

Integrated Pest Management Systems can be used for Promoting Plant Development in Upland Cotton to Combat Reniform Nematode Losses.

by

Justin A. Luangkhot

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 8, 2016

Copyright © 2016 by Justin A. Luangkhot

Approved by

Kathy S. Lawrence, Chair, Professor of Entomology and Plant Pathology
Jeffrey Coleman, Assistant Professor of Entomology and Plant Pathology
Charles C. Mitchell, Extension Specialist and Professor, Dept. of Crop, Soil, and Environmental
Sciences
James D. Spiers, Associate Professor of Horticulture

Abstract

Cotton (*Gossypium hirsutum*) growers of Alabama lost \$8.2 million to the reniform nematode (*Rotylenchulus reniformis*) in 2014. The overall objective for this study was to develop a holistic integrated pest management strategy to enhance plant growth and reduce the yield losses due to *R. reniformis* in upland cotton by 1) evaluating commercially available growth hormones for the enhancement of cotton plant growth; 2) evaluating starter fertilizers for the enhancement of cotton plant growth; 3) evaluating two nematicides for efficacy on *R. reniformis* and; 4) combining the optimum growth hormones and starter fertilizers with the nematicide to determine the most efficient management strategy for *R. reniformis* on cotton. Greenhouse trials and microplot tests were evaluated and field trials were conducted in two Alabama locations in both *R. reniformis* infested soils and non-infested soils. Plant biomass of the entire plant was greater with the addition of Ascend™ (cytokinin 0.09%, gibberellic acid 0.03%, and indolebutyric acid 0.045%) as a seed treatment and as a seed treatment plus foliar spray compared to the untreated control. Ascend™ seed treatment plus foliar spray showed significantly lower nematode eggs per gram of root when compared to the control. Plant height and plant biomass were not increased by any starter fertilizer application in greenhouse trials. Nematode populations were greater ($P < 0.05$) than the untreated control with the addition of starter fertilizers. The nematicides Velum Total™ (Imidacloprid 22.2% and Fluopyram 15.4%) and Vydate C-LV™ (Oxamyl 42%) were evaluated as seed treatments, in-furrow sprays, or foliar sprays. The Velum Total™ seed treatment and in-furrow spray treatments ($P < 0.05$)

reduced *R. reniformis* eggs per gram of root when compared to the control. The best selected plant hormone, starter fertilizers and nematicides treatments from the greenhouse trials were then tested in microplot and field trials individually or as combination treatments. Vydate C-LV™ alone, Vydate C-LV™ + starter fertilizers, and Vydate C-LV™ + plant hormone + starter fertilizers supported increased plant biomass in the presence of *R. reniformis* as compared to the untreated control. *Rotylenchulus reniformis* eggs per gram of root were also lower in the Vydate C-LV™ + starter fertilizers as compared to the control. Trials in *R. reniformis* infested fields found that several treatment combinations supported a larger plant biomass compared to the control. The Velum Total™ and Vydate C-LV™ nematicides alone and in combination with the starter fertilizers and plant hormones reduced *R. reniformis* eggs per gram of root when compared to the untreated control. Increase in cotton yields in the *R. reniformis* infested fields with the addition of Velum Total™ + plant hormone + starter fertilizers ($P < 0.05$) were observed when compared to the control.

Acknowledgments

I would like to express my utmost expression of gratitude to everyone that helped me complete this project. I would like to give a very special thank you to my major professor, Dr. Kathy Lawrence, for her patience, support, guidance, and encouragement through this degree and project. A very special thank you to Drew Schrimsher for triggering my interest and the support of supplying products needed to complete this project. Thank you to my graduate committee members, Dr. Jeffery Coleman, Dr. Charles C. Mitchell, and Dr. James Spiers for their contributions of time and guidance on this project. Thank you to my fellow graduate peers – Amber Smith, Ni Xiang, Caroline Land, Meredith Hall, Daniel Dodge, David Dyer, Stephen Till, and William Groover, and to the undergraduate students – Casie Spencer, Magnolia Wilson, Presley Townsend, Andrew Sikkens, Bruce Faulk, and Cole White, and colleagues Roelof Sikkens and Lam Duong for any and all help that I received towards the completion of this project.

I would like to extend a very special thank you to my colleague, friend, and girlfriend, Amber, for her never ending support and willingness to help in any aspect of this project when I asked. This project would not have been feasible without your help. To my parents and grandmother, I appreciate everything you have done for me with your support, encouragement, and love that you have provided during my time as a graduate student and in life.

Table of Contents

Abstract	ii
Acknowledgements	iv
List of Tables	vii
Chapter I: Review of Literature	1
Introduction	1
Plant Hormones	2
Starter Fertilizers	4
Nematicides	5
<i>Rotylenchulus reniformis</i>	6
Management Strategies	7
Literature cited	9
Chapter II: Greenhouse, Microplot, and Field Trials with Growth Hormone, Starter Fertilizer, and Nematicide Treatments	14
Abstract	14
Introduction	16
Materials and Methods	20
Results	27
Discussion	32
Literature cited	41
Overall Conclusion	46

Appendix 47

List of Tables

Table 1. Growth hormone effects on cotton root fresh weight, plant biomass, <i>Rotylenchulus reniformis</i> total egg numbers, and eggs per gram of root at 49 DAP in greenhouse trials.....	47
Table 2. Starter fertilizer effects on cotton root fresh weight, plant biomass, <i>Rotylenchulus reniformis</i> total egg numbers, and eggs per gram of root at 49 DAP in greenhouse trials.....	48
Table 3. Velum Total™ effects on cotton root fresh weight, plant biomass, <i>Rotylenchulus reniformis</i> total egg numbers, and eggs per gram of root at 49 DAP in greenhouse trials.....	49
Table 4. Vydate C-LV™ effects on cotton root fresh weight, plant biomass, <i>Rotylenchulus reniformis</i> total egg numbers, and eggs per gram of root at 49 DAP in greenhouse trials.....	50
Table 5. Combination effects of growth hormones, starter fertilizers, and nematicides on cotton root fresh weight, plant biomass, and <i>Rotylenchulus reniformis</i> means for eggs per gram of root at 35 DAP in microplot trial.....	51
Table 6. Combination effects of growth hormones, starter fertilizers, and nematicides on cotton root fresh weight, plant biomass, and <i>Rotylenchulus reniformis</i> means for eggs per gram of root at 61 and yields 135 DAP in Microplot Trial.....	52
Table 7. Combination effects of growth hormones, starter fertilizers, and nematicides on cotton root fresh weight, plant biomass, and <i>Rotylenchulus reniformis</i> means for eggs per gram of root at 42 DAP in <i>Rotylenchulus reniformis</i> infested and non-infested field trials.....	53
Table 8. Combination effects of growth hormones, starter fertilizers, and nematicides on cotton root fresh weight, plant biomass, and <i>Rotylenchulus reniformis</i> means for eggs per gram of root at 64 and lint yields 165 DAP in <i>Rotylenchulus reniformis</i> infested and non-infested field trials	54

Chapter I: Review of Literature

Introduction

Rotylenchulus reniformis causes significant economic losses on upland cotton; *Gossypium hirsutum* L. In the 2014 growing season, growers across the cotton belt lost 333,000 bales to *Rotylenchulus reniformis* Lindford and Olivia 1940, (Lawrence et al. 2015). Management strategies for *R. reniformis* have focused on the use of nematicides due to the absence of resistant varieties to *R. reniformis* (Koenning et al., 2004). The industry standard nematicide, Temik 15G™, (Aldicarb) was scheduled in 2011 to be removed from the market voluntarily by 2018 (Wheeler et al., 2014) due to toxicity issues on the environment. Therefore management of *R. reniformis* has relied on other nematicides such as Aeries™ (Thiodicarb), Avicta™ (Abamectin), Vydate C-LV™ (Oxamyl) and crop rotation due to the lack of resistant varieties of upland cotton (Koenning et al., 2004). The use of Vydate C-LV™ was found to reduce losses in yield associated with *R. reniformis* and *Meloidogyne incognita* when applied as foliar sprays (Lawrence and McLean 2000; 2002). New to the market for the 2016 growing season, Velum Total™ (Imidacloprid plus Fluopyram) from Bayer CropScience will be released to growers as another tool for management of *R. reniformis*.

The supplementation of growth hormones to cotton seedlings has resulted in increased early season plant growth. In-furrow applications or seed treatments of gibberellic acid and indolebutyric acid increased lateral root formation and total root length in growth chamber studies (Oosterhuis and Zhao, 1994). The application of cytokinin as a foliar spray increased root masses by twice the size when compared to a control in greenhouse experiments (Burke, 2011). Early root development potentially could reduce losses from *R. reniformis* due to larger

root formation before infection, assisting the plant in overcoming the stresses associated with *R. reniformis* and subsequent reductions in yields. Additionally, starter fertilizer trials have been observed to increase early vigor and growth when applied to the cotton seedlings. Starter fertilizers applied at planting to cotton, either as in-furrow sprays, side dresses, or top-dressed, promotes root growth and plant vigor (Kovar et al., 1994; Toler et al., 2004). Placement of the starter fertilizer, soil type, and nutrient availability can greatly influence the effectiveness of applications (Toler et al., 2004). The efficacy of growth hormones and starter fertilizers in a management program for *R. reniformis* has not been studied to our knowledge. The use of growth hormones and starter fertilizers to stimulate rapid growth to increase plant biomass while using a nematicide as a protectant from *R. reniformis* at planting could be utilized as a management strategy to reduce losses associated with this pathogen. During the first forty days of development, cotton is most susceptible to damage associated with yield losses (Stewart and Faircloth, 2007). Hence utilizing these management tactics could help overcome losses from *R. reniformis*.

Plant Hormones

Artificial plant hormones are used to induce growth during clonal propagation. Of the plant hormones, there are five major hormones that are currently used for increasing plant success: auxin, cytokinin, gibberellin, abscisic acid, and ethylene (Hartman et al., 2011). Each of these hormones is commercially available for use in agriculture, either individually or combined into one solution.

Cytokinin was discovered while researchers were looking for a means to stimulate cellular growth (Miller et al., 1955). Synthetic cytokinin has been used in cotton production to increase yield (Burke, 2011). Synthetic cytokinin is used as a foliar application on reproductive

cotton to increase fruit set and fiber quality (Hedin et al., 1988). In addition to using cytokinin to produce larger yields of cotton, cytokinin is known to increase vegetative and root development of plants (Hedin et al., 1988). The use of cytokinin in correct ratios can promote root growth and shoot development as well as fruiting growth (Taiz et al., 2010).

Just as cytokinin is utilized in promoting growth of plants, gibberellic acid is used in similar ways. Gibberellic acid has been used to break seed dormancy and increase fruit set in horticultural crops (Taiz et al., 2010). In seedless grape production, stalk length is a limiting factor for development of the fruit due to compaction. Once treated with gibberellic acid, the stem elongates allowing fruit set to increase in size (Taiz et al., 2010). Utilizing these known concepts about gibberellic acid, these practices can be applied to cotton plants to potentially produce a larger, healthier plant for testing of increased stress tolerance in the presence of plant parasitic nematodes.

Auxins are used commercially for root development in plant propagation of cuttings. Commercial plant propagation uses indole-3-butyric acid (IBA) for the production of adventitious root development from cuttings (Blazich, 1988). Indole acetic acid, the natural form found in plants has been used in plant propagation; however Indole acetic acid degrades more quickly than IBA because of auxin degrading enzymes (Davies, 1995). This makes the use of IBA more desirable for use in seed application methods. Growth chamber studies conducted by Oosterhuis and Zhao (1994) found that the application of gibberellic acid and indolebutyric acid in the form of PGR-IV produced adventitious early root growth. IBA can be used to stimulate growth of root systems in plants. This knowledge will be applied to an agronomic system for stimulation of cotton plant roots when the pressure of plant parasitic nematodes is present.

Starter Fertilizers

Initially, starter fertilizers have been researched for their abilities to increase yield of cotton plants. Guthrie (1991) stated that yield and lint quality in cotton were sporadic in the use of starter fertilizers in North Carolina. Plant height and stand uniformity has been erratic when starter fertilizers have been applied in upland cotton production (Touchton et al. 1986). Early season development due to the application of starter fertilizers and cotton yield has been found to not be related (Cahill, 2010). Addition of starter fertilizers to cotton plants stimulated early season growth, but effects on yield has been variable (Toler et al., 2004). Various studies have examined starter fertilizers as a way of increasing the yield potential of the cotton plants however significant yield increases have been found to be erratic and unpredictable (Cahill, 2010; Guthrie, 1991). Kovar et al. (1994) found that the addition of starter fertilizers did increase early development of cotton seedlings, but was not related to significant yield increases following their application. Starter fertilizers have been recognized as assisting the cotton plants in overcoming early stresses such as overly damp soils and colder temperatures than favorable for cotton growth (Bednarz et al., 2000). Inconsistencies in the response to starter fertilizers have been observed in multiple field locations across the cotton belt of the United States (Bednarz et al., 2000; Burmester et al., 1994; Kovar et al., 1994; Toler et al., 2004; Touchton et al., 1986). The use of starter fertilizers has not been investigated as a means of overcoming losses on upland cotton associated with *R. reniformis*. The ability of starter fertilizers to promote faster germination and development can potentially prove to be a beneficial strategy for reducing losses when *R. reniformis* is present in the soil.

Nematicides

Control of plant parasitic nematodes is most effectively achieved through the application of nematicides. Nematicides are non-selective, meaning that all nematodes including plant parasites and free-living nematodes present in the soil profile are subject to the toxic effects of nematicides. Chemical nematicide application for control of plant parasitic nematodes is utilized to reduce the primary nematode infection of the plant (Koenning et al., 2004). The intent is to allow the plant adequate time to develop healthy roots before infection of the plant by parasitic nematodes (National Cotton Council, 2014). Nematicides are believed to provide control of nematodes for short periods, usually up to 30 days after application (Somasundaram et al., 1989; Duncan, 1991; Lawrence and McLean, 2000). Currently nematicides are used in six different forms: fumigants, granules, in-furrow spray, seed treatments, foliar sprays, and biologicals for management of plant parasitic nematodes (Koenning et al., 2004).

Seed treatments are the simplest and most cost effective form of nematicide for use by producers. Seed treatment control may be limited when the seed coat is pushed from the soil by the developing plant (Starr et al., 2007). Fumigants provide the largest spectrum of control by moving through the soil until dissipation, making them very effective at nematode control (Starr et al., 2007). The use of granules such as Temik 15G (Aldicarb) is one of the only non-fumigant nematicides that has proven efficacy towards nematodes when planting (Koenning et al., 2004). Granules and sprays applied in-furrow provide a barrier of control until the chemical breaks down in the soil. These options allow the plants to begin development before nematodes infect roots. The foliar applications of nematicides provide protection to the plant once a generation of nematodes has infested the root system. Often the combination of at-planting nematicides

followed by the foliar applications protects the cotton plants from nematode infection over a longer duration of plant development.

Rotylenchus reniformis

In upland cotton, *Rotylenchus reniformis*, commonly known as the reniform nematode, was first found to be a plant pathogen in the southeastern region of the United States in the 1940's (Smith, 1940). Within years of its discovery in the Southeast, the reniform nematode was found to cause economic losses for growers by reducing initial stands, yields, lint quality, and seed quality (Jones, 1959). In Alabama alone, *R. reniformis* caused losses up to 50% in infested fields in 2013 (Lawrence et al., 2014). *Rotylenchulus reniformis* resulted in 193,900 and 333,000 bales lost in 2013 and 2014, respectively (Lawrence et al., 2014; 2015).

Rotylenchus reniformis goes through two molts while inside the egg. The second molt then penetrates the egg shell and the second stage juvenile emerges (Koenning et al. 2004). Upon emerging from the egg the nematode goes through three more molts before feeding starts by the mature female adult. The mature female is the only infective stage of the reniform nematode (Dasgupta and Raski, 1968). Infective females insert the anterior third of the body into the root where it creates a feeding site in the endodermis of the root. The adult female establishes a feeding site and begins to enlarge into the characteristic reniform shape (Lawrence and McLean, 2001). Male reniform nematodes' only purpose is reproduction. Once the female nematode has established the feeding site, the male nematode inseminates the female, and will then perish from starvation. Lifecycle from egg to mature adult for *R. reniformis* is 17-23 days under optimal conditions of 27.2-30°C (Lawrence and McLean, 2001). Each female reniform nematode can lay 75 eggs per egg mass, and can produce multiple egg masses in her life-time. Unhatched eggs

mediate in overwintering for the nematodes because of the ability to resist desiccation in the absence of a host or during long dry periods.

Management strategies

Control of plant parasitic nematodes is most effectively achieved with application of multiple means of control including cultural, biological, and chemical aspects (Koenning et al., 2004). Currently only tolerant cultivars are available for *R. reniformis* management. The use of resistant or tolerant cultivars to the nematode pathogens means that some yield loss will be prevented due to resistance or tolerance bred into the plant for the parasite (Haydock et al., 2006). In addition to using resistant or tolerant cultivars, growers should consider, if feasible, crop rotation to non-hosts the following season to reduce populations (Lawrence and McLean, 2001). Host crops for the *R. reniformis* are somewhat diverse, making crop rotations a feasible control method yet still difficult to manage when using specialized equipment for harvest or planting (Koenning et al., 2004). Biological controls of plant parasitic nematodes can prove to be difficult because of the diverse nature of feeding and sizes associated with nematodes. Some forms of biological control include certain fungal and bacterial species that will infect nematodes as they pass by them and will feed until the host nematode perishes (Viaene et al., 2006). Plant parasitic nematode infested fields should be kept weed and volunteer plant free to reduce alternate host sites for the nematodes. All equipment that passes through infested fields should be thoroughly cleaned as to not move inoculum from infested areas to non-infested areas on farms (Koenning et al., 2004). In the presence of extreme populations, fallowing with field disking is an option as a means of control (Viaene et al., 2006). Fallowed fields must be kept weed free and should be turned using disk implements or plows pulled behind a tractor to assist in desiccation of nematodes present in the soil (Lawrence and McLean, 2001). Resistant or tolerant cultivar

selections along with the use of other cultural practices should be observed when planting (McSorley, 1998; Potter and Dale, 1994; Reese et al., 1988; Seinhorst, 1970; Usery, 2004; Young, 1998). Along with proper techniques, nematicides can be applied to the soil or seeds to provide a chemical control of the nematodes. Nematicides are nonselective, meaning that all nematodes present in the soil profile are subject to extermination where applied (Haydock et al, 2006). In this study, the focus on cotton crop improvement utilizes applications of growth hormones and starter fertilizers to promote early cotton seedling development, while utilizing Velum Total™ or Vydate C-LV™ as a means of protection for the cotton seedling.

The overall hypothesis is that stimulating early cotton growth by applying growth hormones and starter fertilizers will decrease yield losses associated with *R. reniformis*. The supporting objectives for this hypothesis are to: 1) evaluate commercially available growth hormones for the enhancement of cotton plant growth; 2) evaluate starter fertilizers for the enhancement of cotton plant growth; 3) evaluate nematicides for efficacy on *R. reniformis* populations and enhance cotton plant growth; 4) combine the optimum growth hormones and starter fertilizers with the nematicide to determine the most efficient management strategy for *R. reniformis* control on cotton. The overall goal of this project is to increase the plant health of cotton plants as rapidly as possible before infection of the root occurs by *R. reniformis*.

Literature Cited

- Blazich, F. A. 1988. Chemicals and formulations used to promote adventitious rooting. *Advances in Plant Sciences Series (USA) 2*: 132-149.
- Bednarz, C.W., Harris, G.H., and Shurley, W.D. 2000. Agronomic and Economic Analyses of Cotton Starter Fertilizers. *Agronomy Journal 92*:766-771.
- Burke, J.J. 2011. 6-Benzyladenine Enhancement of Cotton. *The Journal of Cotton Science 15*:206-214.
- Burmester, C. H., Patterson, M. G., and Reeves, D. W. 1993. No-till cotton growth characteristics and yield in Alabama. *Proceedings of the 1993 Southern Conservation Tillage Conference for Sustainable Agriculture 93*:30-36.
- Cahill, S., Osmond, D., Gehl, R., Hardy, D., and Crozier, C. 2010. Soil facts. Starter phosphorus fertilizer and additives in NC soils: use, placement, and plant response. In. Vol. AG-439-75W'. North Carolina Cooperative Extension.
- Davies, P. J. 1995. The Plant Hormones: Their Nature, Occurrence, and Functions. Pp 1-12 In *Plant Hormones*.
- Duncan, L. W. 1991. Current Options for Nematode Management. *Annual Review of Phytopathology 29*:469-490.
- Guthrie, D. S. 1991. Cotton response to starter fertilizer placement and planting dates. *Agronomy Journal 83*:836-839.
- Haydock, P. P. J., Woods, S. R., Grove, I. G., and Hare, C. 2006. Chemical control of nematodes. Pp 392-409 in *Plant Nematology*. Eds. Perry and Moens.

Hedin, P. A., Jenkins, J. N., Thompson, A. C., McCarty, Jr., J. C., Smith, D. H., Parrott, W. L., Shepherd, R. L. 1988. Effects of Bioregulators on Flavonoids, Insect Resistance, and Yield of Seed Cotton. *Journal of Agriculture of Food Chemistry* 36:1055-1061.

Jones, J. E., Newsom, L. D., and Finley, E. L. 1959. Effect of the reniform nematode on yield, plant characters, and fiber properties of upland cotton. *Agronomy Journal* 51:353–356.

Koenning, S. R., Kirkpatrick, T. L., Starr, J. L., Wrather, J. A., Walker, N. R., and Mueller, J. D. 2004. Plant-Parasitic Nematodes Attacking Cotton in the United States. *The American Phytopathological Society* 88:100-113.

Kovar, J. L., Funderburg, E. R., and Hutchinson, R. L. 1993. Starter fertilizer can improve growth and yield of cotton. *Better Crops* 78:9-11. International Plant Nutrition Institute. Peachtree Corners, GA.

Lawrence, G. W., and McLean, K. S. 2000. Effect of Foliar Applications of Oxamyl with Aldicarb for the Management of *Rotylenchulus reniformis* on Cotton. *Journal of Nematology* 32:542-549.

Lawrence, G., and McLean, K. 2001. Reniform nematodes. Pp. 42-44 in *Compendium of Cotton Diseases*, 2nd edition. eds. Kirkpatrick, T. L. and Rothrock, C.S. American Phytopathological Society Publications, St. Paul, Minnesota.

Lawrence, G. W., and McLean, K. S. 2002. Foliar Applications of Oxamyl with Aldicarb for the Management of *Meloidogyne incognita* on Cotton. *Nematropica* 32:103-112.

Lawrence, K., Olsen, M., Faske, T., Hutmacher, R., Muller, J., Mario, J., Kemerait, R., Overstreet, C., Sciumbato, G., Lawrence, G., Atwell, S., Thomas, S., Koenning, S., Boman, R.,

Young, H., Woodward, J., and Mehl, H. 2015. Cotton disease loss estimate committee report, 2014. Proceedings of the 2014 Beltwide Cotton Conference Vol. 1:188-190. National Cotton Council of America, Memphis, TN.

Linford, M. B., and Oliveira, J. M. 1940. *Rotylenchulus reniformis*, nov. gen. n. sp., a nematode parasite of roots. Proceedings of the Helminthological Society of Washington 7:35-42.

McSorley, R. 1998. Alternative practices for managing plant parasitic nematodes. American Journal of Alternative Agriculture 13:98–104.

Miller, C., Skoog, F., von Saltza, M. H., Strong, F. M. 1955. Kinetin, a Cell Division Factor from Deoxyribonucleic Acid. Journal of American Chemist Society 77:1392.

National Cotton Council of America. 2014. Cotton production costs and returns per planted acre, excluding Government payments, Southern Seaboard, 2011-2013. <http://www.cotton.org/econ/cropinfo/costsreturns/seaboard.cfm>. Accessed November 2, 2014.

Oosterhuis, D. M. and Zhao, D. 1994. Increased root length and branching in cotton by soil application of the plant growth regulator PGR-IV. Plant and Soil 167:51-56.

Potter, J. W., and Dale, A. 1994. Wild and cultivated strawberries can tolerate or resist root-lesion nematode. Horticulture Science 29:1074– 1077.

Reese, P. F., Boerma, H. R., and Hussey R. S. 1988. Heritability of tolerance to soybean cyst nematode in soybean. Crop Science 28:594– 598.

Seinhorst, J. W. 1970. Dynamics of populations of plant-parasitic nematodes. Annual Review of Phytopathology 8: 131–156.

Somasundaram, L., Coats, J. R., and Racke, K. D. 1989. Degradation of pesticides in soil as influenced by the presence of hydrolysis metabolites. *Journal of Environmental Science and Health* 24:457–478.

Starr, J. L., Koenning, S. R., Kirkpatrick, T. L., Robinson, A. F., Roberts, P. A., and Nichols, R. L. 2007. The Future of Nematode Management in Cotton. *Journal of Nematology* 39: 283-294.

Stewart, S., and Faircloth, J. 2007. The First Forty Days through Fruiting to Finish. *Proceedings from the 2007 Beltwide Cotton Conference* 1:579-586. National Cotton Council of America, Memphis, TN.

Taiz, L. and Zeiger, E. 2010. *Plant Physiology*. Pp. 424-515. Redwood City, CA: The Benjamin/Cummings Publishing Company.

Toler, J. E., Murdock, E. C., and Camberato, J. J. 2004. Starter fertilizer effect on cotton development and weed interference. *The Journal of Cotton Science* 8:33-41.

Touchton, J. T., Rickerl, D. H., Burmester, C. H., and Reeves, D. W. 1986. Starter Fertilizer Combinations and Placement for Conventional and No-tillage Cotton. *Journal of Fertilizer Issues* 3: 91-98.

Usery, S.R., Lawrence, K.S., Lawrence, G.W., and Burmester, C.H. 2005. Evaluation of Cotton cultivars for Resistance and Tolerance to *Rotylenchulus reniformis*. *Nematropica* 35:121-133.

Viaene, N., Coyne, D. L., Kerry, B. R. 2006. Biological and cultural management. Pp 347-370 in *Plant Nematology*. eds. Perry and Moens.

Wright, D. J., Perry, R. M. 2006. Reproduction, physiology and biochemistry. Pp. 187-209 in Plant Nematology. eds. Perry and Moens.

Young, L. D. 1998. Breeding for nematode resistance. Pp. 187–207 in K. R. Barker, G. A. Pederson, and G. L. Windham, eds. Plant nematode interactions. Madison, WI: American Society of Agronomy.

Chapter II: Greenhouse, Microplot, and Field Trials with Growth Hormone, Starter Fertilizer, and Nematicide Treatments

ABSTRACT

Greenhouse trials were conducted to formulate the best combination and application methods of growth hormones (Ascend™), starter fertilizers, and nematicides to create an integrated pest management program that was then tested in microplot and field environments to manage *Rotylenchulus reniformis* in upland cotton, *Gossypium hirsutum* production.

Greenhouse, microplot, and field trials were conducted in both *R. reniformis* infested soils and non-infested soils and all tests were arranged in RCBD with five replications and repeated. In the initial greenhouse screenings, biomass of the entire plant was greater with the addition of Ascend™ as a seed treatment and as a seed treatment + foliar spray compared to the untreated control. Ascend™ seed treatment + foliar spray also supported a lower number ($P < 0.05$) of nematode eggs per gram of root when compared to the control. Plant height and biomass were not increased by any starter fertilizer application in greenhouse trials. Nematode populations were significantly greater with the addition of Sure-K™ + Micro 500™ starter fertilizers. The Velum Total™ seed treatment and in-furrow spray treatments reduced ($P < 0.05$) *R. reniformis* eggs per gram of root. In the microplot trials, Vydate C-LV™ alone, Vydate C-LV™ + starter fertilizers, and Vydate C-LV™ + Ascend™ + starter fertilizers supported increased plant biomass in the presence of *R. reniformis*. The Vydate C-LV™ + starter fertilizers also lowered *R. reniformis* eggs per gram of root. Field trials with *R. reniformis* present in the soil showed that at 40 DAP, Velum Total™ alone, Vydate C-LV™ alone, Velum Total™ + growth hormones, Vydate C-LV™ + growth hormones, Velum Total™ + starter fertilizers, Vydate C-LV™ + starter fertilizers, Velum Total™ + growth hormones + starter fertilizer, and Velum Total™ +

growth hormones + starter fertilizers + Vydate C-LV™ supported a larger plant biomass compared to the control. All treatments that included a nematicide significantly reduced *R. reniformis* eggs per gram of root when compared to the untreated control. Increase in cotton yields in the *R. reniformis* infested fields with the addition of Velum Total™ + Ascend™ + starter fertilizers were observed when compared to the control.

INTRODUCTION

In upland cotton (*Gossypium hirsutum* L.) production, the first forty days of development are the most influential on yield as compared to any other time period (Stewart and Faircloth, 2007). After planting, the cotton seed absorbs water through the chalaza at which point the seed begins germination and hypocotyl elongation (Todaro 1863, 1877; Baranov and Maltzev, 1937). Spieth (1933) documented that in seedling stages of root development sieve tubes and xylem vessels are forming on the outer edges of the stele. At the earliest stages of development the cotton seedlings are at a higher risk of long term effects from *Rotylenchulus reniformis*, Linford and Olivia, (1940) damage due to the establishment of the feeding site in the endodermis region of the root at the margin of the stele. The adult vermiform female is the infective stage of *R. reniformis* (Dasgupta and Raski, 1968). *Rotylenchulus reniformis* infects the cotton root by imbedding herself in the root creating a syncytium in the endodermis (Lawrence and McLean, 2001). The *R. reniformis* nematode is an important pathogen of cotton due to the damaging effects associated with yields losses in infested field sites. Yield reduction associated with *R. reniformis* in the United States in the 2014 growing season totaled 333,000 bales of cotton (Lawrence et al. 2015b).

In plant development, plant hormones regulate plant growth and development (Hartmann et al., 2011). The five major hormones associated in plant development are auxin, cytokinin, gibberellin, abscisic acid, and ethylene (Hartman et al., 2011). In agriculture, the use of plant hormones, both in synthetic or natural forms, is common to influence plant growth; both synthetic and natural forms are recognized as plant growth regulators (Hartman et al., 2011). Cytokinin, gibberellic acid, and indolebutyric acid were selected for the current study for each individual role played in growth promotion of plants. Cytokinin produced in plants is known to

be responsible for cellular growth stimulation (Miller et al., 1955). In plant development, gibberellins are the most influential in plant height determination (Hartman et al., 2011). Gibberellic acid provided to plants as liquid formulations or powder formulations increase stem length formation (Taiz et al., 2010; Hartman et al., 2011). In sugarcane production, gibberellic acid is applied to promote elongation of internode expansion during periods of lower than average daily temperatures (Taiz et al., 2010). Indolebutyric acid is commonly used in vegetative propagation of cuttings as liquid or powder formulations to stimulate adventitious root development (Blazich, 1988; Hartman et al., 2011). Plant growth hormones provided to cotton seedlings have shown mixed results for stimulation of root growth when added as in-furrow sprays or seed treatments (Howard et al., 2001). Oosterhuis and Zhao (1994) stated that in growth chamber tests, early root development was increased with the addition of gibberellic acid and indolebutyric acid. Application of cytokinin to cotton seedlings in the cotyledon stage produced an increase in hypocotyl diameter, breakage of apical dominance, and increases in root mass development (Burke, 2011). Field trials conducted by Burke (2013) focused on yield increases with the application of cytokinin applied to the cotton plant at the cotyledon growth stage. Inconsistent yield increases were observed with the application of cytokinin compared to untreated controls (Guinn, 1986; Cothren and Oosterhuis, 2010). In multiple tests, overall cotton growth and development was increased marginally with the addition of growth hormones in the field but yield increases were not observed (Egilla and Oosterhuis, 1996; Steger and Oosterhuis, 1997; Bassett, 1999; Becker et al., 1999; Oosterhuis and Zhao 1994). Utilizing these growth promotion properties, it is hypothesized that the addition of growth hormones can increase early growth in roots and shoots of cotton seedlings, thereby reducing the damage caused by the presence of *R. reniformis*.

In cotton production, starter fertilizers have been looked at for the potential to increase cotton biomass development and subsequent yields (Toler et al., 2004). Emphasis on early development of cotton seedlings was focused on crop improvement and yield increase via the addition of starter fertilizers (Bednarz et al., 2000; Burmester et al., 1993; Howard et al., 2001; Kovar et al., 1993; Toler et al., 2004). Growth promotion of seedlings has been observed when starter fertilizers have been present, along with increased vigor under environmental stressors such as cool temperatures and overly saturated soils in the earliest developmental stages (Kovar et al., 1993; Toler et al., 2004). Early seedling development of plant biomass of cotton plants was not related to increases in yield when applying starter fertilizers to cotton (Cahill, 2010). Utilizing starter fertilizers to stimulate early plant development for management of losses associated with *R. reniformis* has not been studied in regard to utilizing starter fertilizers for production of early plant development. Utilizing this knowledge of growth promotion with starter fertilizers, cotton plant development could be increased before the infection of *R. reniformis*, allowing the cotton seedling a healthier start to potentially reducing losses associated with the pathogen.

Rotylenchulus reniformis management generally includes the application of nematicides (Koenning et al., 2004). The use of nematicides generally allows for less than 30 days of protection to the root system before the chemical becomes inert or leaches out of the soil (Somasundaram et al., 1989; Duncan, 1991; Lawrence and McLean, 2000). A new pesticide, now to be marketed as a nematicide in the 2016 growing season, is Fluopyram combined with Imidacloprid (Velum Total™) from Bayer CropScience, (Research Triangle Park, NC). Research by the National Cotton Council Nematode Research and Education Committee has indicated Fluopyram plus Imidacloprid has significantly reduced *R. reniformis* populations and enhanced

cotton yields in initial data (Lawrence et al., 2015; Lawrence et al., 2014). Studies conducted by this committee found Velum Total™ + Aeris™ (Fluopyram + Imidacloprid + Thiodicarb) (Bayer CropScience, Research Triangle Park, NC) nematicide treatment reduced nematode populations by 54% and increased yields by 9% over the Gaucho 600™ seed treatment control. The Velum Total™ + Aeris™ treatment was similar to Temik 15G™ (Aldicarb) the old industry standard (Bayer CropScience, Research Triangle Park, NC). The purpose of a nematicide application is to kill the initial population of *R. reniformis* nematodes allowing the cotton seedling a protected region of soil to begin growth and development before plant-parasitic nematodes can infect sensitive young seedling roots.

The overall hypothesis of these studies is that the addition of growth hormones, starter fertilizers, and nematicides can stimulate and protect plant health in the earliest stages of development, which can reduce losses to cotton production associated with *R. reniformis* pressure. The supporting objectives for this hypothesis are: 1) to evaluate commercially available growth hormones for the enhancement of cotton plant growth; 2) to evaluate starter fertilizers for the enhancement of cotton plant growth; 3) to evaluate two nematicides for efficacy on *R. reniformis* populations and enhancement of cotton plant growth; and 4) to combine the optimum growth hormones and starter fertilizers with the nematicide to determine the most efficient management strategy for *R. reniformis* control on cotton. Tests were conducted with the growth hormones and starter fertilizers to assess the optimum combinations and applications, and the effectiveness of combination applications of the multiple products. These tests will serve as a proof of concept that the addition of growth hormones and starter fertilizers with a nematicide will give returns for losses from *R. reniformis* on upland cotton. The overall goal of this project

is to create a management program that utilizes multiple methods of producing a larger cotton plant during the efficacy period of nematode management from nematicides.

MATERIALS AND METHODS

Trials were conducted to test growth hormones, starter fertilizers, and nematicides for the production of plant biomass on *G. hirsutum* in the presence of *R. reniformis*. Growth hormones and starter fertilizers commercially available to growers were tested with various applications and combinations to determine their ability to increase cotton biomass. Nematicides evaluated were tested for their efficacy in reducing *R. reniformis* population densities on cotton. The first set of trials were set up in the greenhouse and conducted at the Plant Science Research Center of Auburn University. Cotton cultivar selected in all experiments was FiberMax 1944 GLB2 (Bayer CropScience, Research Triangle Park, NC). All seeds were pretreated by Bayer CropScience with the standard fungicide and insecticide package which consists of the fungicides Thiram at 0.002 mg a.i./seed, Metalaxyl at 0.0003 mg a.i./seed, and Ipconazole at 0.0001 mg a.i./seed for management of seedling disease plus the insecticide Imidacloprid 0.34 mg a.i./seed to control for thrips damage in early plant development (Bayer CropScience, Research Triangle Park, NC).

Plots in the greenhouse trials consisted of 983cc container (Stuewe and Sons, Tangent, OR) filled with pasteurized surface soils from a Kalmia loamy sand (fine-loamy over sandy or sandy skeletal, siliceous, semiactive, thermic Typic Hapludults) comprised of 80% sand, 10% silt, and 10% clay mixed as 2 parts field soil with 1 part sand. All soil was mixed with 45mg/kg N as equivalent to the standard recommendation of 101 kilograms of N per hectare and the equivalent of 3.7 metric tons per hectare ground limestone. Individual tests were wrapped in a reflective foam board to equalize temperature gradients among plots. Each plot received 3 cotton

seeds upon planting. Plants were thinned after one week and were allowed to grow for forty-nine days. Plots were all hydrated prior to planting, irrigated immediately following planting, and immediately inoculated with 10,000 *R. reniformis* eggs plus vermiform life stages.

Rotylenchulus reniformis inoculum preparation

Rotylenchulus reniformis used as inoculum for all trials were extracted from 500 cm³ polystyrene stock cultures maintained in greenhouse conditions that were allowed to grow for 60 days on cotton to increase inoculum levels. Reniform nematode eggs were extracted from the roots of the stock cotton plants by placing the root systems in a 0.625% NaOCl solution and shaking for 4 minutes at 140 rpm, followed by a water rinse and physical scrubbing of root systems (Hussey and Barker, 1973). Eggs were collected on a 25 µm pore sieve washed into a 50 mL centrifuge tube, and centrifuged at 1400 rpm for 1 minute in sucrose (sp. gravity 1.14) similar to the method described by Jenkins, (1964). Eggs were recollected on a 25 µm pore sieve, enumerated using the Nikon TSX 100 inverted microscope at 40x magnification and standardized to 10,000 eggs and vermiform life stages per 983 cm³ of soil. Nematodes were added to all containers by pipetting 2 mL of the nematode suspension into each container and covering the soil to eliminate desiccation of the nematodes. In the field microplots, nematode samples were taken to quantify the initial *R. reniformis* populations, and additional nematodes were added to microplots at planting to standardize populations. Field site locations were sampled for initial *R. reniformis* populations at planting, and additional nematodes were added to plots to standardize populations.

Greenhouse trials growth hormone application evaluations

Growth hormone trials tested the effect of a commercial blend of plant growth hormones, Ascend PGR™ (Agri-AFC, Decatur, AL) on *R. reniformis* population development and cotton biomass production with single and multiple application methods in controlled greenhouse environmental conditions. Ascend PGR™ is comprised of cytokinin 0.090%, gibberellic acid 0.03%, and indole butyric acid 0.045%. Plant hormones treatments consisted of (1) an untreated control, (2) Ascend™ seed treatment (ST 88.7 mL/CWT), (3) Ascend™ in-furrow spray (IFS 233.7 mL/ha), (4) Ascend™ foliar spray (FS 233.7 mL/ha) applied at 2 true leaf stage, (5) Ascend™ ST + IFS, (6) Ascend™ ST + FS, (7) Ascend™ IFS + FS applied at 2 true leaf stage, and (8) Ascend™ ST + IFS + FS.

Greenhouse trials starter fertilizer combinations evaluations

Starter fertilizer trials tested for the effect of the single and multiple starter fertilizers on cotton plant biomass when infected with *R. reniformis*. Starter fertilizers were selected based on availability to growers in our region, and were all applied as an in-furrow spray (IFS) at planting or top dressed (TD). Fertilizers selected in these trials were Sure-K™ (2-1-6) (Agroliquid, St. Johns, MI), Pro-germinator™ (9-24-3) (Agroliquid, St. Johns, MI), Micro 500™ (B 0.02%, Cu 0.25%, Fe 0.37%, Mn 1.2%, and Zn 1.8%) (Agroliquid, St. Johns, MI), and ammonium polyphosphate (10-34-0) (Faithway Feed Co. LLC, Guntersville, AL). Starter fertilizer treatments were (1) an untreated control, (2) Sure-K™ IFS (9.28 liter/ha), (3) Pro-germinator™ IFS (4.64 liter/ha), (4) Micro 500™ IFS (2.32 liter/ha), (5) ammonium polyphosphate top dressed (TD 1.16 liter/ha), (6) Sure-K™ IFS + Micro 500™ IFS, (7) Pro-germinator™ IFS + Micro 500™ IFS, and (8) ammonium polyphosphate TD + Micro 500™ IFS.

Greenhouse trials nematicide applications evaluations

Trials testing the efficacy of nematicides to manage *R. reniformis* populations were installed using Velum Total™ (Fluopyram 15.4% + Imidacloprid 22.2%). Velum Total™ was selected based on it being a newly available nematicide to growers in the 2016 growing season. Treatments in nematicide trials were (1) untreated control, (2) Gaucho 600™ (0.375 mg a.i./seed), (3) Velum Total™ seed treatment (ST 1.0 mg a.i./seed), and (4) Velum Total™ in-furrow spray (IFS 1 L/hectare).

Management of *R. reniformis* was tested using a second set of nematicide treatments containing Vydate C-LV™ (Oxamyl 42%) (DuPont, Wilmington, DE). The second set of nematicide treatments were (1) untreated control, (2) Vydate C-LV™ applied as an IFS (1.2 L/hectare), (3) Vydate C-LV™ IFS (0.6 L/hectare), (4) Vydate C-LV™ FS at 4 true leaves (1.2 L/hectare), and (5) Vydate C-LV™ IFS (0.6 L/hectare) + Vydate C-LV™ FS at 4 true leaves (0.6 L/hectare).

Experimental design

Each individual growth hormone, starter fertilizer, and nematicide trial was arranged in randomized complete block design with five replications and repeated twice for a total of 120 experimental units across all tests. Greenhouse temperatures were 24 to 35°C, averaging 29°C throughout the tests. Soil moisture was kept between 40 and 60% of the field capacity. Targeted destructive harvesting was at 49 DAP. In all greenhouse trials, parameters measured at the termination of the trials included: plant height, shoot and root fresh weights, *R. reniformis* eggs per plot, and eggs per gram of root. Plant heights and fresh weights were immediately recorded.

Rotylenchulus reniformis population densities were extracted and evaluated as previously described.

Data collected in all trials were analyzed in SAS 9.4 (SAS Institute, Inc. Cary NC) using the PROC GLIMMIX procedure. Dependent variables included root fresh weight, plant biomass, eggs per gram of root, and yield. Fixed effects were growth hormone, starter fertilizer, or nematicides treatments and the random effects included replications and time. The assumptions of normality and homogeneity were evaluated using the studentized residual plots obtained from the SAS option PLOTS=STUDENTSPANEL. The normal distribution assumption was evaluated with the student panel graphs. All parameters were analyzed using a normal distribution. The critical *P*-value of 0.10 was used for testing treatment effect, and determination of differences in least-squares means was based on adjusted *P*-value obtained by using the SAS option ADJUST=DUNNETT in the LSMEANS statement. Dunnett's *P* values are presented in tables to determine statistical differences. Pearson correlation coefficients were used to measure the strength and linear relationship between variables measured in greenhouse trials.

Microplot evaluations

The optimum application and combination treatments were selected from controlled atmosphere greenhouse trials before being tested in microplots and before testing in variable field sites. Treatments in the microplot trial were (1) untreated control, (2) Velum Total™ IFS (1 liter/ha), (3) Vydate C-LV™ IFS (1.2 liter/ha), (4) Velum Total™ IFS + Ascend™ ST + FS (88.7 mL/CWT + 233.7 mL/ha), (5) Vydate C-LV™ IFS + Ascend™ ST +FS (6) Velum Total™ IFS + Sure-K™ IFS (9.28 liter/ha) + Micro 500™ IFS (2.32 liter/ha), (7) Vydate C-LV™ IFS + Sure-K™ IFS + Micro 500™ IFS, (8) Velum Total™ IFS + Ascend™ ST + FS + Sure-K™ IFS

+ Micro 500™ IFS, (9) Vydate C-LV™ IFS + Ascend™ ST + FS + Sure-K™ IFS + Micro 500™ IFS, and (10) Velum Total™ IFS + Ascend™ ST + FS + Sure-K™ IFS + Micro 500™ IFS + Vydate C-LV™ FS (1.2 liter/ha). Microplot plots represent 0.3m of row in the field and consisted of two 25 L plastic tree pots nested one on top of the other with a brick in between to limit root growth by air pruning. Plots were filled with the surface soil from a Kalmia loamy sand with a soil:water (1:1) pH 5.4. All soil was mixed with 45mg/kg N as equivalent to the standard recommendation of 101 kilograms of N per hectare and the equivalent of 3.7 metric tons per hectare ground limestone mixed into each plot prior to planting. Plots were hand planted with FiberMax 1944 GLB2 at a rate of five seeds per 0.3m row in each microplot. In-furrow sprays were pipetted in seed furrow to represent field application at a rate of 37 liter/ha. Foliar sprays were applied using a Solo™ pump up backpack sprayer (Newport News, VA) calibrated to deliver a total volume of 75 L per ha at cotton pin head square developmental stage. The microplot trial was set up as a randomized complete block design with five replications for a total of 50 experimental units and was repeated.

Field evaluations

Rotylenchulus reniformis field trials were implemented to test efficacy of treatments selected from greenhouse trials at the E.V. Smith Research Center (EVS), Shorter, AL and the Tennessee Valley Research and Extension Center (TVREC), Belle Mina, AL. Fields were artificially infested with *R. reniformis* annually since 2007. Soil at EVS is Orangeburg sandy loam (Fine-loamy, kaolintic, thermic Typic Kandiudults) (% sand, silt, clay at 80-11-9, pH 6.0). Soil at TVREC is a Decatur silt loam (fine, kaolinitic, thermic Rhodic Paleudults) (% sand, silt, clay at 24-49-28, pH 6.0). Trials were replicated at these locations without *R. reniformis* present in the soil to test treatment efficacy on cotton development and yields. The soil type without *R.*

reniformis present is identical to that of the trials with the *R. reniformis* infested soil.

Rotylenchulus reniformis infested field test conducted at EVS were located at 32°25'21"N, 85°53'21"W whereas the non-infested *R. reniformis* location was at 32°25'17"N, 85°53'8"W on the adjacent tier with a 30 m distance between the test plots. The *R. reniformis* trial conducted at TVREC was located at 34°41'7"N, 86°52'57"W and the non-infested *R. reniformis* trial was conducted at 34°41'5"N, 86°53'16"W on the adjacent tier with a 30m distance between the test sites. Treatments tested in field trials were identical to the microplot treatments. The field trials in each location were structured as a randomized complete block design with five replications for a total of 200 experimental units. Each plot consisted of four rows at a length of 7.62m each with 0.9m of spacing between rows and a 1.8m alley between replications. One hundred seeds per row were planted using a John Deere MaxEmerge™ planter (Moline, Illinois) with Almaco cone planters™ (Nevada, Iowa). Trials were planted at TVREC and EVS on May 6 and 12, 2015, respectively.

Experimental design and parameters measured in the microplot and field trials

Cotton plant growth parameters evaluated were (1) populations at 21 DAP, (2) plant height at 42 DAP, (3) shoot and root fresh weights at 42 DAP, (4) canopy width (after foliar spray) at 63 DAP, and (5) seed cotton yield at 170 DAP. *R. reniformis* parameters measured were nematode egg density at 42 and 63 DAP and nematode eggs per gram of root at 42 and 63 DAP. Harvest of yields at EVS and TVREC occurred at 159 DAP and 170 DAP respectively.

RESULTS

Greenhouse trials growth hormone applications evaluations

Screening of application methods of the growth hormone blend displayed no statistical differences in treatments compared to the control in root fresh weights (Table 1). Pearson correlations between root and shoot fresh weight indicated a significant positive correlation ($R^2 = -0.42187$; $P \leq 0.05$) in overall cotton plant formation. When the Dunnett's option was conducted in the LSMEANS statement, cotton plant biomass was significantly increased by the Ascend™ ST alone and Ascend™ ST + FS by 20 and 23% respectively, when compared to the untreated control (Table 1). A negative relationship ($R^2 = -0.30435$; $P \leq 0.05$) was observed between biomass and *R. reniformis* eggs per gram of root. The addition of Ascend™ as a ST or IFS did not increase *R. reniformis* total egg production or eggs per gram of root. Ascend™ ST + FS decreased *R. reniformis* eggs per gram of root by 41% when compared to the control ($P \leq 0.10$) (Table 1). The addition of Ascend™ FS however, increased *R. reniformis* total eggs by 51% when compared to the untreated control (Table 1).

Greenhouse trials starter fertilizer evaluations

All starter fertilizers applied individually or in combinations produced similar root fresh weights and plant biomasses compared to the control at the 49 DAP harvest date (Table 2). When the Dunnett's option was conducted in the LSMEANS statement, the application of Sure-K™ IFS + Micro 500™ IFS significantly increased the total number of *R. reniformis* eggs and *R. reniformis* eggs per gram of root compared to the control by 134% and 77%, respectively (Table 2). Pearson correlations between biomass formation and *R. reniformis* eggs per gram of root showed that a moderate negative correlation was observed ($R^2 = -0.39189$; $P \leq 0.05$). Stimulated

growth from the starter fertilizer treatments showed that as the plant biomass increased, *R. reniformis* eggs per gram of root decreased.

Greenhouse trials Velum Total™ evaluations

Pearson correlations disclosed *R. reniformis* eggs per gram of root ratios were negatively correlated with plant biomass ($R^2 = -0.41812$; $P \leq 0.05$). The correlation between *R. reniformis* eggs per gram of root and plant biomass indicates that as the plant biomass increases the number of *R. reniformis* eggs per gram of root decreases. The Dunnett's option when conducted in the LSMEANS statement indicated the addition of Velum Total™ reduced the total number of *R. reniformis* eggs produced on the cotton plant when added as either a seed treatment or as an in-furrow spray by 93 to 98%, respectively, which is a significant reduction when compared to the control (Table 3). The same trend is seen in the *R. reniformis* eggs per gram of root with the addition of Velum Total™ as either a seed treatment or as an in-furrow spray in reducing populations by 95% and 99% respectively (Table 3). Plant biomass weight was significantly increased in the Gaucho 600™ ST by 23% compared to the untreated control (Table 3). Differences were not detected among any treatments compared to the untreated control in root fresh weight (Table 3).

Greenhouse trials Vydate C-LV™ evaluations

Pearson correlations between root fresh weight and *R. reniformis* eggs per gram of root had a moderate negative correlation ($R^2 = -0.45472$; $P \leq 0.05$). As the root fresh weight increased among treatments *R. reniformis* eggs per gram of roots was negatively influenced. Plant biomass formation and *R. reniformis* eggs per gram of root followed the same trend with a moderate negative correlation of ($R^2 = -0.44467$; $P \leq 0.05$). When Vydate C-LV™ was applied and plant

biomass increased, the *R. reniformis* eggs per gram of root decreased. There were no differences in either *R. reniformis* total eggs or eggs per gram of root among Vydate C-LV™ treatments compared to the untreated control using the Dunnett's option of the LSMEANS statement (Table 4.) Vydate C-LV™ treatments did not affect root fresh weight or plant biomass production (Table 4).

Microplot trials evaluations

The Dunnett's option of the LSMEANS statement indicated that the nematicide Vydate C-LV™ IFS combined with starter fertilizers, Sure-K™ IFS + Micro 500™ IFS, supported a significant increase in root fresh weight when compared to the control (Table 5). Root fresh weights were not increased by any of the other nematicide, starter fertilizer, and growth hormone treatments (Table 5). Plant biomass was increased by Vydate C-LV™ IFS (71% increase), Vydate C-LV™ IFS + Sure-K™ IFS + Micro 500™ IFS (75% increase), and Vydate C-LV™ IFS + Ascend™ ST + FS + Sure-K™ IFS + Micro 500™ IFS (73% increase) (Table 5). Vydate C-LV™ IFS + Sure-K™ IFS + Micro 500™ IFS not only increased plant biomass but also supported lower of *R. reniformis* eggs per gram of root as compared to the untreated control (Table 5).

At 42 days after planting, Ascend™ foliar treatments and the Vydate C-LV™ foliar spray treatment were applied to the microplot trial. In regards to root fresh weight when evaluated with the Dunnett's option of LSMEANS statement, treatments that were significantly higher than the control were Velum Total™ IFS (122% increase), Vydate C-LV™ IFS + Ascend™ ST + FS (176% increase), Vydate C-LV™ IFS + Sure-K™ IFS + Micro 500™ IFS (133% increase), Velum Total™ IFS + Ascend™ ST + FS + Sure-K™ IFS + Micro 500™ IFS (143% increase),

and Velum Total™ IFS + Ascend™ ST + FS + Sure-K™ IFS + Micro 500™ IFS + Vydate C-LV™ FS (155% increase) (Table 6). Velum Total™ IFS, Vydate C-LV™ IFS + Ascend™ ST + FS, Velum Total™ IFS + Ascend™ ST + FS + Sure-K™ IFS + Micro 500™ IFS, and Velum Total™ IFS + Ascend™ ST + FS + Sure-K™ IFS + Micro 500™ IFS + Vydate C-LV™ FS were significantly larger than the untreated control in biomass formation by 98%, 127%, 97% and 195% respectively (Table 6). Vydate C-LV™ IFS supported a significantly greater population density of *R. reniformis* eggs per gram of root as compared to the untreated control by 61 DAP (Table 6). No yield differences in grams per plot were detected among any treatments when compared to the untreated control.

Field trials evaluations

When evaluated using Dunnett's option of the LSMEANS statement, significant increases in root fresh weights were observed as compared to the control when *R. reniformis* was present in the soil. Both nematicides alone, Velum Total™ IFS (47% increase), Vydate C-LV™ IFS (74% increase), and when combined with the starter fertilizers, Velum Total™ IFS + Sure-K™ IFS + Micro 500™ IFS (49% increase), Vydate C-LV™ IFS + Sure-K™ IFS + Micro 500™ IFS (59% increase), increased root fresh weights. Velum Total™ combined with the starter fertilizer and growth hormones, and also with the additional Vydate C-LV™ spray increased root fresh weights by 79% and 58% respectively (Table 7). In the presence of *R. reniformis*, the nematicides alone, or combined with the starter fertilizer or growth hormone produced plants with a larger biomass than the untreated control: Velum Total™ IFS (36% increase), Vydate C-LV™ IFS (52% increase), Velum Total™ IFS + Ascend™ ST + Ascend™ FS (39% increase), Vydate C-LV™ IFS + Ascend™ ST + Ascend™ FS (42% increase), Velum Total™ IFS + Sure-K™ IFS + Micro 500™ IFS (48% increase), Vydate C-LV™ IFS + Sure-

KTM IFS + Micro 500TM IFS (47% increase). The total combination of Velum TotalTM IFS + AscendTM ST + FS + Sure-KTM IFS + Micro 500TM IFS increased biomass by 73% however the further addition of the second nematicide, Vydate C-LVTM, produced only a 46% increase in biomass (Table 7). The population densities of *R. reniformis* eggs per gram of root were lower in all the nematicide alone and combination treatments as compared to the untreated control. Reductions of nematode numbers ranged from 56% to 88%. No differences were detected among root fresh weight and plant biomass in treatments when *R. reniformis* was not present in the soil (Table 7).

AscendTM foliar treatments and the Vydate C-LVTM foliar spray treatment were applied to cotton at the pin head square or 6 to 8 leaf growth stages in the field trials. Vydate C-LVTM IFS alone and Velum TotalTM IFS + Sure-KTM IFS + Micro 500TM IFS increased root fresh weight as compared to the untreated control by 27% and 30%, respectively, when *R. reniformis* was not present in the fields (Table 8). In the fields with *R. reniformis*, the three and four way combination treatments of Velum TotalTM IFS + AscendTM ST + FS + Sure-KTM IFS + Micro 500TM IFS and Velum TotalTM IFS + AscendTM ST + FS + Sure-KTM IFS + Micro 500TM IFS + Vydate C-LVTM FS resulted in greater root fresh weights when compared to the control by 27% and 26% respectively (Table 8). All treatments were statistically similar to the untreated control in regards to *R. reniformis* eggs per gram of root at 64 DAP. Vydate C-LVTM IFS (12% increase), Velum TotalTM IFS + Sure-KTM IFS + Micro 500TM IFS (11% increase), and Vydate C-LVTM IFS + AscendTM ST + AscendTM FS + Sure-KTM IFS + Micro 500TM IFS (14% increase) were statistically higher yielding than the control when *R. reniformis* was not present in the soil (Table 8). Velum TotalTM IFS + AscendTM ST + AscendTM FS + Sure-KTM IFS + Micro 500TM IFS yielded 17% higher than the control in the presence of *R. reniformis* in the soil (Table 8).

DISCUSSION

Growth hormone trials

Growth hormones did affect the plants' abilities to grow larger plant masses in the presence of *R. reniformis*. While no significant differences were detected in root fresh weights, there were differences detected among plant biomass formation. Studies conducted by Oosterhuis and Zhao (1994) showed that the addition of PGR-IV™ hormone blend IFS increased root development in the first 3 weeks, but after 5 weeks, growth differences were not detected compared to the control. Our results were similar as the root mass was not affected compared to the control by the addition of Ascend™ hormone blend at the 7 week harvest date which was necessary for the nematode development. *Rotylenchulus reniformis* egg population density was similar to the control for all Ascend™ growth hormone treatments applied at planting, but egg populations were greater when Ascend™ FS was applied 33 days after planting. These earlier exposures to growth hormones could have lessened the chance for *R. reniformis* to become established before adequate root growth developed. The application of cytokinin is known to increase production of lateral roots and improve the performance of cotton plants with a foliar application (Burke, 2011). This could lessen severity of the *R. reniformis* infection. Ascend™ ST + FS had significantly higher plant biomass and lower *R. reniformis* eggs per gram of root production thus the increase in plant growth may have reduced the effect of the nematode. Burke (2011) showed that increased root growth was observed within three weeks of application of cytokinin as foliar sprays, prompting cotton plants to increase root mass for greater exploration of the soil profile, and these differences were not confirmed due to longer growing time period for nematode reproduction in this study. The seed treatment provided immediate exposure to the growth hormones upon the seed being planted, and the foliar spray

that was applied 33 days after planting could have given an additional boost to the growth of the plant to help manage losses obtained against the plant due to *R. reniformis* infection by stimulating new plant growth.

Starter fertilizer trials

The starter fertilizer evaluations conducted in greenhouse trials showed statistical similarities between the control and all treatments except for Sure-K™ IFS + Micro 500™ IFS in regards to total nematode egg populations, as well as eggs per gram of root. Although this combination had higher nematode populations, it consistently performed numerically better than the control in the aspects of root fresh weight and plant biomass production when comparing Sure-K™ treatments alone, however it was not statistically greater than the control. These results were repeated in a separate set of trials with *Meloidogyne incognita* (Luangkhot, unpublished data). During germination and early growth of cotton seedlings, the addition of starter fertilizers has been found to promote root growth (Kovar et al., 1994; Toler et al., 2004). This combination was selected to take to field studies because of the ease of application that could be done by producers. No-till cotton production was observed to have the best response to plant growth and yields from the addition of starter fertilizers (Burmester et al., 1993; Kovar et al., 1994). All treatments performed numerically better than the control in root fresh weight and plant biomass except for Pro-Germinator™ IFS + Micro 500™ IFS however no statistical differences were observed. Kovar et al. (1994) stated that the addition of starter fertilizers to cotton seedlings produces variable responses in shoot and root formation. Sure-K™ IFS seemed to be the most consistent compound across all treatments in the production of plant biomass, and performed similarly when Micro 500™ IFS was added; therefore this was the compound chosen for further microplot and field studies. Correlations between biomass formation and *R. reniformis* eggs per

gram of root were negative; therefore as plant biomass formation increased, *R. reniformis* eggs per gram of root decreased.

Velum Total™ trials

Velum Total™ was applied as both a seed treatment and as an in-furrow spray and compared to an untreated control. A negative correlation indicated that *R. reniformis* eggs per gram of root decreased as plant biomass increased within this test. Velum Total™ applied as a seed treatment or in-furrow spray dramatically decreased *R. reniformis* populations, both in total egg and eggs per gram of root. Lawrence et al. (2014) also reported Velum Total™ with Poncho/Votivo™ + Aeris™ significantly reduced *R. reniformis* and *M. incognita* populations in 3 field locations. The addition of Velum Total™ + Aeris™ reduced nematode populations by 54% compared to the Gaucho 600™ control in field trials conducted in the 2014 growing season (Lawrence et al., 2015a). Although both Velum Total™ treatments were almost identical in plant biomass formation, both were lower than the Gaucho 600™ seed treatment but higher than the untreated control. Both Velum Total™ treatments were similar to the control in root fresh weights, but sustained a much lower population of nematodes. The reduction of *R. reniformis* populations were similar as the field trials completed by the Cotton Disease Council research on Velum Total™ (Lawrence et al., 2015a).

Vydate C-LV™ trials

Full and half rates of Vydate C-LV™ were tested as in-furrow sprays, foliar sprays, and a combination of in-furrow and foliar sprays. Trial results indicated that the half rate of Vydate C-LV™ IFS showed numerically higher root fresh weight, biomass production, lower total egg counts, and lower eggs per gram of root than the control. Full rates of Vydate C-LV™ IFS

showed lower numerical total egg counts and eggs per gram of root than the control, but had less biomass and root fresh weight than the control. This is inconsistent with other test results as positive responses from the full rate of Vydate C-LV™ IFS against *M. incognita* and higher biomass formation and root fresh weights when compared with the untreated control (Luangkhot, unpublished data). Lorenz et al. (1998) observed the addition of Vydate C-LV™ as foliar spray applications did not reduce *R. reniformis* populations. A two year field study with the application of Vydate C-LV™ foliar sprays + Temik 15G™ indicated that *R. reniformis* populations collected from soil samples were reduced (Lawrence and McLean, 2000). *Meloidogyne incognita* trials utilizing Temik 15G™ + Vydate C-LV™ saw that the two year mean was reduced with the application of Vydate C-LV™ (Lawrence and McLean, 2002). This could be due to the fact that Vydate C-LV™ is mobile in soil and these plots were watered twice per day in the present study, enabling the product to leach beyond the root zone of the plants. DuPont labeling for Vydate C-LV™ states that the product can seep or leach in the soil, and this is especially true for loamy sands. The soil used in the greenhouse trials is consistent with high probability of leaching due to the mobility of Vydate C-LV™. Correlations between root fresh weight and *R. reniformis* eggs per gram of root in addition to biomass formation and *R. reniformis* eggs per gram of root were negative, indicating that as root fresh weights and biomass formations increase, *R. reniformis* eggs per gram of root decrease.

Microplot trials

Microplot trials consisted of combinations of the high-performing treatments in previous tests conducted under greenhouse conditions. Treatments included Velum Total™ IFS and Vydate C-LV™ IFS as nematicidal treatments; Ascend™ ST + Ascend™ FS as the growth hormone treatment; and Sure-K™ IFS + Micro 500™ IFS as the starter fertilizer treatment, and

combinations of all three. Vydate C-LV™ was used as a FS to supplement Velum Total™ IFS in the last treatment as a measure of all possible products being used together. Vydate C-LV™ IFS + Sure-K™ IFS + Micro 500™ IFS was the superior treatment in this trial with increased root fresh weight, biomass production, and decreased *R. reniformis* eggs per gram of root in comparison to the untreated control. Vydate C-LV™ as foliar applications in addition to in-furrow applications of nematicides has been observed as an effective means of control on plant parasitic nematodes (Lawrence and McLean, 2000; Lawrence and McLean, 2002). Vydate C-LV™ IFS alone, Vydate C-LV™ IFS + Sure-K™ IFS + Micro 500™ IFS, and Vydate C-LV™ IFS + Ascend™ ST + FS + Sure-K™ IFS + Micro 500™ IFS all produced greater plant biomass in comparison to the control. Plant biomass increases compared to the control could be attributed to the application of Vydate C-LV™, Sure-K™ + Micro 500™, or Ascend™. Significant increases in plants heights when Vydate C-LV™ was applied as foliar applications following the in-furrow application of a nematicide have been observed (Lawrence and McLean, 2000; Lawrence and McLean, 2002). Early promotion of growth from applications of growth hormones to cotton seeds has been observed to increase overall plant development, indicating that the plant may be able to use available nutrients more efficiently for quicker development and increased yields (Oosterhuis and Zhao, 1994; Oosterhuis, 1994). Application of starter fertilizers has shown inconsistent results when looking at early season development (Kovar et al., 1994; Burmester et al., 1993; Touchton et al., 1986; Toler et al., 2004).

At 42 days after planting, the Ascend™ and Vydate C-LV™ foliar treatments were applied and plots were sampled at mid-season to test the effects of foliar treatments. Velum Total™ IFS alone, Vydate C-LV™ IFS + Ascend™ ST + FS, Vydate C-LV™ IFS + Sure-K™ IFS + Micro 500™ IFS, Velum Total™ IFS + Ascend™ ST + FS + Sure-K™ IFS + Micro

500™ IFS, and a combination of Velum Total™ IFS + Ascend™ ST + FS + Sure-K™ IFS + Micro 500™ IFS+ Vydate C-LV™ FS showed increases in root fresh weight in comparison to the control. Velum Total™ IFS alone, Vydate C-LV™ IFS + Ascend™ ST + FS, Velum Total™ IFS + Ascend™ ST + FS + Sure-K™ IFS + Micro 500™ IFS, and a combination of Velum Total™ IFS + Ascend™ ST + FS + Sure-K™ IFS + Micro 500™ IFS + Vydate C-LV™ FS treatments showed increases in plant biomass formation in comparison to the control. Increases in plant development could be attributed to the addition of the foliar spray of Ascend™. Later applications of growth hormones have been found to increase yield (Oosterhuis, 1994). The initial development from the starter fertilizers could have led to the increased plant biomass with the addition of the nematicides as a protecting agent, allowing the plants to develop longer before nematode infection occurred. This is indicative that nematicides in addition to starter fertilizers as well as growth hormones have potential to increase plant biomass and root mass to help combat losses due to nematode infection. Velum Total™ nematicide plus the starter fertilizers had a 20% higher yield than the control; however, it was not statistically significant due to the variability of the plot data. Interestingly, an additional study with *M. incognita* showed the same yield response with Velum Total™ nematicide plus the starter fertilizers with a 38% yield increase over the control (Luangkhot, unpublished data). These inconsistencies align with inconsistencies observed in other trials with starter fertilizers and growth hormones versus cotton yield data when testing their individual efficacies (Howard et al., 2001; Oosterhuis and Zhao, 2000; Oosterhuis and Robertson, 2000; Kovar et al., 1994; Burmester et al., 1993). Statistical differences in yields have been observed in both Velum Total™ trials and Vydate C-LV™ trials conducted by Lawrence et al. (2014 and 2015a). In trials conducted on *R. reniformis* and *M. incognita* testing afore mentioned treatments, none of the other treatments showed as dramatic of

a yield increase over the control as with the Velum Total™ plus starter fertilizers added however these yields were not significantly greater than the control.

Field trials

The same nematicides, growth hormone, starter fertilizer, and combination treatments that were tested in microplot trials were moved to field trials for testing on a larger scale. Trials were tested in *R. reniformis* infested fields in addition to non-infested fields. In non-infested fields, root fresh weights and biomass productions showed no differences in comparison to the control. Researchers in Alabama, Arkansas, Louisiana, and Tennessee found inconsistencies with early growth and yield improvements with either growth hormones or starter fertilizers (Burmester et al., 1993; Howard et al., 2001; Kovar et al., 1994; Oosterhuis and Robertson, 2000; Oosterhuis and Zhao, 2000). In the presence of *R. reniformis*, positive results were found for both root fresh weight and biomass production among certain treatments. Velum Total™ alone, Vydate C-LV™ alone, Velum Total™ in combination with starter fertilizers, Vydate C-LV™ in combination with starter fertilizers, and Velum Total™ in combination with Ascend™ and starter fertilizers all showed significant increases in root fresh weights and plant biomass production over the untreated control. Growth promotion of cotton has been observed with the addition of plant growth hormones when multiple applications were made, such as seed treatments, in-furrow sprays, and foliar sprays, and yield increases have also been observed due to repeated foliar applications (Oosterhuis, 1994). Variable responses have been seen in other trials with the addition of starter fertilizers, but Toler et al. (2004) found that over the row applications did increase early cotton development. Lawrence et al. (2014 and 2015a) found that vigor of cotton seedlings was increased with the addition of nematicides when applied at planting. Velum Total™ or Vydate C-LV™ plus the plant growth hormones and the combination

of both Velum Total™ and Vydate C-LV™ nematicides, plant hormones, and starter fertilizer increased plant biomass production over the control. These findings that early applications of growth hormones, starter fertilizers, and or nematicides can improve cotton seedling development were similar to the findings of other researchers (Burke, 2011; Burke, 2013; Kovar et al., 1994; Lawrence et al., 2014; Lawrence et al., 2015a; Oosterhuis, 1994; Oosterhuis and Zhao, 1994). All treatments significantly decreased *R. reniformis* eggs per gram of root compared to the untreated control. The addition of Velum Total™ and Vydate C-LV™ has been shown to reduce nematode populations (Lawrence et al., 2014; Lawrence et al., 2015; Lawrence and McLean, 2000; Lawrence and McLean, 2002). The addition of nematicides was proven to suppress nematode populations when used alone and in combination with Ascend™ treatments and starter fertilizers at 42 DAP.

At 42 days after planting, Ascend™ and Vydate C-LV™ foliar sprays were applied to field trials and mid-season nematode samples were taken. In non-infested fields, Vydate C-LV™ alone and Velum Total™ with the starter fertilizers produced significantly higher root fresh weights than the control. In the presence of *R. reniformis*, Velum Total™ plus plant hormones and starter fertilizers produced increased root fresh weights in comparison to the control. The significant increases in root fresh weight could be attributed to the foliar spray of Ascend™, but that result was not consistent across all other treatments containing a foliar spray of Ascend™. This result is supported by work done by Oosterhuis and Zhao (2000) that in-furrow applications and seed treatments do stimulate root development, but foliar applications of some growth hormones do not positively affect yields and have no effect on plant height. No significant differences were found among *R. reniformis* eggs per gram of root. Lawrence and McLean (2000) found that multiple foliar applications of Vydate C-LV™ reduced *R. reniformis*

populations following an early season nematicide. Those results were not supported in these findings following the sampling period after the foliar application of Vydate C-LV™. In the presence of *R. reniformis*, Velum Total™ plus plant hormones and starter fertilizers significantly increased cotton yields by 301 kg/ha over the untreated control; however, when nematodes were absent, both nematicides plus plant hormones and starter fertilizers significantly increased yield by 295 kg/ha over the untreated control. While the treatments that performed best were not identical in the non-infested and the *R. reniformis* infested fields, some differences in yield compared to the untreated control were observed. The Velum Total™ nematicide plus growth hormones and starter fertilizer was the best performing in the *R. reniformis* infested field, but could not completely recover the losses associated with the pathogen when comparing yield with the non-infested control that produced 356 kg/ha more lint cotton. This is indicative that a program utilizing a nematicide + growth hormones + starter fertilizers can increase yields over untreated plots.

LITERATURE CITED

- Barnov, P.A., and Maltzev, A. M. 1937. The structure and development of the cotton plant: An Atlas. Ogis-Isogis, Moscow-Leningrad.
- Bednarz, C.W., Harris G. H., and Shurley W. D. 2000. Agronomic and economic analyses of cotton starter fertilizers. *Agronomy Journal* 92:766-771.
- Blazich, F. A. 1988. Chemicals and formulations used to promote adventitious rooting. *Advances in plant sciences series (USA)* 2:132-149.
- Burke, J.J. 2011. 6-Benzyladenine enhancement of cotton. *The Journal of Cotton Science* 15: 206-214.
- Burke, J. J. 2014. 6-Benzyladenine enhancement of cotton yields. *The Journal of Cotton Science* 17: 1-8.
- Burmester, C. H., Patterson, M. G., and Reeves, D. W. 1993. No-till cotton growth characteristics and yield in Alabama. *Proceedings of the 1993 Southern Conservation Tillage Conference for Sustainable Agriculture* 93:30-36.
- Cothren, J. T., and Oosterhuis, D. M. 2010. Use of growth regulators in cotton production. *Physiology of Cotton*: 287-303.
- Dasgupta, D. R., and Raski, D. J. 1968. The biology of *Rotylenchulus parvus*. *Nematologica* 14:429-440.
- Duncan, L. W. 1991. Current options for nematode management. *Annual Review of Phytopathology* 29:469-490.

Guinn, G. 1986. Hormonal relations during reproduction. The Cotton Foundation: 113-136.

Hartmann, H. T., Kester, D. E., Davis Jr, F. T., Geneve, R. L. 2011. Hartmann & Kester's Plant Propagation Principles and Practices eighth edition. Upper Saddle River, NJ. Pearson Education, Inc., Pub: Prentice Hall.

Howard, D. D., Gwathmey, C. O., Lessman, G. M., and Roberts, R. K. 2001. Fertilizer additive rate and plant growth regulator effects on cotton. The Journal of Cotton Science 5:42-52.

Huessy, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne spp.* including a new technique. Plant Disease Reporter 57:1025-1028.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Koenning, S. R., Kirkpatrick, T. L., Starr, J. L., Wrather, J. A., Walker, N. R., and Mueller, J. D. 2004. Plant-parasitic nematodes attacking cotton in the United States. The American Phytopathological Society. 88:100-113.

Kovar, J.L., Funderburg, E.R., and Hutchinson, R.L. 1993. Starter fertilizer can improve growth and yield of cotton. Better Crops 78:9-11. International Plant Nutrition Institute. Peachtree Corners, GA.

Lawrence, G. W., Lawrence, K. S., and Cacerus, J. 2007. Options after the furrow is closed: applications of Vydate CLV and Temik 15G. Proceedings from the 2007 Beltwide

Conference: 598-601. National Cotton Council of America, Memphis, TN.

<<http://www.cotton.org/beltwide/proceedings>>

Lawrence, G. W., and McLean, K. S. 2000. Effect of Foliar Applications of Oxamyl with Aldicarb for the Management of *Rotylenchulus reniformis* on Cotton. The Society of Nematologists 32:542-549.

Lawrence, G., and McLean, K. 2001. Reniform nematodes. T.L. Kirkpatrick and Rothrock, C.S., eds. Compendium of Cotton Diseases. 2nd edition 42-44. St. Paul, MN: APS Press.

Lawrence, G. W., and McLean, K. S. 2002. Foliar Applications of Oxamyl with Aldicarb for the Management of *Meloidogyne incognita* on Cotton. Nematropica 32:103-112.

Lawrence, K., Lawrence, G., Faske, T., Overstreet, C., Wheeler, T., Young, H., Koenning, S., Muller, J., Kemerait, R., and Mehl, H. 2014. Cotton Variety and Nematicide Combinations for Reniform and Root knot Management Across the Cotton Belt. Proceedings from the 2014 Beltwide Cotton Conference: 295-301. National Cotton Council of America, Memphis, TN. <<http://www.cotton.org/beltwide/proceedings>>

Lawrence, K., Huang, P., Lawrence, G., Faske, T., Overstreet, C., Wheeler, T., Young, H., Koenning, S., Muller, J., Kemerait, R., and Mehl, H. 2015a. Beltwide Nematode Research and Education Committee 2014 Nematode Research Report Cotton Varietal and Nematicide Responses in Nematode Soils. Proceedings from the 2015 Beltwide Cotton Conference: 739-742. National Cotton Council of America, Memphis, TN.

Lawrence, K., Olsen, M., Faske, T., Hutmacher, R., Muller, J., Mario, J., Kemerait, R., Overstreet, C., Sciumbato, G., Lawrence, G., Atwell, S., Thomas, S., Koenning, S., Boman, R., Young, H., Woodward, J., and Mehl, H. 2015b. Cotton disease loss estimate committee report, 2014. Proceedings of the 2014 Beltwide Cotton Conference 1:188-190. National Cotton Council of America, Memphis, TN.

Linford, M. B., and Oliveira, J. M. 1940. *Rotylenchulus reniformis*, nov. gen. n. sp., a nematode parasite of roots. Proceedings of the Helminthological Society of Washington 7:35-42.

Lorenz, G. M., Kirkpatrick, T., Robbins, R., Rorie, K., and Fisher, A. 1998. Suppression of Reniform and Root-knot Nematode with Temik and Vydate. Proceedings from the 1998 Beltwide Cotton Conference 1:138-139. National Cotton Council of America, Memphis, TN.

Miller, C., Skoog, F., von Saltza, M. H., Strong, F. M. 1955. Kinetin, a cell division factor from deoxyribonucleic acid. Journal of American Chemistry Society. 1955, 77, 1392.

Oosterhuis, D.M. 1994. Effects of PGR-IV on the growth and yield of cotton: A Review. Proceedings of 1994 World Cotton Conference.

Oosterhuis, D. M., Steger, A., and McConnell, J.S. 1997. Field evaluation of plant growth regulators in 1996. Special report 183:133-135. Arkansas Agricultural Experiment Station. University of Arkansas. Fayetteville, Arkansas.

Oosterhuis, D. M. and Zhao, D. 1994. Increased root length and branching in cotton by soil application of the plant growth regulator PGR-IV. Plant and Soil 167:51-56.

Stewart, S., and Faircloth, J. 2007. The first forty days through fruiting to finish. Proceedings from the 2007 Beltwide Cotton Conference 1:579-586. National Cotton Council of America, Memphis, TN.

Somasundaram, L., Coats, J. R., and Racke, K. D. 1989. Degradation of pesticides in soil as influenced by the presence of hydrolysis metabolites. *Journal of Environmental Science and Health* 24: 457-478.

Spieth, A. M. 1933. Anatomy of the transition region in *Gossypium*. *Botanical Gazette*. 95:338-347.

Taiz, L. and Zeiger, E. 2010. *Plant physiology*. Pp. 424-515. Redwood City, CA: The Benjamin/Cummings Publishing Company.

Toler, J. E., Murdock, E. C., and Camberato, J.J. 2004. Starter fertilizer effect on cotton development and weed interference. *The Journal of Cotton Science* 8: 33-41.

Touchton, J.T., Rickerl, D. H., Burmester, C. H., and Reeves, D.W. 1986. Starter fertilizer combinations and placement for conventional and no-tillage cotton. *Journal of Fertilizer Issues* 3:91-98.

OVERALL CONCLUSION

The application of growth hormones, different starter fertilizers, and two separate nematicides were evaluated for their efficacy in the management of *Rotylenchulus reniformis* in upland cotton production. Growth hormones applied to cotton seedlings has been explored by many researchers in the past. The theories behind why they should be used are good; however the real world translation of growth hormone applications is inconsistent and hard to predict. Starter fertilizers have been investigated in many different formulations for best timing, application method, and which compounds to apply. The outside factors – such as soil type and planting temperatures – play a major role in the efficacy of starter fertilizers to increase cotton seedling development and potentially yield. Velum Total™ as a nematicide was found to be a very effective nematicide in managing *R. reniformis* in the earliest part of the season. Vydate C-LV™ evaluations exhibited good efficacy when applied as an in-furrow spray, but this method for application is not labeled and cannot currently be recommended. A program focused on crop rotation, nematicides, starter fertilizers, and possibly growth hormones could achieve successful management of a *R. reniformis* infestation.

APPENDIX

Tables

Table 1. Growth hormone effects on cotton root fresh weight, plant biomass, *Rotylenchulus reniformis* total egg numbers, and eggs per gram of root at 49 DAP in greenhouse trials.

Treatment	Rate	Root fresh weight (g)	Plant biomass (g)	<i>Rotylenchulus reniformis</i>	
				Total eggs	Eggs/g root
Control ^z	---	5.78 ^y	11.81	66899	12594
Ascend™ ST	88.7 mL/cwt	6.85	14.21**	67826	9582
Ascend™ IFS	233.7 mL/ha	5.41	11.78	53303	11438
Ascend™ FS	233.7 mL/ha	6.77	13.70	101043**	15255
Ascend™ ST + IFS	88.7 mL/cwt + 233.7 mL/ha	6.54	13.64	70452	11006
Ascend™ ST + FS	88.7 mL/cwt + 233.7 mL/ha	7.16	14.56**	52839	7443*
Ascend™ IFS + FS	233.7 mL/ha + 233.7 mL/ha	6.51	13.37	76555	11795
Ascend™ ST + IFS + FS	88.7 mL/cwt + 233.7 mL/ha + 233.7 mL/ha	6.26	12.76	58633	10358

^zAscend™ is comprised of cytokinin 0.090%, gibberellic acid 0.03%, indole butyric acid 0.045%. Ascend™ ST is a seed treatment, Ascend™ IFS is an in-furrow spray, Ascend™ FS is a foliar spray applied at 2 true leaf stage.

^yMeans in the same column followed by * $P < 0.10$; ** $P < 0.05$ according to Dunnett's P values compared to the control are significantly different.

Table 2. Starter fertilizer effects on cotton root fresh weight, plant biomass, *Rotylenchulus reniformis* total egg numbers, and eggs per gram of root at 49 DAP in greenhouse trials.

Treatment	Rate	Root fresh weight (g)	Plant biomass (g)	<i>Rotylenchulus reniformis</i>	
				Total eggs	Eggs/g root
Control	---	4.06 ^y	11.77	28359	9434
Sure-K TM IFS ^z	9.28 L/ha	4.40	12.15	23792	5974
Pro-Germinator TM					
IFS	4.64 L/ ha	4.87	13.59	26801	6154
Micro 500 TM IFS	2.32 L/ ha	4.44	12.28	27788	8874
Ammonium Poly					
Phosphate TD	1.16 L/ ha	5.33	14.32	33267	8483
Sure-K TM IFS +	9.28 L/ ha +				
Micro 500 TM IFS	2.32 L/ha	4.24	12.15	66246**	16683*
Pro-Germinator TM					
IFS + Micro 500 TM	4.64 L/ ha +				
IFS	2.32 L/ha	3.63	11.10	25504	8035
Ammonium					
Polyphosphate TD	1.16 L/ ha +				
+ Micro 500 TM IFS	2.32 L/ha	4.09	12.43	15469	4055

^zFertilizers selected in these trials were Sure-KTM (2-1-6), Pro-germinatorTM (9-24-3), Micro 500TM (B 0.02%, Cu 0.25%, Fe 0.37%, Mn 1.2%, and Zn 1.8%), and Ammonium polyphosphate (10-34-0). IFS stands for in furrow spray application at planting and TD stands for top dressed at planting.

^yMeans in the same column followed by* $P < 0.10$; ** $P < 0.05$ according to Dunnett's P values compared to the control are significantly different.

Table 3. Velum Total™ effects on cotton root fresh weight, plant biomass, *Rotylenchulus reniformis* total egg numbers, and eggs per gram of root at 49 DAP in greenhouse trials.

Treatment	Rate	Root fresh weight (g)	Plant biomass (g)	<i>Rotylenchulus reniformis</i>	
				Total Eggs	Eggs/g Root
Control ^z	---	8.44 ^y	22.42	27099	5886
Gaucho 600™ ST	0.375 mg a.i./seed	10.55	27.67*	29973	3093
Velum Total™ ST	1.0 mg a.i./seed	8.39	23.15	1903***	269**
Velum Total™ IFS	1 L/ha	9.01	23.45	564***	82**

^zGaucho 600™ contains Imidacloprid, Velum Total™ contains Fluopyram and Imidacloprid. ST denotes seed treatment and IFS denotes in-furrow spray.

^yMeans in the same column followed by * $P < 0.10$; ** $P < 0.05$; *** $P < 0.001$ according to Dunnett's P values compared to the control are significantly different.

Table 4. Vydate C-LV™ effects on cotton root fresh weight, plant biomass, *Rotylenchulus reniformis* total egg numbers, and eggs per gram of root at 49 DAP in greenhouse trials.

Treatment	Rate	Root fresh weight (g)	Plant biomass (g)	<i>Rotylenchulus reniformis</i>	
				Total Eggs	Eggs/g Root
Control ²	---	8.04	24.22	13770	1933
Vydate C-LV™					
IFS	1.2 L/ha	7.09	22.15	10265	1660
Vydate C-LV™					
IFS	0.6 L/ha	10.18	28.72	11153	1297
Vydate C-LV™					
FS	1.2 L/ha	5.67	20.33	12206	2677
Vydate C-LV™	0.6L/ ha +				
IFS + FS	0.6 L/ha	8.01	23.27	8391	1382

²Vydate C-LV™ contains Oxamyl. IFS denotes, in-furrow spray at planting and FS denotes foliar spray at 4 leaf stage.

Table 5. Combination effects of growth hormones, starter fertilizers, and nematicides on cotton root fresh weight, plant biomass, and *Rotylenchulus reniformis* means for eggs per gram of root at 35 DAP in microplot trial.

Treatment	Rate	Root fresh weight (g)	Plant biomass (g)	Reniform Eggs/g Root
Control ^z	---	3.48 ^y	40.64	744
Velum Total TM IFS	1 L/ha	4.96	52.44	446
Vydate C-LV TM IFS	1.2 L/ha	5.2	69.52*	528
Velum Total IFS TM + Ascend TM ST + Ascend TM FS	1 L/ha + 88.7 mL/cwt + 233.7 mL/ha	5.24	55.48	533
Vydate C-LV TM IFS + Ascend TM ST + FS	1.2 L/ha + 88.7 mL/cwt + 233.7 mL/ha	4.24	51.44	533
Velum Total TM IFS + Sure-K TM IFS + Micro 500 TM IFS	1 L/ha + 9.28 L/ha + 2.32 L/ha	3.28	37.12	516
Vydate C-LV TM IFS + Sure-K TM IFS + Micro 500 TM IFS	1.2 L/ha + 9.28 L/ha + 2.32 L/ha	5.72*	71.00**	331*
Velum Total TM IFS + Ascend TM ST + FS + Sure-K TM IFS + Micro 500 TM IFS	1 L/ha + 88.7 mL/cwt + 233.7 mL/ha + 9.28 L/ha + 2.32 L/ha	3.52	47.32	454
Vydate C-LV TM IFS + Ascend TM ST + FS + Sure-K TM IFS + Micro 500 TM IFS	1.2 L/ha + 88.7 mL/cwt + 233.7 mL/ha + 9.28 L/ha + 2.32 L/ha	5.28	70.20*	412
Velum Total TM IFS + Ascend TM ST + FS + Sure-K TM IFS + Micro 500 TM IFS + Vydate C-LV TM FS	1 L/ha + 88.7 mL/cwt + 233.7 mL/ha + 9.28 L/ha + 2.32 L/ha + 1.2 L/ha	4.28	47.44	621

^zAscendTM is comprised of cytokinin 0.090%, gibberellic acid 0.03%, indole butyric acid 0.045%, Sure-KTM (2-1-6), Micro 500TM (B 0.02%, Cu 0.25%, Fe 0.37%, Mn 1.2%, and Zn 1.8%), Velum TotalTM contains Fluopyram and Imidacloprid, and Vydate C-LVTM contains Oxamyl. IFS stands for in furrow spray application at planting ST is a seed treatment, FS is a foliar spray.

^yMeans in the same column followed by * $P < 0.10$; ** $P < 0.05$ according to Dunnett's P values compared to the control are significantly different.

Table 6. Combination effects of growth hormones, starter fertilizers, and nematicides on cotton root fresh weight, plant biomass, and *Rotylenchulus reniformis* means for eggs per gram of root at 61^z and yields 135^y DAP in Microplot Trial.

Treatment	Rate	Root fresh weight ^z (g)	Plant biomass ^z (g)	<i>R. reniformis</i> eggs/g root ^z	Yield Grams/plot ^y
Control ^x	---	6.65 ^w	72.38	109	169.07
Velum Total TM					
IFS	1 L/ha	14.77*	143.51*	111	157.48
Vydate C-LV TM					
IFS	1.2 L/ha	13.45	115.69	439**	173.07
Velum Total					
IFS TM +	1 L/ha +				
Ascend TM ST +	88.7 mL/cwt +				
Ascend TM FS	233.7 mL/ha	12.45	139.04	271	147.58
Vydate C-LV TM	1.2 L/ha +				
IFS + Ascend TM	88.7 mL/cwt +				
ST + FS	233.7 mL/ha	18.38**	164.20**	200	156.15
Velum Total TM					
IFS + Sure-K TM	1 L/ha +				
IFS + Micro	9.28 L/ ha +				
500 TM IFS	2.32 L/ha	9.34	102.60	160	202.11
Vydate C-LV TM					
IFS + Sure-K TM	1.2 L/ha+				
IFS + Micro	9.28 L/ ha +				
500 TM IFS	2.32 L/ha	15.51**	130.57	183	131.51
Velum Total TM					
IFS + Ascend TM	1 L/ha +				
ST + FS + Sure-	88.7 mL/cwt +				
K TM IFS + Micro	233.7 mL/ha +				
500 TM IFS	9.28 L/ ha +	16.15**	142.70*	151	176.86
Vydate C-LV TM	1.2 L/ha +				
IFS + Ascend TM	88.7 mL/cwt +				
ST + FS + Sure-	233.7 mL/ha +				
K TM IFS + Micro	9.28 L/ ha +				
500 TM IFS	2.32 L/ha	13.15	132.61	176	153.86
Velum Total TM					
IFS + Ascend TM	1 L/ha +				
ST + FS + Sure-	88.7 mL/cwt +				
K TM IFS + Micro	233.7 mL/ha +				
500 TM IFS +	9.28 L/ ha +				
Vydate C-LV TM	2.32 L/ha +				
FS	1.2 L/ha	16.98*	213.61**	182	163.93

^z61 days after planting Root fresh weight, Plant biomass, *R. reniformis* eggs/g root

^y 135 days after planting Grams/plot

^xAscendTM is comprised of cytokinin 0.090%, gibberellic acid 0.03%, indole butyric acid 0.045%, Sure-KTM (2-1-6), Micro 500TM (B 0.02%, Cu 0.25%, Fe 0.37%, Mn 1.2%, and Zn 1.8%), Velum TotalTM contains Fluopyram and Imidacloprid, and Vydate C-LVTM contains Oxamyl. IFS stands for in furrow spray application at planting ST is a seed treatment, FS is a foliar spray.

^wMeans in the same column followed by * $P < 0.10$; ** $P < 0.05$ according to Dunnett's P values compared to the control are significantly different.

Table 7. Combination effects of growth hormones, starter fertilizers, and nematicides on cotton root fresh weight, plant biomass, and *Rotylenchulus reniformis* means for eggs per gram of root at 42 DAP in *Rotylenchulus reniformis* infested and non-infested field trials.

Treatment	Rate	Root fresh weight (g)		Plant biomass (g)		<i>R. reniformis</i> eggs/g root
		W/o RR	W/ RR	W/o RR	W/ RR	
Control ^z	---	2.42 ^y	2.86	36.06	29.89	1543
Velum Total TM IFS	1 L/ha	2.43	4.19*	35.75	40.56*	190***
Vydate C-LV TM IFS	1.2 L/ha	3.25	4.98**	46.87	45.47**	213***
Velum Total IFS TM + Ascend TM ST + Ascend TM FS	1 L/ha + 88.7 mL/cwt + 233.7 mL/ha	2.87	3.74	42.55	41.62*	359***
Vydate C-LV TM IFS + Ascend TM ST + FS	1.2 L/ha + 88.7 mL/cwt + 233.7 mL/ha	2.57	4.01	37.00	42.54**	680**
Velum Total TM IFS + Sure-K TM IFS + Micro 500 TM IFS	1 L/ha + 9.28 L/ ha + 2.32 L/ha	3.20	4.25*	43.82	44.15**	552**
Vydate C-LV TM IFS + Sure-K TM IFS + Micro 500 TM IFS	1.2 L/ha + 9.28 L/ ha + 2.32 L/ha	2.67	4.48**	39.94	44.00**	313***
Velum Total TM IFS + Ascend TM ST + FS + Sure-K TM IFS + Micro 500 TM IFS	1 L/ha + 88.7 mL/cwt + 233.7 mL/ha + 9.28 L/ ha + 2.32 L/ha	2.82	5.11**	40.49	51.78***	534**
Vydate C-LV TM IFS + Ascend TM ST + FS + Sure-K TM IFS + Micro 500 TM IFS	1.2 L/ha + 88.7 mL/cwt + 233.7 mL/ha + 9.28 L/ ha + 2.32 L/ha	2.55	3.68	38.97	37.34	455***
Velum Total TM IFS + Ascend TM ST + FS + Sure-K TM IFS + Micro 500 TM IFS + Vydate C-LV TM FS	1 L/ha + 88.7 mL/cwt + 233.7 mL/ha + 9.28 L/ ha + 2.32 L/ha + 1.2 L/ha	2.84	4.54**	43.21	43.58**	541**

^zAscendTM is comprised of cytokinin 0.090%, gibberellic acid 0.03%, indole butyric acid 0.045%, Sure-KTM (2-1-6), Micro 500TM (B 0.02%, Cu 0.25%, Fe 0.37%, Mn 1.2%, and Zn 1.8%), Velum TotalTM contains Fluopyram and Imidacloprid, and Vydate C-LVTM contains Oxamyl. IFS stands for in furrow spray application at planting ST is a seed treatment, FS is a foliar spray.

^yMeans in the same column followed by * $P < 0.10$; ** $P < 0.05$; *** $P < 0.001$ according to Dunnett's P values compared to the control.

Table 8. Combination effects of growth hormones, starter fertilizers, and nematicides on cotton root fresh weight, plant biomass, and *Rotylenchulus reniformis* means for eggs per gram of root at 64^z and lint yields 165^y DAP in *Rotylenchulus reniformis* infested and non-infested field trials.

Treatment	Rate	Root fresh weight ^z (g)		<i>R. reniformis</i> eggs/g root ^z	Lint Yields kg/ha ^y	
		W/o RR	W/ RR		W/o RR	W/ RR
Control ^x	---	22.14 ^w	23.57	60	2379	1722
Velum Total TM IFS	1 L/ha	23.92	22.37	87	2481	1874
Vydate C-LV TM IFS	1.2 L/ha	28.16*	22.05	102	2670*	1911
Velum Total IFS TM + Ascend TM ST + Ascend TM FS	1 L/ha + 88.7 mL/cwt + 233.7 mL/ha	24.14	21.49	84	2591	1831
Vydate C-LV TM IFS + Ascend TM ST + FS	1.2 L/ha + 88.7 mL/cwt + 233.7 mL/ha	25.82	22.65	93	2538	1819
Velum Total TM IFS + Sure-K TM IFS + Micro 500 TM IFS	1 L/ha + 9.28 L/ ha + 2.32 L/ha	28.84*	24.18	63	2639*	1854
Vydate C-LV TM IFS + Sure-K TM IFS + Micro 500 TM IFS	1.2 L/ha + 9.28 L/ ha + 2.32 L/ha	26.55	23.27	61	2405	1922
Velum Total TM IFS + Ascend TM ST + FS + Sure-K TM IFS + Micro 500 TM IFS	1 L/ha + 88.7 mL/cwt + 233.7 mL/ha + 9.28 L/ ha + 2.32 L/ha	27.19	29.97*	79	2594	2023*
Vydate C-LV TM IFS + Ascend TM ST + FS + Sure-K TM IFS + Micro 500 TM IFS	1.2 L/ha + 88.7 mL/cwt + 233.7 mL/ha + 9.28 L/ ha + 2.32 L/ha	27.01	22.22	73	2713**	1800
Velum Total TM IFS + Ascend TM ST + FS + Sure-K TM IFS + Micro 500 TM IFS + Vydate C-LV TM FS	1 L/ha + 88.7 mL/cwt + 233.7 mL/ha + 9.28 L/ ha + 2.32 L/ha + 1.2 L/ha	21.85	29.60*	64	2612	1923

^z61 days after planting Root fresh weight and *R. reniformis* eggs/g root

^x165 days after planting Lint Yields kg/ha

^y AscendTM is comprised of cytokinin 0.090%, gibberellic acid 0.03%, indole butyric acid 0.045%, Sure-KTM (2-1-6), Micro 500TM (B 0.02%, Cu 0.25%, Fe 0.37%, Mn 1.2%, and Zn 1.8%), Velum TotalTM contains Fluopyram and Imidacloprid, and Vydate C-LVTM contains Oxamyl. IFS stands for in furrow spray application at planting ST is a seed treatment, FS is a foliar spray.

^wMeans in the same column followed by * $P < 0.10$; ** $P < 0.05$; *** $P < 0.001$ according to Dunnett's P values compared to the control.