 nozzles; Spraying Systems Co.; Wheaton, IL) calibrated to deliver 140 L ha^{-1} at 160 kPa.

Crop tolerance sites were kept weed free throughout the season with residual at plat herbicides and hand weeding. At each site, cotton was evaluated for stand, visual chlorosis or necrosis, height, percent late-season boll opening and boll morphology, yield, and lint fiber quality. Cotton stand was measured by counting the number of plants per m at 7 and 14 days after planting while visual crop injury evaluations were made 7 to 14 days after each application using a scale of 0 = no injury and 100 = crop death. Plant heights were recorded on 10 plants at 5 and 8 wk after planting. Boll opening was determined by counting the number of opened and unopened bolls when the GlyTol plus LibertyLink control visually appeared to have 50% open bolls. At the same time, 25 bolls per plot were compared to the control for uniformity of shape. The center two rows of each plot were mechanically harvested. A 200-g sample of harvested seed cotton was collected from each plot and used for lint percentage and fiber quality determinations. Seed cotton was ginned on a laboratory gin and fiber length, fiber length uniformity, fiber strength, and mironaire were determined by high volume instrumentation testing (Sasser, 1981). At the weed control locations, percent control was visually estimated 7 to 13 days after each application and again prior to cotton defoliation. Weed control was estimated using a scale of 0 to 100, where 0 = no weed control and 100 = complete weed control (Frans et al., 1986).

Data were analyzed using SAS ProcMixed due to experiment design and evaluation as a single experiment (SAS Institute, 2003). Treatments were considered main effects, while replication and site were considered as random effects. Data were also analyzed using orthogonal contrasts providing treatment comparisons within cultivars.

Results and Discussion

As there was no significant treatment-by-site interaction for the crop tolerance experiment, all cotton variables were combined across the eight locations. Herbicide treatments did not visually injure cotton at any time during the season at any location (Table Appendix 1). This level of tolerance is similar to currently available herbicide-tolerant cotton (Blair-Kerth at al. 2001; Jones and Snipes 1999; Nida et al. 1996). Similar results were noted with GlyTol plus LibertyLink cotton tolerance to glyphosate and glufosinate across the MidSouth states during 2008 (Irby et al. 2009). These results suggest that Glytol plus LibertyLink cotton confers a greater level of tolerance than does Phytogen'sWidestrike™ cotton (Culpepper et al. 2009).

Herbicide systems also had no impact on cotton stand (7.7 plants per m at 14 d after planting) or cotton heights (73 to 78 cm tall at 46 to 54 d after 1 to 3-leaf application). GlyTol plus LibertyLink cotton emergence was 19% greater and cotton heights were 0.8 and 3.4 cm larger when compared to GlyTol cotton at these times. Boll opening and boll morphology were similar across cultivars and were not impacted by herbicide treatments.

Similar to cotton development, herbicide systems did not impact cotton yields (Table Appendix 2). In GlyTol cotton, yields from the control (1040 kg ha^{-1}) were similar to the system receiving four glyphosate applications (1060 kg ha^{-1}). GlyTol plus LibertyLink cotton yields ranged only 5% from 1120 kg ha^{-1} to 1170 kg ha^{-1} when comparing the control with four applications of glyphosate, glufosinate, mixtures of glyphosate plus glufosinate, or alternating applications of glyphosate and glufosinate. When contrasting cultivars, the GlyTol plus LibertyLink 'FiberMax 958' cultivar produced at least 60 kg ha^{-1} more cotton than did the GlyTol 'Coker 312' cultivar.

Fiber length, micronaire, strength, and uniformity were similar within each cultivar regardless of herbicide systems (Table Appendix 3). Within the GlyTol plus LibertyLink cultivar, fiber quality variations ranged from 2.99 to 3.04 cm for fiber length, 4.49 to 4.52 for micronaire, 32.9 to 33.7 g tex^{-1} for fiber strength, and 83.7 to 84.1% for fiber uniformity for all systems. Although herbicide systems did not impact fiber quality, differences were noted when comparing cultivars. Averaged over herbicide options within cultivar, GlyTol plus LibertyLink cotton had 11.8% lower micronaire, 2.4% lower fiber length, 3.3% lower fiber strength, and 1.7% lower fiber uniformity when compared to GlyTol cotton. Fiber quality is often impacted by environments and cotton cultivars planted (Shurley et al. 2004) but rarely are they impacted by herbicide systems when yields are not influenced (Keeling et al., 1996; York, 1983).

Weed control sites were not combined, as they were evaluating control of a GR and a glyphosate-sensitive biotype of Palmer amaranth. Control of glyphosate-sensitive Palmer amaranth was complete throughout the season with all herbicide systems (Table Appendix 4). Glyphosate remains one of the most effective management tactics to control glyphosate-senstive Palmer amaranth (Bond et al., 2006; Corbett et al., 2004; Starke and Oliver, 1998) but is no longer effective in controlling GR Palmer amaranth (Culpepper et al., 2006). At the GR Palmer amaranth location, three applications of glyphosate provided only 15% control late in the season (Table Appendix 4). This population of GR Palmer amaranth has been shown to be highly resistant to glyphosate (Culpepper et al., 2006). Applying glyphosate to 1- to 3-leaf cotton, glufosinate to 6- to 8-leaf cotton, and glyphosate to 14- to 16-leaf cotton only provided 48% late season control. After the initial application of glyphosate failed to slow the growth of Palmer amaranth, the Palmer amaranth became too large, 30 cm, to control with glufosinate. Glufosinate must be applied to small (<10 cm) Palmer amaranth for consistent control (Anonymous, 2006b; Coetzer et al., 2002; Corbett et al., 2004). In the present study, complete Palmer amaranth control was noted with all systems containing glufosinate applied first in the system when Palmer amaranth was 7 cm or less in height and when cotton was in the 1- to 3-leaf stage.

In this experiment, GlyTol plus LibertyLink cotton was tolerant to sequential applications of glyphosate, glufosinate, and even mixtures of glyphosate plus glufosinate. This technology, once implemented in high yielding cultivars, will offer cotton producers the ability to apply both glyphosate and glufosinate topically to cotton without crop injury and yield loss concerns. Although cotton cultivars tolerant to topical applications of both glyphosate and glufosinate will offer growers much flexibility, it will be essential that specific weed management programs with timely applications be developed to control troublesome weeds as noted with GR Palmer amaranth in this experiment.

Table Appendix 1. GlyTol Coker 312 and GlyTol plus LibertyLink FiberMax 958 cotton growth and development as impacted by topical glyphosate and/or glufosinate applications across five Southeastern States [z]

Cultivar technology	Herbicide application (cotton stage of growth)[y]				Injury	Stand	Height	Open Boll
	1-3 leaf	6-8 leaf	14-16 leaf	50% boll crack	(%)	(plant/m)	(cm)	(%)
GlyTol	None	None	None	None	0	7.7	73.8	48.4
GlyTol	Glyphosate	Glyphosate	Glyphosate	Glyphosate	0	7.7	73.4	48.1
GlyTol + LL[x]	None	None	None	None	0	9.4	77.6	49.7
GlyTol + LL	Glyphosate	Glyphosate	Glyphosate	Glyphosate	0	9.4	76.1	49.1
GlyTol + LL	Glufosinate	Glufosinate	Glufosinate	Glufosinate	0	9.4	76.7	48.4
GlyTol + LL	Glufosinate	Glyphosate	Glufosinate	Glyphosate	0	9.4	77.5	47.5
GlyTol + LL	Glyphosate	Glufosinate	Glyphosate	Glufosinate	0	9.4	77.2	47.8
GlyTol + LL	Glyphosate + Glufosinate	Glyphosate + Glufosinate	Glyphosate + Glufosinate	Glyphosate + Glufosinate	0	9.4	77.0	46.6
Contrasts[w]					Pr > F	Pr > F	Pr > F	Pr > F
GlyTol vs. GlyTol + LL						<0.0001	<0.0001	0.9133
Compare within GlyTol						0.7719	0.8107	0.8504
Treatments vs. GlyTol + LL	None	None	None	None		0.8162	0.5100	0.1596
Gyltol + LL nontreated vs. GlyTol + LL	Glyphosate	Glyphosate	Glyphosate	Glyphosate		0.8490	0.2990	
Gyltol + LL nontreated vs. GlyTol + LL	Glufosinate	Glufosinate	Glufosinate	Glufosinate		0.9965	0.4938	
GlyTol + LL nontreated vs. GlyTol + LL	Glufosinate	Glyphosate	Glufosinate	Glyphosate		0.9414	0.9298	
GlyTol + LL nontreated vs. GlyTol + LL	Glyphosate	Glufosinate	Glyphosate	Glufosinate		0.7162	0.7657	
GlyTol + LL nontreated vs. GlyTol + LL	Glyphosate + Glufosinate	Glyphosate + Glufosinate	Glyphosate + Glufosinate	Glyphosate + Glufosinate		0.7818	0.6583	

[z] Data averaged over eight locations.

[y] Glyphosate applied at 1.3 kg ae ha^{-1}, glufosinate applied at 0.68 kg ae ha^{-1}

[x] LL - LibteryLink

[w] Orthogonal contrasts for cultivars and treatments

Table Appendix 2. GlyTol Coker 312 and GlyTol plus LibertyLink FiberMax 958 cotton lint yield as impacted by topical glyphosate and/or glufosinate applications across eight locations in five Southeastern States [z]

Cultivar technology	Herbicide application (cotton stage of growth)[y]				Yield (kg/ha)
	1-3 leaf	6-8 leaf	14-16 leaf	50% boll crack	
GlyTol	**None**	**None**	**None**	**None**	**1040**
GlyTol	**Glyphosate**	**Glyphosate**	**Glyphosate**	**Glyphosate**	**1060**
GlyTol + LL[x]	**None**	**None**	**None**	**None**	**1170**
GlyTol + LL	**Glyphosate**	**Glyphosate**	**Glyphosate**	**Glyphosate**	**1130**
GlyTol + LL	**Glufosinate**	**Glufosinate**	**Glufosinate**	**Glufosinate**	**1130**
GlyTol + LL	**Glufosinate**	**Glyphosate**	**Glufosinate**	**Glyphosate**	**1150**
GlyTol + LL	**Glyphosate**	**Glufosinate**	**Glyphosate**	**Glufosinate**	**1150**
GlyTol + LL	**Glyphosate + Glufosinate**	**Glyphosate + Glufosinate**	**Glyphosate + Glufosinate**	**Glyphosate + Glufosinate**	**1120**
Contrasts[w]					**Pr > F**
GlyTol vs. GlyTol + LL					**<0.001**
Compare within GlyTol					**0.6412**
Treatments vs. GlyTol + LL	**None**	**None**	**None**	**None**	**0.2152**
GlyTol + LL nontreated vs. GlyTol + LL	**Glyphosate**	**Glyphosate**	**Glyphosate**	**Glyphosate**	**0.3024**
GlyTol + LL nontreated vs. GlyTol + LL	**Glufosinate**	**Glufosinate**	**Glufosinate**	**Glufosinate**	**0.3091**
GlyTol + LL nontreated vs. GlyTol + LL	**Glufosinate**	**Glyphosate**	**Glufosinate**	**Glyphosate**	**0.5198**
GlyTol + LL nontreated vs. GlyTol + LL	**Glyphosate**	**Glufosinate**	**Glyphosate**	**Glufosinate**	**0.4678**
GlyTol + LL nontreated vs. GlyTol + LL	**Glyphosate + Glufosinate**	**Glyphosate + Glufosinate**	**Glyphosate + Glufosinate**	**Glyphosate + Glufosinate**	**0.151**

[z] **Data averaged over eight locations.**

[y] **Glyphosate applied at 1.3 kg ae ha^{-1}, glufosinate applied at 0.68 kg ae ha^{-1}**

[x] **LL - LibteryLink**

[w] **Orthogonal contrasts for cultivars and treatments**

Table Appendix 3. GlyTol Coker 312 and GlyTol plus LibertyLink FiberMax 958 cotton lint quality as impacted by topical glyphosate or glufosinate applications across eight locations in five Southeastern States [z]

Cultivar technology	Herbicide application (cotton stage of growth)[y]				Fiber length (cm)	Micronaire	Strength (g/tex)	Uniformity (%)
	1-3 leaf	6-8 leaf	14-16 leaf	50% boll crack				
GlyTol	None	None	None	None	3.08	5.08	34.3	85.4
GlyTol	Glyphosate	Glyphosate	Glyphosate	Glyphosate	3.09	5.12	34.4	85.2
GlyTol + LL[x]	None	None	None	None	3.01	4.49	32.9	83.9
GlyTol + LL	Glyphosate	Glyphosate	Glyphosate	Glyphosate	2.99	4.5	33.5	83.7
GlyTol + LL	Glufosinate	Glufosinate	Glufosinate	Glufosinate	3.04	4.52	33.1	83.9
GlyTol + LL	Glufosinate	Glyphosate	Glufosinate	Glyphosate	3.00	4.48	33.0	84.0
GlyTol + LL	Glyphosate	Glufosinate	Glyphosate	Glufosinate	3.01	4.48	33.0	83.7
GlyTol + LL	Glyphosate + Glufosinate	Glyphosate + Glufosinate	Glyphosate + Glufosinate	Glyphosate + Glufosinate	3.02	4.51	33.7	84.1
Contrasts[w]					Pr > F	Pr > F	Pr > F	Pr > F
GlyTol vs. GlyTol + LL					<0.0001	<0.0001	<0.0001	<0.0001
Compare within GlyTol					0.6364	0.7652	0.5137	0.2576
Treatments vs. GlyTol + LL	None	None	None	None	0.9844	0.3595	0.8816	0.8247
GlyTol + LL nontreated vs. GlyTol + LL	Glyphosate	Glyphosate	Glyphosate	Glyphosate	0.385	0.206	0.8853	0.2244
GlyTol + LL nontreated vs. GlyTol + LL	Glufosinate	Glufosinate	Glufosinate	Glufosinate	0.213	0.6696	0.6308	0.9876
GlyTol + LL nontreated vs. GlyTol + LL	Glufosinate	Glyphosate	Glufosinate	Glyphosate	0.5708	0.8634	0.8853	0.7082
GlyTol + LL nontreated vs. GlyTol + LL	Glyphosate	Glufosinate	Glyphosate	Glufosinate	0.8798	0.9451	0.8101	0.3339
GlyTol + LL nontreated vs. GlyTol + LL	Glyphosate + Glufosinate	Glyphosate + Glufosinate	Glyphosate + Glufosinate	Glyphosate + Glufosinate	0.6776	0.1067	0.7365	0.3339

[z] Data averaged over eight locations.

[y] Glyphosate applied at 1.3 kg ae ha^{-1}, glufosinate applied at 0.68 kg ae ha^{-1}

[x] LL - LibteryLink

[w] Orthogonal contrasts for cultivars and treatments

Table Appendix 4. Glyphosate-resistant (GR) and sensitive (GS) Palmer amaranth response to glyphosate and/or glufosinate systems in GlyTol Coker 312 and GlyTol plus LibertyLink FiberMax 958 cotton[z]

Cultivar technology	Herbicide application (cotton stage of growth)[y]			GR Palmer amaranth	GR Palmer amaranth	GS Palmer amaranth	GS Palmer amaranth
	1-3 leaf	6-8 leaf	14-16 leaf	25 DAFA[x]	13 DALA[x]	21 DAA	30 DALA
GlyTol	None	None	None	0 d	0 d	0 b	0 b
GlyTol	Glyphosate	Glyphosate	Glyphosate	10 c	15 c	100 a	100 a
GlyTol + LL[w]	None	None	None	0 d	0 d	0 b	0 b
GlyTol + LL	Glyphosate	Glyphosate	Glyphosate	10 c	15 c	100 a	100 a
GlyTol + LL	Glufosinate	Glufosinate	Glufosinate	100 a	100 a	99 a	99 a
GlyTol + LL	Glufosinate	Glyphosate	Glufosinate	100 a	100 a	100 a	100 a
GlyTol + LL	Glyphosate	Glufosinate	Glyphosate	70 b	48 b	100 a	100 a
GlyTol + LL	Glyphosate + Glufosinate	Glyphosate + Glufosinate	Glyphosate + Glufosinate	100 a	100 a	99 a	99 a
Contrasts[v]				Pr > F	Pr > F	Pr > F	Pr > F
GlyTol vs. GlyTol + LL				<0.0001	<0.0001	<0.0001	<0.0001
Compare within GlyTol				<0.0001	<0.0001	<0.0001	<0.0001
Treatments vs. GlyTol + LL	None	None	None	<0.0001	<0.0001	<0.0001	<0.0001
Glytol + LL nontreated vs. Glytol + LL	Glyphosate	Glyphosate	Glyphosate	<0.0001	<0.0001	<0.0001	<0.0001
Glytol + LL nontreated vs. Glytol + LL	Glufosinate	Glufosinate	Glufosinate	<0.0001	<0.0001	<0.0001	<0.0001
Glytol + LL nontreated vs. Glytol + LL	Glufosinate	Glyphosate	Glufosinate	<0.0001	<0.0001	<0.0001	<0.0001
Glytol + LL nontreated vs. Glytol + LL	Glyphosate	Glufosinate	Glyphosate	<0.0001	<0.0001	<0.0001	<0.0001
Glytol + LL nontreated vs. Glytol + LL	Glyphosate + Glufosinate	Glyphosate + Glufosinate	Glyphosate + Glufosinate	<0.0001	<0.0001	<0.0001	<0.0001

[z] Glyphosate-resistant palmer amaranth location Garden Valley, GA; Glyphosate-sensitive palmer amaranth location Rocky mount, NC.

[y] Glyphosate applied at 1.3 kg ae ha^{-1}, glufosinate applied at 0.68 kg ae ha^{-1}

[x] DAFA – Days After First Application (1-3 leaf); DALA – Days After Last Application (14-16 leaf)[w] LL - LibteryLink

[v] Orthogonal contrasts for cultivars and treatments