Volume 25 2012

TEXAS JOURNAL OF AGRICULTURE AND NATURAL RESOURCES

A Publication of the Agriculture Consortium of Texas

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ISSN 0891-5466

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Susceptibility of *Helicoverpa zea* to Commercial Insecticides Used in Green Bean Production on Texas High Plains[1]

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ABSTRACT

Susceptibility levels of adult *Helicoverpa zea* (Boddie), collected from green bean growing areas of Texas and New Mexico using sex-pheromone baited traps, were tested against some of the most common commercial insecticides that are extensively used in green bean production. Insecticide vial bioassays indicated variation in the levels of tolerance over the season with highest LC_{50} values during mid-season in 2002 and 2003. Studies included three active ingredients from two insecticide classes. Zeta-cypermethrin, bifenthrin, and methomyl were tested at nine different concentrations. Data from 2002 showed a statistically significant progressive decrease in susceptibility levels between the generations that were tested with bifenthrin and methomyl. In 2003, these variations in susceptibility levels were different though not significant. Differences in bifenthrin susceptibility were substantial between the locations in the middle of the 2003 season for third generation moths. Increased insecticide use in 2002 compared to 2003 might have accounted for higher tolerance levels in 2002.

KEY WORDS: *Helicoverpa zea*, corn, green beans, insecticide resistance

INTRODUCTION

Green beans, *(Phaseolus vulgaris* L.), also referred to as common, snap, wax, and field beans, are grown throughout the world for consumption as immature pods. In 2002, the United States alone had a market value for green beans of $127 million, with

[1]Acknowledgements: We thank Dr. Buddy Laing for providing equipment and office space. Also, we are very thankful to Doug Dillon (Allen Canning Company) for providing previous efficacy and other empirical data.

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100,000 acres producing 6 million tons for the fresh market, while an additional 210,100 acres produced 831,260 tons for the processing market (USDA-NASS 2003). Texas ranked 14th in both fresh and processing markets in 2002. Its green bean production contributes around $429 million to the United States economy ($391 million and $38 million from fresh and processing market, respectively).

Helicoverpa zea (Boddie), referred to as cotton bollworm, corn earworm (CEW), or soybean pod borer, is considered an important pest of green beans because of its extensive tunneling of the pods, which leads to rejection at the processing plant. Insecticidal control is the primary pest management strategy for this pest in almost all cases. Bollworm has developed resistance to many insecticides (Winteringham and Hewlett, 1964), including organophosphates (methyl parathion in cotton) and organochlorines (Wolfenbarger 1971; Sparks 1981). Resistance is considered a key factor for the pest status of bollworm (Sparks 1981; Sparks et al., 1993). Tolerance to pyrethroids such as cypermethrin, permethrin, and methomyl were documented in *Helicoverpa zea* (Stadelbacher et al., 1990; Hsu and Yu 1991). Also, in South Carolina, widespread application of pyrethroids resulted in a drastic decline in efficacy against corn earworm (Brown et al., 1997). The excess use of insecticides, especially synthetic pyrethroids, might have triggered the development of higher tolerance in bollworm observed in Texas in 1986 (Kanga and Plapp 1996). Sparks (1981) reported that synthetic pyrethroids were effective against Texas bollworms. However, recent statewide bioassays have shown marked increase in tolerance in some areas, and pyrethroids have been abandoned during part of the growing season in some counties. In spite of recent changes in susceptibility to pyrethroids in field populations of *H. zea*, little information is available on possible mechanisms of pyrethroid resistance (Graves et al., 1993; Bagwell et al., 1996; Kanga et al., 1996). Because bollworm infestation in cotton often occurs along with tobacco budworm infestation, higher insecticide doses and more frequent applications used for budworm may cause an increase in the selection pressure on bollworm populations (Kanga et al., 1996). Thus, frequent monitoring of bollworm susceptibility to insecticides used in green bean production, along with efficacy assessment against bollworm populations may help in predicting possible outbreaks of resistance.

Reduced control of the bollworm populations because of the increase in tolerance might be relatively acceptable in cotton or other row crop production. However, green beans produced for the canning industry must be almost *H. zea* free, with an economic threshold of about one larva per 10,000 pods. Therefore, even moderate increase in insecticide tolerance poses a significant threat to green bean growers and the canning industry.

The objectives of this study are: (1) to determine the levels of susceptibility of *H. zea* to different insecticides used in green bean production; (2) to study the year to year variation in susceptibility levels; and (3) to discuss the likely efficacy of pyrethroids against bollworm in green beans as influenced by use of pyrethroids in the local cropping systems on Texas High Plains. Data from this study could be very useful to growers in making decisions based on the historic data on bollworms in the area.

MATERIALS AND METHODS

Studies were conducted in 2002 and 2003 in Parmer County, Texas, located on the Texas-New Mexico border (Figure 1). Most of the green beans grown in this area were under contract with a canning company based in Arkansas.

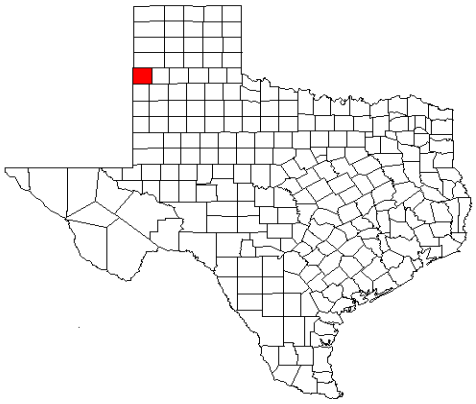

Figure 1. Location of Parmer County in Texas.

Two locations, Lazbuddie and Oklahoma Lane, were selected for the 2002 study. The nearby locations, Lariat and Hub, were selected in 2003 as crop sites used in the previous year were subjected to severe hail damage. All the study sites selected were within a 30-mile area (Figure 2).

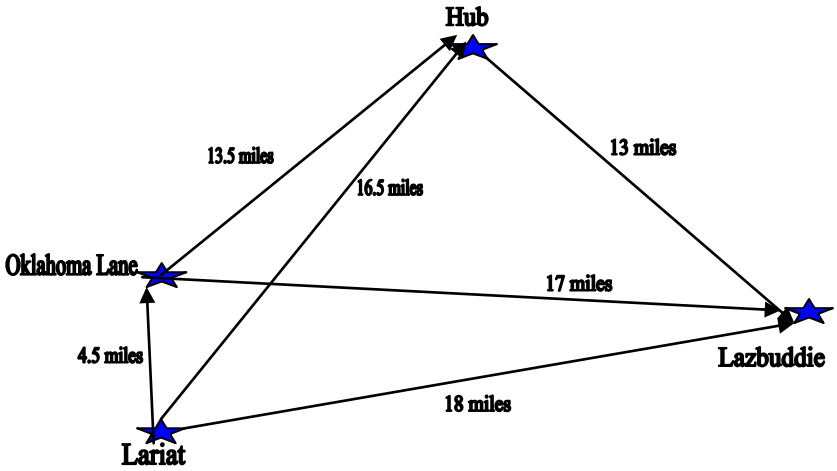

Figure 2. Relative distance between trap locations in 2002-2003, Parmer County, Texas.

Insecticides and insect treatment. Technical grade (>90% purity) bifenthrin, zeta-cypermethrin, and methomyl were used in the study. Vials with 9 different doses of each insecticide were prepared at New Mexico State University in Las Cruces and transferred to the test area. Concentrations (μg/vial) included for bifenthrin and zeta-cypermethrin were the same (0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.2, and 12.8) while methomyl differed from the other two in lacking concentration of 0.05 μg/vial and having an extra

concentration of 25 µg/vial. The treatments in adult vial bioassay were conducted using 20 ml wide-mouth glass scintillation vials coated on the inside with insecticides (Kanga and Plapp 1992). A 0.5 m1 stock solution was added to the vials and the vials were rotated on a rack to get a uniform coating on the inner surface until they dried. Adult moths were collected using sex-pheromone baited Hartstack wire mesh traps (Hartstack et al., 1979), which were placed near cornfields surrounding green beans growing area. Fresh moths collected from the traps in the morning hours were treated by placing two moths per vial. Only healthy looking, non-rubbed adults of similar body size were included in the test (Figure 3). The vials containing the moths were then kept in a cooler with a small amount of ice to prevent excessively high temperatures.

Figure 3. Insecticide-treated vials representing different concentrations.

To avoid any influence of low temperatures on the efficacy of the insecticides, care was taken to avoid direct contact between the vials and ice; using Styrofoam and paper towels. Vials were transported as quickly as possible to the laboratory where they were held at room temperature until mortality data was obtained at 8 h post-exposure. Mortality was assessed by prodding the thoracic region with a pencil to check for survival.

Data analysis. Mortality rates were calculated by applying Abbott's (1925) formula. Data obtained from different generations were pooled and analyzed by means of probit analysis (Russell et al., 1977) using POLO-PLUS (LeOra Software 2003). The differences among the populations, in responses to insecticides, were considered not significant if the 95% confidence intervals of LC_{50} or LC_{90} overlapped (Robertson and Preisler 1992).

RESULTS

Insecticidal bioassay study showed variation in the levels of susceptibility. A significant decrease in susceptibility to both bifenthrin and methomyl was observed between the generations during the year 2002. However, populations from different generations in the year 2003 showed an increase in susceptibility levels.

Bifenthrin in Year 2002. The adult moths showed a significant decrease in susceptibility to bifenthrin as the 2002 growing season progressed (Table 1). LC_{50} values at Lazbuddie were 0.22 µg/vial in the June flight and 2.05 µg/vial in the August/September flight.

Table 1. Toxicity of bifenthrin to corn earworm moths in a bioassay conducted at Lazbuddie and Oklahoma Lane, Texas in 2002.

Generation	n[a]	Slope ± SE	LC_{50}[b] (95% CI)[c]	LC_{90}[b] (95% CI)[c]	χ^2
			Lazbuddie		
June	280	1.79 ± 0.25	0.22 (0.15-0.30)	1.16 (0.79-2.08)	3.97
Aug/Sept	314	1.97 ± 0.20	2.05 (1.09-4.79)	9.2 (4.16-85.04)	33.47*
			Oklahoma Lane		
June	176	1.45 ± 0.27	0.24 (0.004-0.99)	1.86 (0.59-2.53)	11.23
Aug/Sept	380	2.12 ± 0.26	2.51 (1.59-3.75)	10.07 (6.17-25.8)	12.76*

[a] Number of corn earworms moths tested. [b] Lethal concentrations, expressed in micrograms of insecticide per vial. [c] 95% confidence limits shown beneath each LC_{50} and LC_{90}. * Significant.

Similarly, a significant reduction in susceptibility from 0.24 to 2.51 µg/vial was observed at Oklahoma Lane between the June and August/September flights. The differences in LC_{50} values between the generations at Lazbuddie (χ^2 = 3.97, 33.47, p < 0.05) as well as Oklahoma Lane (χ^2 = 11.23, 12.76; p < 0.05) were statistically significant.

Based on the overlapping confidence limits, the susceptibility level of moths tested at the two locations was not different between the two locations in the study.

Bifenthrin in Year 2003. The populations in 2003 showed a significant increase in susceptibility of the adults (Table 2). LC_{50} values declined significantly between first and the third generation (1.01 to 0.39 µg/vial) (χ^2 = 4.66, 3.95; p < 0.05) for moths collected at Lariat.

However, LC_{90} values from June-August (4.33 to 2.05 µg/vial) were not significantly different. Adult populations at Hub showed no significant variation in either LC_{50} or LC_{90} levels (χ^2 = 0.92, 0.97; p > 0.05). Comparison of the populations over the generations between the locations showed a significant difference in both LC_{50} and LC_{90} (non-overlapping of CI limits) values for third generation adults.

Methomyl in Year 2002. Adults tested for Methomyl showed a decreasing trend in susceptibility levels (Table 3). LC_{90} values increased between the generations and were also significantly different between Lazbuddie and Oklahoma Lane. LC_{50} values varied from 0.91-4.02 µg/vial and 1.46-5.38 µg/vial respectively (χ^2 = 6.68, 11.94 and 4.22,

2.28; $p < 0.05$). LC_{90} values followed similar increase (2.35-17.72 and 7.68-27.84 µg/vial respectively).

Comparison of the susceptibility levels at the two locations showed the LC_{50} values did not differ (overlapping CI limits). A significant difference was observed in the LC_{90} values just in first generation flights. The adult moths were found to be less susceptible in the latter half of the season.

Table 2. Toxicity of bifenthrin to corn earworm moths in a bioassay conducted at Lariat and Hub, Texas in 2003.

Generation	n[a]	Slope± SE	LC_{50}[b] (95% CI)[c]	LC_{90}[b] (95% CI)[c]	χ^2
			Lariat		
June	320	1.79 ± 0.25	1.01 (0.73-1.30)	4.33 (3.19-6.67)	4.66*
July	320	1.97 ± 0.20	0.46 (0.09-0.95)	2.63 (1.29-11.52)	10.25
Aug	320	1.79 ± 0.24	0.39 (0.25-0.54)	2.05 (1.47-3.33)	3.95*
			Hub		
June	320	1.97 ± 0.22	0.92 (0.73-1.15)	4.15 (3.01-6.58)	3.06
July	320	2.19 ± 0.33	0.63 (0.40-0.88)	2.44 (1.77-3.82)	2.24
Aug	320	1.75 ± 0.21	0.97 (0.66-1.34)	5.23 (3.64-8.65)	3.98

[a] Number of corn earworms moths tested. [b] Lethal concentrations, expressed in micrograms of insecticide per vial. [c] 95% confidence limits shown beneath each LC_{50} and LC_{90}. * Significant.

Methomyl in Year 2003. Moths collected at Lariat and Hub showed no difference in susceptibility to methomyl among the collections in the June, July, and August (LC_{50} of 2.15-1.82 and 3.09-1.57 µg/vial) (Table 4).

All three generations were not significantly different in levels between generations as well as locations except that the second generation moths varied in susceptibility between locations (6.62 and 9.65 µg/vial; $\chi^2 = 4.26$ & 6.37). Adults tested in 2003 adults were more susceptible as the season progressed.

Zeta-Cypermethrin in Year 2003. Moths tested for Zeta-cypermethrin in 2003 showed no significant variation in the susceptibility levels. LC_{50} and LC_{90} values were not different between generations or between locations tested. Overlapping CI limits evidenced the non-significance in the variations over the season with LC_{50} varying across the season (Lariat: 2.11-1.03; Hub: 1.93-1.2 µg/vial; $p>0.05$) between the June and August generations (Table 5).

LC_{90} values at Lariat declined from 6.18 in the first generation to 3.74 µg/vial (non-significant) in third generation. As this insecticide was not tested in the year 2002, the susceptibility levels were not compared between the years.

Table 3. Toxicity of methomyl to corn earworm moths in a bioassay conducted at Lazbuddie and Oklahoma Lane, Texas in 2002.

Generation	n[a]	Slope ± SE	LC_{50}[b] (95% CI)[c]	LC_{90}[b] (95% CI)[c]	χ^2
			Lazbuddie		
June	320	3.10 ± 0.05	0.91 (0.56-1.25)	2.35 (1.66-4.48)	6.68
Aug/Sept	292	1.99 ± 0.28	4.02 (2.44-10.92)	17.72 (7.7-269.03)	11.94*
			Oklahoma Lane		
June	204	1.78 ± 0.31	1.46 (0.96-2.16)	7.68 (4.6-19.2)	4.22
Aug/Sept	342	1.79 ± 0.42	5.38 (3.79-9.8)	27.84 (13.5-158.8)	2.28

[a] Number of corn earworms moths tested. [b] Lethal concentrations, expressed in micrograms of insecticide per vial. [c] 95% confidence limits shown beneath each LC_{50} and LC_{90}. * Significant.

Table 4. Toxicity of methomyl to corn earworm moths in a bioassay conducted at Lariat and Hub, Texas in 2003.

Generation	n[a]	Slope ± SE	LC_{50}[b] (95% CI)[c]	LC_{90}[b] (95% CI)[c]	χ^2
			Lariat		
June	320	1.93 ± 0.27	2.15 (0.71-3.81)	9.86 (5.56-9.98)	6.63
July	320	2.62 ± 0.47	2.15 (0.92-3.37)	6.62 (4.20-6.14)	4.26
Aug	320	2.63 ± 0.36	1.82 (0.91-2.77)	5.6 (3.61-13.60)	9.54
			Hub		
June	320	2.27 ± 0.47	3.09 (1.58-4.41)	11.31 (8.20-19.3)	2.98
July	320	2.36 ± 0.41	2.77 (1.10-4.34)	9.65 (6.20-22.61)	6.37
Aug	320	1.94 ± 0.20	1.57 (0.94-2.46)	7.19 (4.19-20.6)	11.25*

[a] Number of corn earworms moths tested. [b] Lethal concentrations, expressed in micrograms of insecticide per vial. [c] 95% confidence limits shown beneath each LC_{50} and LC_{90}. * Significant.

Table 5. Toxicity of zeta-cypermethrin to corn earworm moths in a bioassay conducted at Lariat and Hub, Texas in 2003.

Generation	n[a]	Slope ± SE	LC_{50}[b] (95% CI)[c]	LC_{90}[b] (95% CI)[c]	χ^2
			Lariat		
June	320	2.75 ± 1.03	2.11 (0.00-4.50)	6.18 (3.08-235)	24.42*
July	320	1.65 ± 0.27	1.1 (0.58-1.67)	6.51 (4.31-12.2)	4
Aug	320	2.29 ± 0.42	1.03 (0.63-1.4)	3.74 (2.75-6.22)	3.99
			Hub		
June	320	2.48 ± 0.31	1.93 (1.00-3.06)	6.31 (3.86-17.2)	10.92
July	320	1.95 ± 0.42	2.07 (1.02-3.04)	9.38 (6.35-19.34)	2.54
Aug	320	1.98 ± 0.21	1.20 (0.96-1.51)	5.34 (3.82-8.65)	3.44

[a] Number of corn earworms moths tested. [b] Lethal concentrations, expressed in micrograms of insecticide per vial. [c] 95% confidence limits shown beneath each LC_{50} and LC_{90}. * Significant.

DISCUSSION

Adult moth populations of *Helicoverpa zea* collected on the Texas-New Mexico border, a major green bean producing area, were subjected to adult vial tests to estimate the susceptibility levels to different insecticide chemistries, because of the intensive and continuous use of pesticides in these areas. Moths collected from pheromone traps over the course of the growing season showed variations in the levels of susceptibility. In both years' study, the susceptibility levels tended to decrease (though not always significantly) in the mid-season, followed by an increase later in the season. The decline in adult susceptibility over the season is, to some extent, due to the in-season insecticide use in both green beans and cotton. The increased application of insecticides in cotton and corn, in addition to green beans in this area during middle of summer, could be one reason for an increase in LC values from 0.63 µg/vial in July to 0.97 µg/via1 in August for bifenthrin during July and August. Conversely, exposure of adults to transgenic crops with *Bacillus thuringiensis* in the surrounding areas may be one reason for increased tolerance to insecticides. A study in *Helicoverpa armigera* has shown that the exposure of bollworm to sub lethal doses of *B. thuringiensis* produced a decline in tolerance to pyrethroids (Wang et al., 1994 and Tan et al., 1998). However, metabolic and non-metabolic resistance, cytochrome P450 dependent metabolism, and synthetic insecticides cannot be discounted as mechanisms for conferring resistance (Samir et al., 1993; Cohen et al., 1992; Wang and Hobbs 1995). Previous studies on *Helicoverpa armigera* indicated esterase and monooxygenase activity in resistance mechanisms (Gunning 1994 and Kranthi 1997). Also, research showed that the *H. zea* exposed to allelochemicals had decreased susceptibility to alpha-cypermethrin due to detoxification by a P450 mediated

response (Li et al., 2000). Rose et al. (1992) identified an allelochemical-resistant strain of *H. virescens* with 2.0 to 2.5 fold resistance to quercetin, a flavonoid present in its host plants, exhibited similar levels of elevated resistance to the organophosphate methyl parathion, the carbamate methomyl, and the pyrethroid fenvalerate.

One key factor responsible for the change in the susceptible levels of the adults over the season is the constant mixing of the populations between areas. Polyphagy and migratory nature of this pest (Fitt 1989; Dent 1991) resulted in the immigration of moths with varying levels of tolerance into the region in the early part of the season, followed by a decrease due to the mating with the local populations. Although migration, transgenic crops, and alternative hosts were not investigated in this study, these factors warrant further study to determine their roles as a means of altering susceptibility levels in this pest. *H. zea*, a major pest of concern in most of the field crops today, has a broad host range and strong mobility to extend from southern to northern areas of the United States. The frequent movement of the adult moths between locations and fields is a major influence on this study.

A study conducted in 2004 across the entire state of Texas showed that the tolerance levels were relatively high in South Texas (Pietrantonio et al., 2004). The populations survived insecticidal concentrations of 30-60 µg/vial. Thus, given the migratory nature of this pest, this study highlights the possibility of population mixing in North Texas, resulting in lowered susceptibility in the beginning of the season. This migration plays a key role in carrying the resistant or susceptible alleles. Over the season, these moths mix with the other individuals resulting in dilution of the resistant alleles if they exist at all. The homozygous resistant adults mate with susceptible adults, thereby producing heterozygote offspring, which show tolerance. Discriminating doses should be based on this knowledge, so a more precise monitoring system can be developed. This would lead to more appropriate control strategies for this pest.

The frequency of the nerve insensitivity gene is expected to increase in the field populations if selection pressure to pyrethroid is continued (Kranthi et al., 2001). Since this mechanism is the most difficult to eliminate, thus appropriate management strategies need to be devised to further reduce selection pressure otherwise pyrethroid resistance may become more unmanageable in the future. A study conducted in Australia showed that the reduction in the pyrethroid selection pressure resulted in a shift in pyrethroid resistance mechanisms from nerve insensitivity to oxidative metabolism (Forrester et al., 1993). Hence, reduction of selection pressure could play a key role in diluting the contribution of nerve insensitivity to pyrethroid resistance.

Thus, it is highly advisable to avoid the use of pyrethroids against the first few generations and restrict their use to later generations of bollworm to prevent the potentially worsening resistance problem. Growers in the Texas high plains have already started shifting to zeta-cypermethrin (Porter, personal communication) due to lower efficacy of bifenthrin in 2001 and 2002. It is recommended to switch to another class of insecticides in the late season. These results need further investigation both at molecular and cellular levels to better understand the biochemical mechanisms (high esterase, nerve insensitivity, gene frequency, and cytochrome P450 monoxygenases) involved in tolerance development. Also, differences in the susceptibility levels between locations indicate the need for more frequent resistance monitoring at multiple locations. Extension entomologists, along with the processing industries and chemical industries are closely monitoring insecticide resistance to avoid control failures of this pest. The study indicated that bollworm control in green beans is not under direct threat, but needs the

grower's attention and cooperation to keep the insecticide selection pressures low by adjusting the timing and frequency of applications.

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The Texas Journal of Agriculture and Natural Resource 25:13-23 (2012)

Effect of the Easiflo Cottonseed Processing Method on Recovery of *Xanthomonas axonopodis* pv. *malvacearum*

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ABSTRACT

Laboratory studies were conducted to compare the effects of acid delinting and Easiflo treated cottonseed on the survival of *Xanthomonas axonopodis* pv. *malvacearum* (*Xam*), causal agent of bacterial blight of cotton. Symptomatic bolls of the susceptible cultivars Paymaster 2326 RR and/or All-Tex Xpress RR were sampled from plots artificially inoculated with *Xam*. In 2007, seed of Deltapine 164B2RF, were obtained from a field naturally infested with *Xam*. Seeds from each cultivar were either mechanically delinted and treated with the Easiflo coating or subjected to acid delinting and placed on potato carrot agar. Resulting yellow, mucoid colonies characteristic of *Xam* were tested for pathogenicity on susceptible cotton seedlings. In 2006, seed from artificially inoculated bolls, receiving the Easiflo treatment had a greater frequency of *Xam* (2.7%), than seed receiving the acid treatment (0%). No differences in *Xam* isolation frequency were observed between mechanically delinted seed treated with Easiflo and acid delinted seed, when bolls were naturally infested with *Xam*. Overall, neither method completely eradicated *Xam* from the seed, which may serve as initial inoculum in the development of bacterial blight in the field.

KEY WORDS: *Xanthomonas axonopodis* pv. *malvacearum*, angular leaf spot, bacterial blight, black arm, seed borne

INTRODUCTION

Bacterial blight, caused by *Xanthomonas axonopodis* pv. *malvacearum* (*Xam*) (synonym = *Xanthomonas campestris* pv. *malvacearum* (Smith) Dye) (Vauterin et al., 2000) is an important disease of cotton (*Gossypium hirsutum* L.) that causes economic damage throughout the world (Hillocks 1992). The bacterium is capable of infecting

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cotton at all stages of growth. Symptoms such as seedling blight, angular leaf spot (Figure 1), black arm (Figure 2), and boll rot (Figure 3) are associated with specific developmental stages (Verma et al., 1986). The disease was first reported in the United States in 1891 (Atkinson 1891). Bacterial blight losses in the United States are typically below 1% (Blasingame 2000). However, yield losses of 10 to 50% have been reported (Thaxton and El-Zik 2001; Verma 1986). Although sporadic, severe Bacterial blight epidemics have been observed on the Southern High Plains of Texas (Boman, personal observation).

Bacterial blight management is achieved through the use of resistant cultivars (Bayles and Verhalen 2007), and cultural methods such as sanitation and acid-delinting of seeds (Bain 1939). The initial development of resistant cultivars was slow due to the complex race structure of *Xam.* At present, 19 races of *Xam* have been identified (Ruano and Mohan 1982), with race 18 being most prevalent (Allen and West 1991; Hussian 1984; Thaxton et al., 2001; Verma and Singh 1975). *Gossypium. hirsustum* lines immune to bacterial blight have been identified (Bird 1960; Bird 1962); however, a large number of commercial cultivars currently being grown are susceptible to infection (Nichols et al., 2007; Sagaram et al., 2003; Thaxton et al., 2001; Wheeler et al., 2007).

The bacterium can survive in the field on debris from previously harvested crops. However, initial inoculum can also be seed borne (Mohan 1983). Viable propagules of *Xam* can be recovered from cottonseed for periods of more than two years when stored at 5^{o}C (Mehta et al., 2005). Studies have reported that seed infection rates as low as 2% can lead to destructive epidemics within a field (Brinkerhoff and Hunter 1963). The use of fungicides such as mancozeb and copper oxychloride were previously evaluated to aid in the elimination *Xam* from cottonseed. However, the results were inconclusive and cost prohibitive (Jeyachandran and Shanmugan 1979). Currently, seed companies in the U. S. acid delint gin run cottonseed to remove remaining lint on the seeds in preparation for planting. The process of acid delinting was first used for disease control in 1911 (Gregory et al., 1999).

Figure 1. Characteristic foliar symptoms of bacterial blight of cotton. Note appearance of angular lesions on the lower leaf surface.

Figure 2. Systemic infection of *Xanthomonas axonopodis* pv. *malvacearum* resulting in black arm symptoms on cotton.

Figure 3. Characteristic boll rot symptoms caused by *Xanthomonas axonopodis* pv. *malvacearum*. Note the water-soaked appearance of the boll.

Acid delinting is the process of exposing gin run cottonseed to a wet sulfuric acid (H_2SO_4), or a gaseous hydrochloric acid (HCl) solution (Cherry and Leffler 1984; Delouche 1986). This process facilitates the removal of low quality and less dense seed, and allows the seeds to be more uniformly coated with seed treatments. In addition, this process aids in the flowability of the seeds through air and vacuum planters, and is believed to kill most microorganisms found on the seed surface (Bain 1939). However, acid delinting uses large quantities of concentrated acid (Fink 1964) and is considered a source of environmental concern (Gregory et al., 1999). Concerns due to worker protection standards and hazardous waste disposal have resulted in an increased interest

in safer processing methods. One such system is the Easiflo cottonseed processing system.

Easiflo coated cottonseed was first designed as an alternative to fuzzy cottonseed feed for better handling, storage, and shipping characteristics (Laird et al., 1997). The Easiflo seed coating process is a combination of mechanical delinting and the addition of a polymer-based coating (Olivier 2005). Studies have reported that varying rates of corn starch have little to no effect on germination (Williams et al., 1999). Studies evaluating the field performance of Easiflo treated cottonseed have been conducted. Olivier (2005) determined that seedling emergence was reduced with the Easiflo system than with acid delinted seeds. However, final stands for the two treatments were similar (McMichael et al., 2004). Since the Easiflo seed processing system circumvents the use of acid, there is an increased potential for a resurgence of seed borne pathogens, such as *Xam*.

The objective of this research was to determine the survival of *Xam* on cottonseed treated with the Easiflo seed processing system compared to the more standard acid delinting procedure.

MATERIALS AND METHODS

Field Parameter. The glyphosate-tolerant cotton cultivars 'All-Tex Xpress RR' and 'Paymaster 2326 RR' were chosen because of their varying levels of genetic resistance to bacterial blight. All-Tex Xpress RR is considered a moderately susceptible cultivar; whereas, Paymaster 2336RR is highly susceptible (Sagaram et al., 2003). Field trials were conducted at the Texas AgriLife Research Station at Halfway, TX, in 2006 and 2007. The field had no history of bacterial blight in the previous three years. Plots were four rows wide by the length of the field (~0.2 ha per cultivar). Plots were sprayed with a pathogenic *Xam*, race 18 isolate at a rate of 1×10^6 colony forming units/ml (CFU). The organosilicone surfactant Silwet L77 (0.2% v:v, Helena Chemical Co., Collierville, TN) was added to the solution and 470 L of solution applied/ha (Wheeler et al., 2007). This procedure was developed for leaf symptom development, and was not optimized for boll infection. Applications of *Xam* were made on 10-July, 19-July, 4-Aug., and 18-Aug. in 2006. In 2007, applications were made on 20-July and 10-Aug. However, there was a problem with tank contamination on the first application resulting in lack of symptom development. To obtain infected seeds in 2007, bolls were injected with *Xam* at a concentration of 1×10^6, 1×10^7, and 1×10^8 CFU/mL. A syringe (Becton, Dickinson and Co. 1 mL Tuberculin Slip Tip, Franklin Lakes, NJ) was used to inject the bolls with 0.2 mL of each *Xam* concentration. Six hundred bolls were injected with the 1×10^7 concentration on 30-Aug., while 200 bolls were injected with the 1×10^6 and 1×10^8 on 5-Sept. Following the applications of *Xam* inoculum, bolls were tagged and subsequent selections were made on the basis of boll infection. In 2007, cotton lint and seeds were bulk harvested from a production field near Midkiff, TX, in which a natural bacterial blight epidemic was observed. The susceptible cultivar 'Deltapine 164 B2RF' was planted at this location. With 2007 being the only growing season that this cultivar was utilized, two separate seed lots (n = 1,000) were tested to determine the reliability of the results. Tagged bolls were hand harvested on November 18 and November 7 in 2006 and 2007, respectively. Boll samples were stored at ambient temperature in paper bags in the laboratory prior to processing on a 10-saw laboratory gin to obtain the fuzzy seeds. Seeds from the multiple sampling dates were combined in order to have enough seed to

administer each treatment. Fuzzy seeds for each cultivar were divided into two separate portions (approximately 454 g), and were acid delinted, or mechanically delinted followed by the application of Easiflo.

Seed Preparation. The acid delinted seeds were processed at the Bayer CropScience laboratory in Idalou, TX. The process involved placing the fuzzy, gin-run cottonseed in acid-resistant mesh bags, and then coating the seeds with a dilute 19% H_2SO_4 solution. The wetted seeds were then placed in a tumbling dryer for approximately 40 min at a temperature of 60°C. The delinted seeds were exposed to sodium carbonate (Na_2CO_3) to neutralize the remaining H_2SO_4 on the seed coat. Seeds were placed back into the tumbling dryer and dried at 49°C until the seeds obtained a suitable moisture level (5-10%).

The Easiflo treatment on the cottonseed was applied at the Seed Physiology Laboratory at Texas Tech University in Lubbock, TX. The fuzzy, gin-run cottonseed were placed in a proprietary mechanical delinter (Cotton Incorporated, Cary, NC) until >90% of the fuzz was removed from the body of the seeds. For the Easiflo process to be efficient, it is desirable to have the only remaining fuzz on the micropilar and chalazal caps of the cottonseed. After the seeds were mechanically delinted, the seeds were weighed and a talc, corn starch, and water mixture (2.0%, 0.5%, and 5.0% by weight, respectively) was applied to the seed using a modified Hege seed treater. Seeds were dried for approximately five minutes using a forced air blower maintained at 40°C.

Bacterial Isolation. To test individual seeds for *Xam* infection, each seed was placed on potato carrot dextrose agar with peptone and yeast extract (PCA) composed of $MgSO_4$ (0.3 g), $CaCO_3$ (0.2 g), technical grade agar (10.0 g), commercial grade potato dextrose agar (40.0 g), peptone (2.5 g), commercially canned carrot juice (15 mL), and yeast extract (0.5 g) per liter of distilled water. Bacterial colonies from individual seeds (900 seeds/treatment in 2006 and 1,000 seeds/treatment in 2007) were streak plated onto a fresh PCA plate. Bacterial colonies from the streaked plates were serial diluted by placing a loop of bacteria into a vial filled with 10 mL sterile distilled water. The serial dilution process resulted in a 1×10^{-7} stock solution. A 0.1 mL aliquot of the stock solution was pipetted onto a PCA plate, spread over the plate using a sterilized glass rod and incubated at room temperature on a laboratory bench for 48 to 72 hours. Transfers from pure colonies were placed in a vial of permanent, freezable media consisting of tryptone (10 g), yeast extract (5 g), NaCl (0.5 g), K_2HPO_4 (6.3 g), KH_2PO_4 (1.8 g), sodium citrate (0.45 g), $MgSO_4\cdot7H_2O$ (0.09 g), $(NH_4)_2SO_4$ (0.09 g), glycerol (50.6 g) per liter of distilled water. Each vial was placed on a wrist action shaker (Burrell Scientific, Pittsburg, PA). Sub-samples of the bacterial isolates were streaked onto additional PCA plates for confirmation, and vials were frozen for storage at -18°C.

Pathogenicity Tests. Pathogenicity tests were conducted using the yellow, mucoid bacterial colonies characteristic of *Xam* (Bradbury 1986) recovered from field infested or inoculated seed. Cotton seedlings (Paymaster 2326RR) grown in flats and placed in a growth chamber (Percival Scientific Inc. Model AR-75L, Perry, IA) maintained at 27 ± 2°C. Following cotyledon emergence, the plants were removed from the growth chamber and divided into three replicates for each sample. This process was repeated for each location from both growing seasons. An autoclaved toothpick was used to scratch each cotyledon with a sample of each bacterial isolate. Following inoculation, the plants were

placed in a dew chamber (Percival Scientific Inc. Model I-35D2L, Perry, IA) maintained at 24°C and >90% RH for 24 hours and then transferred back into the growth chamber. After a two-week incubation period, plants were rated on the basis of symptom development using a qualitative scale, where a positive reaction was determined if angular, water-soaked lesions had developed away from the inoculation point; whereas, a negative reaction was characterized by discoloration of the inoculation point. All plant material was discarded after being rated.

Data Analysis. The experimental design was a binomial test consisting of an infected or non-infected proportion. Data were analyzed using a chi-squared analysis in the Mixed Procedure of SAS (v.9.1, SAS Institute, Inc., Cary, NC). A test of proportions ($\alpha = 0.05$) was utilized to test the infected proportion versus the non-infected proportion of a cultivar. Specifically, a proportions test performs two hypothesis tests of the difference between two binomial proportions. The output includes two types of hypothesis tests, one based on a normal approximation; whereas, the other is Fisher's exact test.

RESULTS AND DISCUSSION

Significant differences ($P \leq 0.05$) were observed between the acid and Easiflo treatments for Paymaster 2326RR in 2006 for all bacterial isolates obtained from yellow, mucoid colonies that were associated with the seeds, and for pathogenic *Xam* isolates (Table 1). Acid delinting eliminated all yellow, mucoid colonies from the seeds tested; whereas, colonies were recovered from 3.0% of the seed treated with Easiflo. The isolation frequency of *Xam* recovered from seed, as exhibited by a positive reaction in the pathogenicity test, was 2.7% (Table 1). In 2007, the isolation frequency of bacteria for the two treatments was similar for Paymaster 2326RR. However, a greater number (2.4%) of yellow, mucoid colonies were recovered from acid delinted seed when compared to Easiflo for All-Tex Xpress RR (Table 1). Likewise, there was a 2.0% increase in the number of *Xam* isolates identified from the pathogenicity test for acid delinted seed than Easiflo treated seed (Table 1). The bacteria were injected directly into the bolls for this set of seed, which may have bypassed the partial resistance exhibited by All-Tex Xpress RR to the disease (Sagaram et al., 2003). It is possible that the distribution of the bacteria was different within the seed for the two inoculation techniques. The leaf spray may have resulted in more of an external infestation of seed and the syringe inoculation may have caused more internal infection of seed. No differences in isolation frequency were observed between treatments for either of the Deltapine 164 B2RF seed lots obtained from the natural epidemic from 2007 (Table 1). The overall seed infection was low for the natural epidemic, where bolls were collected without regards to Bacterial blight symptoms.

Although sporadic in nature, severe bacterial blight epidemics can occur. In 2011, widespread occurrences of the disease were reported in Arkansas (Rothrock et al., 2012) and Mississippi (Allen 2012). Spray inoculations of *Xam* inoculum are effective at inciting temporary leaf symptoms and low percentages of boll rot (Wheeler et al., 2007). Adequate disease development was observed following spray inoculations in 2006. However, spray applications were unsuccessful in initiating disease in 2007 due to a chemical contamination in the spray tank. Therefore, bolls were artificially infected by injecting *Xam* concentrations with a hypodermic syringe. Overall, injecting bolls with *Xam* was detrimental to boll retention with 58.3 to 72.0% of the bolls abscising (data not

presented). As a result, inoculation rates were combined to provide a sufficient amount of seeds with which to administer treatments. Populations of *Xam* were generally greater when bolls were injected compared to spray inoculations. There is evidence that *Xam* can reside on the seed surface, or below the seed coat (Bain 1939; Brinkerhoff and Hunter 1963). Furthermore, acid delinting is capable of decreasing *Xam* on the seed surface; however, the overall effect on internal infections is unclear. It appears that internal seed infections resulted from the boll injections, as neither treatment with acid nor Easiflo affected the isolation frequency. In contrast, acid delinting had a significant effect on *Xam* isolation frequency from bolls collected from plots receiving spray inoculation. Although no differences between the treatments were observed in either of the seed lots from the natural epidemic, *Xam* populations were consistently greater for the Easiflo treated seed. With such low infection frequencies, it may have been necessary to isolate from more seed to obtain significant differences.

Based on this study, Easiflo treated seed does not appear to differ from acid delinting in negating the transmission of *Xam* in infested planting seed. The data from 2006 show that in a year with relatively unfavorable environmental conditions for disease development, acid delinting can reduce the likelihood of disease development (Table 1). This is due to the fact that the poor disease development conditions did not lead to a systemic infection that would infect the seed internally. Therefore, the pathogen affected the seed externally, and was removable with the use of acid delinting, but not the Easiflo process.

The data collected in 2007 shows drastic differences from those seen in 2006 (Table 1). This may be attributed to above average precipitation during the growing season in 2007. Observations from the West Texas Mesonet Plainview Station (www.mesonet.ttu.edu/mesonet-precipitation.htm) show that rainfall received during 2006 was similar to the 10-year average; whereas, rainfall amounts in 2007 were 48% above the long-term average. The acid delinted portion of all three of the cultivars tested resulted in greater infection rates than those experienced in 2006, and in one case had a greater infection rate than the Easiflo treated seed of the same cultivar (All-Tex Xpress RR). With that said, the same conclusion concerning the Easiflo system can be made in that it does not eliminate transmission of *Xam* on infested cottonseed. While both treatments experienced greater infection rates in 2007, the results exhibited by the seed treatments in 2006 are evidence of the ability of the acid delinting process to remove external infestations while the Easiflo system was not able to remove external *Xam* either year, or probable internal infection that was encountered in 2007. Also, acid delinting did not appear to impact internal *Xam* infections, as the bacterium was likely protected within the seed.

Table 1. Effect of two cottonseed processing methods on isolation frequency of *Xanthomonas axonopodis* pv. *malvacearum* (*Xam*) from seed artificially or naturally infested in 2006 and 2007.

Year, cultivar	Yellow, mucoid colonies			Positive pathogenicity test (*Xam*)		
	Acid delinted	Easiflo treated	*P*-value	Acid delinted	Easiflo treated	*P*-value
	- - - - - - (%) - - - - - -			- - - - - - (%) - - - - - -		
2006[a] Paymaster 2326 RR	0.0	3.0	<0.0001	0.0	2.7	<0.0001
2007[b] Paymaster 2326 RR	2.2	1.8	NS	1.8	1.1	NS
2007 All-Tex Xpress RR	3.8	1.4	0.001	3.1	1.1	0.003
2007 Deltapine 164 B2RF (Lot 1)[c]	0.7	1.4	NS	0.6	0.9	NS
2007 Deltapine 164 B2RF (Lot 2) [c]	0.5	1.2	NS	0.4	1.0	NS

[a] A total of 900 seeds were tested for each treatment. Seeds were obtained from artificially infecting plants via foliar applications of *Xam* inoculum as described by Wheeler et al. (2007).
[b] A total of 1,000 seeds were tested for each treatment. Seeds were obtained from bolls injected with *Xam* concentrations.
[c] Two separate seed lots of Deltapine were tested from a naturally infested field to determine reproducibility.

CONCLUSIONS

Evaluations from these studies indicate that bacterial blight development in bolls differs greatly by the type of inoculation method used. While severe bacterial blight symptoms can be observed following natural infections, the sporadic nature of the disease often requires artificial inoculation to ensure disease development. Spray inoculation with *Xam* suspensions were effective at inciting bacterial blight on leaves, but have poor penetration within bolls. Furthermore, the sensitivity of *Xam* to tank contaminants experienced in this study necessitated the need for an additional inoculation method. Injections of *Xam* should result in more severe boll rot, as the bacterial inoculum is introduced directly to the developing boll. In these studies, injections with concentrations of *Xam* did result in adequate levels of boll rot development; however, the injections themselves reduced boll retention. Additional research in the different inoculation methods is warranted, to better understand the mechanism of boll infection by *Xam.*

Overall, results from the comparisons made between acid delinting and the Easiflo seed coating system were inconclusive. The use of Easiflo did not appear to differ from the acid delinting process as it relates to reducing *Xam* populations on infested seed. The initial low populations of the bacterium on seed from 2006 were completely removed via acid delinting. The use of the Easiflo system did not reduce *Xam* populations below levels that could result in field epidemics. When internal seed infection occurred under favorable environmental conditions, and/or boll injections with *Xam*, as was the case in 2007, neither acid delinting, nor treatment with Easiflo had any effect on isolation frequency of *Xam* from Paymaster 2326RR. Contradictory to what was observed in 2006 for Paymaster 2326RR, the isolation frequency of *Xam* from All-Tex Xpress RR was greater for acid delinted seed than for Easiflo-treated seed. Differences in reaction to the treatments between Paymaster 2326RR and All-Tex Xpress RR cultivars during the 2007 trial could be attributed to varying response between the cultivars to injections. This could potentially be explained by differences in boll size, carpel wall thickness, or sensitivity to breach in the carpel wall of the two cultivars. These factors could influence *Xam* development independently, thus differences in boll infection may have been observed. Although no observable differences between treatments for the two naturally infected Deltapine 164 B2RF seed lots were observed, isolation frequencies of *Xam* were numerically lower from seed treated with acid. Additional studies utilizing naturally infected seed with a larger number of observations need to be conducted.

Further research is warranted to better understand the effect of Easiflo on cottonseed infected with *Xam*. Another area of interest that should be examined is the possible contamination of Easiflo seed coating equipment by *Xam* infected seed during the Easiflo coating process. Additional studies are also necessary to examine the impact of Easiflo on other seed transmitted pathogens.

The current logic that acid delinting completely removes *Xam* from infected cottonseed appears to only apply to infestations of the seed coat by the bacterium. The development of an assay to test internal portions of the seed, such as the embryo, is needed to determine the extent of *Xam* infections. Strategies to minimize the spread of *Xam* must include sanitation and the use of high quality pathogen-free seed. Fields known to be infested with *Xam*, or exhibiting symptoms of bacterial blight should not be harvested for seed, nor should gin trash from such fields be land applied for disposal.

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The Texas Journal of Agriculture and Natural Resources 25:24-33 (2012)

Effects of Fat Content and Source on Consumption Time in Two-Year-Old Quarter Horses

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ABSTRACT

Twelve two-year-old quarter horses were used in this study to determine if the amount and source of fat supplement influenced consumption of grain. The experiment took place at Sam Houston State University's Equine Center in Huntsville, Texas. Horses were allotted into one of three treatment groups. All horses received 1.5% BW in hay and 1% BW in grain twice per day throughout the study. Treatment group one received no additional top-dressed fat to the diet and was considered the no added oil (NO) treatment group. Treatment group two received 0.099 kg of additional top-dressed soybean oil (SO) to the diet. Treatment group three received 0.099 kg of additional top-dressed fish oil (FO) to the diet. Elapsed time for horses to consume the treatment rations was recorded at each feeding from d1 to d21. Results of this study indicate that over the entire 21-day treatment period, horses in the SO and FO treatment groups took longer ($P < 0.001$) to consume their rations than horses in the NO treatment group. Further, SO and FO were consumed at a similar rate ($P > 0.05$) over the entire study. Similar trends were observed when comparing mean daily and weekly consumption times between treatments.

KEY WORDS: fish oil, soybean oil, quarter horse

INTRODUCTION

In recent years, fat supplementation has become common in the typical equine diet which is different from the early 1970s, where little fat was added. It has been determined that horses can digest fats very efficiently which is surprising since their natural diet is low in fat (Pilliner and Davies 2004).

Fats provide a source of essential fatty acids to the horse's diet. Essential fatty acids include linoleic acid (an omega-6 fatty acid) and alpha-linolenic acid (an omega-3 fatty acid). Adding fat in the diet may assist with a more efficient utilization of fat-soluble vitamins A, D, E, and K (Warren 2004).

Since the horse's total daily intake is limited, additional fat in the diet can result in a substantial increase in calories without the requirement of more feed. Due to the fact

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that fat contains more energy than carbohydrates and is available to horses, fat is an ideal supplement for increased gains in thin horses as well as maintaining body condition of older horses (Warren 2004).

Meeting the energy demands of working and growing horses may require large amounts of grain to be fed that is high in starch. Horses have limited capacity for starch digestion. The more starch that escapes duodenal digestion, the more that may be fermented in the hindgut which could potentially lead to a decrease in gut pH, disruption of normal microbial populations, and an increased risk for digestive disturbances. Replacing some of the grain with fat, which contains no starch, aids in minimizing the risk of digestive upsets. Adaptation to dietary fat may spare muscle glycogen by increasing the use of fatty acids as fuel and reducing the amount of liver and muscle glycogen used for energy needs (Warren 2004).

According to Warren (2004), fat supplementation also reduced the horse's thermal load. The lower heat load associated with feeding fat lessens the need for evaporative heat loss therefore reducing the water and electrolyte loss. Fat may favorably alter the horse's behavior. Warren (2004) found that feeding horses a high fat diet caused insulin production to be suppressed reducing the "sugar high." Furthermore, increasing dietary fat and decreasing dietary starch resulted in beneficial effects in horses with polysaccharide storage myopathy and recurrent exertional rhabdomyolysis (Valberg and McKenzie 2005). Likewise, Rich (2004) stated that feeding fat gave gloss to the hair coat and improved skin health.

Animal and vegetable-based fats are the major sources of fat available for equine consumption (Valberg and McKenzie 2005). Vegetable oils tend to be higher in unsaturated fatty acids than animal fats (Warren 2004). Vegetable oils are also highly digestible at 90% to 100% and are very energy dense. The most palatable and commonly used sources of these vegetable oils tend to be corn and soybean oil (Valberg and McKenzie 2005). These sources of fat are attractive to feed companies because they are readily available and are generally the most economical sources of fat.

While vegetable fats tend to be very digestible, animal fats vary in digestibility between 75% and 90% (Valberg and McKenzie 2005). Traditional animal sources of fat include beef tallow, lard, and fish oil (Warren 2004). Tallow and lard are no longer used in horse feeds due to the rise of bovine spongiform encephalopathy. In the past, problems with fish oil included low palatability due to impurities causing foul taste and smell. However, recent manufacturing methods have been somewhat successful in removing many of these impurities increasing palatability (Rich 2004).

While soy and corn oil are commonly used in the equine feeding industry, these products are highest in linoleic acids when compared to other fat sources. Fish oil and linseed oil, on the other hand, tend to be very high in linolenic acid (Rich 2004). Fish oil is also an excellent source of eicosapentaenoic acid and docosahexaenoic acid. Other benefits of fish oil supplementation include lower heart rates throughout exercise tests, lower packed cell volumes, and lower free fatty acids (O'Connor et al., 2004).

The primary omega-6 fatty acid is linoleic acid whereas the primary omega-3 fatty acid is alpha-linolenic acid (Rich 2004). Once in the body, these essential fatty acids are further metabolized to produce other fatty acids. Arachidonic acid is the predominant product of linoleic acid metabolism whereas eicosapentaenoic acid is the major product of linolenic metabolism. Arachidonic acid and eicosapentaenoic acid are both metabolized into eicosanoids which are potent regulators of vital body functions. Grain

products typically contain linoleic acid, whereas forage contains predominantly linolenic acid (Warren 2004).

Omega-6 fatty acids tend to increase blood clotting and increase inflammatory response. On the other hand, omega-3 fatty acids tend to decrease blood clotting and decrease inflammatory response. There is growing interest in omega-3 fatty acids as they are thought to possibly be useful in treating heaves, recurrent uveitis, and hives (Warren 2004). Omega-3 fatty acids may be useful in preventing or treating upper airway diseases, degenerative joint diseases, and laminitis (Warren 2004).

Natural feed ingredients generally contain less than 3% fat. However, the equine digestive tract can handle greater amounts of dietary fat when introduced gradually (Rich 2004). The ideal amount of fat supplementation has not been determined and likely differs based on the horse's intended use, amount of grain replacement desired, and the horse's energy expenditure. A horse can tolerate up to 20% of its diet as fat but 10% is generally a reasonable limit (Warren 2004).

The objective of this study is to determine the effects of fat source and amount on consumption time when feeding two-year-old quarter horses. There are many benefits of adding fat to the equine diet. The results of this study will provide insight into the differences in palatability, and therefore utility, of fats from vegetable versus animal sources.

MATERIAL AND METHODS

Twelve two-year-old quarter horses were used to determine consumption time when fed different sources and amounts of fat in their diets. All treatment groups were blocked by sex and location in the barn. Prior to arriving to the barn, the horses were maintained in a natural pasture setting together as a uniform group. Genetics among horses were similar and all were born within three months of each other. Upon arrival to the research facility, all horses were weighed, dewormed with a common commercial anthelmintic, and randomly placed in 3.048 m x 4.267 m box stalls where they were housed for the remainder of the experiment. Stalls were bedded with pine wood shavings with access to clean, fresh water and salt at all times. Throughout the experiment, horses were exercised daily which typically consisted of 30 minutes to an hour of riding or lunging.

Upon initiation of the study, horses were divided into three treatment groups. Each treatment group received approximately 1% BW of a commercially produced, textured, sweet feed referred to as grain and 1.5% BW of Coastal bermudagrass hay (*Cynodon dactylon)* per day. All horses were fed grain and hay twice daily. Treatment group 1 was considered the control group and therefore no additional oil was added to the grain ration. This group is identified as the no oil (NO) treatment group. Treatment group 2 received an additional 0.099 kg of soybean oil every feeding as a top-dress to the grain ration. This group was identified as the soybean oil (SO) treatment group. Treatment group 3 received an additional 0.099 kg of fish oil every feeding as a top-dress to the grain ration. This group was identified as the fish oil (FO) treatment group. Rations for treatments 2 and 3 were balanced to provide approximately 8.5% total fat in the diet from the grain/oil top-dress source. The soybean oil used in the experiment as a top-dress had no additional flavor enhancer. The fish oil was flavor-enhanced by the manufacturer to aid in consumption. Nutrient analysis of grain, hay, SO, and FO are listed in Table 1 as analyzed by Dairy One Forage Testing Laboratory (Ithaca, New York).

Table 1. Nutrient Analysis of Grain, Hay, FO and SO.[a]

Item	Grain	Hay	SO	FO
DM, %	90.7	95	0.1	0.1
CP, %	15.1	9.9	-	-
CF, %	5.8	1.9	100	99
ADF, %	8.6	39.2	-	-
NDF, %	0.18	72.8	-	-
Ca, %	0.01	0	-	-
P, %	0.01	0	-	-
DE, Mcal/Kg	3.48	1.83	15.16	12.15

[a] Dry Matter Basis.

Table 2 illustrates the amount of fat provided by each treatment group. Treatment 1 contained 3.45% total fat in the diet compared to treatments 2 and 3 which contained approximately 4.54% total fat in the diet. Treatment 1 received approximately 5.78% of fat from the grain, whereas treatments 2 and 3 received approximately 8.52% from the grain and oil. Typical high fat grain rations that are provided to horses contain a minimum 7% crude fat. Diets were formulated and adjusted so that the top-dress treatments were above this value and the control treatment was below this value. Warren (2004) stated that horses can receive up to 20% total fat in the diet, therefore total fat content in diets were believed to not affect consumption.

Table 2. Amount of Fat Consumed per Day by Treatment.[a]

	NO	SO	FO
Grain, kg	0.21	0.21	0.21
Hay, kg	0.103	0.103	0.103
SO, kg	0	0.099	0
FO, kg	0	0	0.099
Total Fat Consumed, kg	0.313	0.412	0.412
Fat Consumed from Grain + Oil, %	5.78	8.52	8.52
Fat Consumed in Total Diet, %	3.45	4.54	4.54

[a] Dry Matter Basis.

Table 3 illustrates the Daily Digestible Energy Nutrient Requirements for horses at 24 months of age (National Research Council 2007). This table is based on horses that will have a mature body weight of 500 kg. The table indicates the different digestible energy (DE) requirements for horses 24 months of age under maintenance, light, moderate, heavy, and very heavy exercise.

Table 4 reports the daily consumption of DE each horse received in each treatment group. Total amount of DE consumed daily for each treatment group was: NO = 22.6 Mcal, SO = 24.10 Mcal, and FO = 23.82 Mcal. Referring back to values in Table 3, all treatment groups received a level of digestible energy that met the requirements for a 24-month-old horse receiving light to moderate exercise.

Table 3. NRC Daily Digestible Energy Requirements for Horses 24 Months of Age.[a]

Type	DE, Mcal
Maintenance	18.7
Light Exercise	21.8
Moderate Exercise	24.8
Heavy Exercise	27.9
Very Heavy Exercise	32.5

[a] Mature Body Weight of 500kg.

Table 4. Daily Consumption of Digestible Energy on a Dry Matter Basis.

Source (Mcal/d)	NO	SO	FO
Grain	12.64	12.64	12.64
Hay	9.96	9.96	9.96
Fish Oil	-	-	1.20
Soybean Oil	-	1.50	-
Total	22.6	24.10	23.80

The horses were fed at 07:00 and 18:30 hours every day throughout the trial. After feeding, horses were monitored to determine length of time necessary to consume each treatment diet. Times were recorded up to 300 minutes. After 300 minutes elapsed, horses were no longer observed and received a 300 for consumption time. The statistical analysis was conducted using SPSS (2009) one-way ANOVA to determine differences in consumption rates.

RESULTS

All horses remained healthy and vigorous throughout the study and showed no signs of colic. Initial and ending weights are reported in Table 5. All weights were similar ($P > 0.05$) among treatment groups at the beginning and end of the trial.

Table 5. Mean Initial and Ending Weights of Horses by Treatment, kg.

Weight	NO	SO	FO	P-value
Initial	398.70	373.80	389.60	0.145
Ending	391.00	375.60	387.40	0.334

In Table 6, mean consumption time over the entire trial by treatment group is reported. The NO group consumed the entire diet in a shorter period of time ($P < 0.001$) than the SO and FO treatment groups. The FO and SO treatments were similar ($P > 0.05$) in consumption time throughout the entire study.

Mean consumption times of NO, SO, and FO from weeks 1, 2, and 3 are reported in Table 7. Consumption time for NO was similar ($P > 0.05$) for weeks 1 and 2, but consumption time increased and was significantly different ($P < 0.001$) in week 3.

Consumption time for SO and FO increased significantly from week 1 to 2 ($P < 0.001$) and from week 2 to 3 ($P < 0.001$).

Table 6. Overall Mean Consumption Time for NO, SO, and FO Diets, Minutes.

Days	NO	SO	FO	P-value
21	36.70[a]	152.90[b]	161.4[b]	< 0.001

[a, b] means in the same row without a common superscript are significantly different.

Table 7. Weekly Mean Consumption Times within Treatment for NO, SO, and FO Diets, Minutes.

Week	NO	SO	FO
1	21.43[a]	29.00[a]	38.02[a]
2	19.88[a]	129.61[b]	150.61[b]
3	68.82[b]	300.00[c]	295.61[c]
P-value	< 0.001	< 0.001	< 0.001

[a,b,c] means in the same column without a common superscript are significantly different.

Weekly mean consumption times for all treatments are reported in Table 8. Mean consumption times were compared across treatments. In week 1, consumption time for NO was significantly shorter ($P = 0.015$) than that of FO. Consumption time for SO was similar to the other two treatments. In week 2, NO was consumed significantly faster ($P < 0.001$) than SO and FO, but no difference existed in consumption time of SO and FO. In week 3, NO was again consumed significantly faster ($P < 0.002$) than SO and FO, and SO and FO were consumed at a similar rate.

Table 8. Weekly Mean Consumption Times between Treatments for NO, SO, and FO Diets, Minutes.

Week	NO	SO	FO	P-value
1	21.43[a]	29.00[a,b]	38.02[b]	0.015
2	19.88[a]	129.61[b]	150.61[b]	< 0.001
3	68.82[a]	300.00[b]	295.61[b]	< 0.002

[a,b] means in the same row without a common superscript are significantly different.

Daily mean consumption time for NO, SO, and FO diets are reported in Table 9. Consumption time for the NO diet was similar ($P = 0.239$) for d 1 to d 21. From d 1 to 9, SO consumption time was similar ($P > 0.05$). Consumption time at d 10 significantly increased ($P < 0.001$) through d 14 for SO. From d 14 to 21, consumption time for SO was 300 minutes. For FO, consumption time was similar ($P > 0.05$) for d 1 to 8. Consumption time at d 9 significantly increased ($P < 0.001$) through d 14 for FO. At d 14, consumption time for FO was 284 minutes or greater. Consumption time for FO from d 14 to 21 was similar ($P > 0.05$).

The daily mean consumption time for NO, SO, and FO is reported in Table 10. Consumption time for NO, SO, and FO are similar from d 1 to 7. However, NO was consumed faster ($P = 0.006$) than FO on d 8. Also, consumption time on d 8 for SO was

intermediate and similar (P > 0.05) to both NO and FO. On d 9 and 10, all diets were consumed at a similar rate (P > 0.05). On d 11, NO was consumed faster than FO (P = 0.009), but SO was consumed at a similar rate to both NO and FO (P > 0.05). On d 12, NO was consumed faster (P = 0.016) than SO and FO. From d 13 to 21, NO was again consumed faster (P < 0.001) than SO and FO.

Table 9. Daily Mean Consumption Times within Treatment for NO, SO, and FO Diets, Minutes.

Day	NO	SO	FO
1	34.75	46.25^{a,b}	66.63^{b}
2	19.38	38.75^{a,b}	61.25^{a,b}
3	18.00	27.88^{a}	22.50^{a}
4	18.63	22.50^{a}	39.13^{a,b}
5	20.50	21.75^{a}	27.63^{a,b}
6	19.63	23.13^{a}	24.25^{a}
7	19.13	22.75^{a}	24.25^{a}
8	18.75	22.88^{a}	27.75^{a,b}
9	20.75	37.75^{a,b}	65.00^{b}
10	20.88	71.63^{b,c}	42.88^{a,b}
11	17.25	103.75^{c}	198.38^{c,d}
12	17.75	160.13^{d}	186.25^{c}
13	19.75	211.13^{e}	239.50^{d}
14	24.00	300.00^{f}	294.50^{e}
15	55.38	300.00^{f}	300.00^{e}
16	71.75	300.00^{f}	300.00^{e}
17	73.88	300.00^{f}	284.50^{d,e}
18	56.00	300.00^{f}	300.00^{e}
19	79.25	300.00^{f}	300.00^{e}
20	74.38	300.00^{f}	294.38^{e}
21	71.13	300.00^{f}	290.38^{e}
P-value	0.239	< 0.001	< 0.001

^{a,b,c,d,e,f} means in the same column without a common superscript are significantly different.

Table 10. Daily Mean Consumption Times between Treatments for NO, SO, and FO Diets, Minutes.

Day	NO	SO	FO	P-value
1	34.75	46.25	66.63	0.332
2	19.38	38.75	61.25	0.371
3	18	27.88	22.5	0.173
4	18.63	22.5	39.13	0.246
5	20.5	21.75	27.63	0.181
6	19.63	23.13	24.25	0.178
7	19.13	22.75	24.25	0.104
8	18.75^{a}	$22.88^{a,b}$	27.75^{b}	0.006
9	20.75	37.75	65	0.073
10	20.88	71.63	42.88	0.207
11	17.25^{a}	$103.75^{a,b}$	198.38^{b}	0.009
12	17.75^{a}	160.13^{b}	186.25^{b}	0.016
13	19.75^{a}	211.13^{b}	239.50^{b}	< 0.001
14	24.00^{a}	300.00^{b}	294.50^{b}	< 0.001
15	55.38^{a}	300.00^{b}	300.00^{b}	< 0.001
16	71.75^{a}	300.00^{b}	300.00^{b}	< 0.001
17	73.88^{a}	300.00^{b}	284.50^{b}	< 0.001
18	56.00^{a}	300.00^{b}	300.00^{b}	< 0.001
19	79.25^{a}	300.00^{b}	300.00^{b}	< 0.001
20	74.38^{a}	300.00^{b}	294.38^{b}	< 0.001
21	71.13^{a}	300.00^{b}	290.38^{b}	< 0.001

^{a,b} means in the same row without a common superscript are significantly different.

DISCUSSION

Warren (2004) stated that horses can consume up to 20% fat in the diet with 10% being ideal. In this study, the percentage of fat in the total diet was between 3.45% and 5.45%. Therefore, rate of consumption should not have been affected due to the amount of fat in the diet.

All horses remained healthy with no signs of illness or colic throughout the duration of the study. The initial and ending weights of horses were similar ($P > 0.05$). However, mean weights from the beginning to the end of trial showed trends of minor weight loss. This may have been due to the daily exercise that the horses were receiving. Upon initiation of the study, digestible energy amounts from all feed sources were analyzed to determine the amount of digestible energy each treatment provided so that consumption of excessive energy would not affect ration consumption. According to the National Research Council (2007), the digestible energy the horses were consuming was

within the digestible energy requirements for a 24-month-old horse in light to moderate training.

Results of this study indicate that two-year-old quarter horses consuming a typical grain ration without an oil top-dress will consume the grain ration in approximately 30 minutes. When soy or fish oil is top-dressed on the grain ration, consumption time will increase to approximately 2.5 hours. Rich (2004) stated that vegetable sources of fat tend to be more palatable to horses than animal sources. She further stated that most vegetable sources including soy oil were quite palatable. This study disagrees with Rich (2004) due to the significant increase in time that the soy oil top-dressed ration was consumed when compared to the control ration. This study further disagrees with the statement from Rich (2004) that vegetable sources were more palatable than animal sources since the study indicates that the soy oil top-dressed ration was consumed similarly to the fish oil top-dressed ration over the entirety of the trial. However, it is important to note that consumption of soy oil and fish oil may have been affected by the flavor enhancer added by the manufacturer to the fish oil. Soy oil was not flavor-enhanced.

When considering weekly consumption of diets, the NO diet was consumed in approximately 20 minutes for the first two weeks. However, in week 3, the diet was consumed in approximately 68 minutes which was significantly longer than the first two weeks. An explanation for this is unknown. Assumptions can be made that the feed may have been from a different batch that was manufactured from the feed company causing palatability issues. When considering SO and FO, both treatment group consumption times increased drastically from week 1 to week 2, and week 3. Assumptions can be made that the increased oil included in the grain rations caused a decrease in consumption, again disagreeing with Rich (2004).

When comparing the treatment groups within each week, SO was consumed in approximately 30 minutes and was similar in consumption time to both NO and FO which were consumed in 21 and 38 minutes, respectively. It is interesting to note that all diets in the first week were consumed in less than 40 minutes. When comparing diets in weeks 2 and 3, results are different. SO and FO in weeks 2 and 3 were consumed at a similar rate. In week 2, they were consumed in approximately 2 to 2.5 hours whereas NO was consumed in approximately 20 minutes. In week 3, SO and FO were consumed in 5 hours indicating that the horses at this point were refusing to consume the entire grain source and oil top-dress provided to them. In week 3, NO consumption time was approximately 68 minutes which was a much longer consumption time when compared to the first two weeks of NO, but was still significantly lower than SO and FO in week 3. Conclusions from the increased consumption time can include a change in feed ration makeup and/or lack of palatability of oil top-dressed rations.

Daily consumption times of NO from d 1 to d 21 were not different. However, referring back to the weekly analysis, there were significant differences between weeks 1 and 2 when compared to week 3. When observing mean values over the 21-d period, mean consumption times tended to be low from d 1 to d 14. From d 16 to d 21, mean values appear higher. Therefore, it can be concluded that day means may have not had enough observations to show significant differences. When evaluating SO and FO consumption rate from d 1 to d 21, consumption times increased significantly. When comparing NO, SO, and FO within day of treatment, no differences existed between treatments from d 1 to d 7. From d 12 to d 21, NO consumption time was much lower

than that of SO and FO. Consumption times for SO and FO eventually reached five hours or more.

According to a study completed by Hayes and Kouba (2007), horses receiving flavored fish oil diets tended to have a lower consumption time between d 4 and d 7 whereas 91.6% of horses had been consuming all grain on d 3. Similar to the results in the Hayes and Kouba (2007) study, a lower consumption time was documented by d 13 in this study. Feeding FO with an added flavor, as in the previous mentioned study by Hayes and Kouba (2007), could be a potential method to increase the consumption time and palatability of fish oil in the diet. Rich (2004) stated that FO had a lower palatability due to impurities causing a foul taste and smell when compared to NO and SO. This could have affected the horses consumption time. However, horses in this study on the SO treatment had a significant increase in consumption time as well. The SO and FO groups were also similar in that they both had an increase in consumption time over the duration of the experiment. A possible method in reducing the odor of the fish oil could be to clean feeders daily in order to reduce the odor of fish oil from the previous day(s) or by feeding a grain source that will help to minimize the taste such as a textured sweet feed. Conclusions from this study are that adding oil to a typical grain ration increases consumption time, but soy oil and fish oil added to a grain ration are consumed similarly. Further research needs to be conducted with longer feeding periods, different grain sources, texture of the fat (oil vs. dry), flavoring, and percent of fat in diet to determine which factors have the most influence on consumption of fat supplements.

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The Texas Journal of Agriculture and Natural Resources 25:34-45 (2012)

The Impact of Income Tax Laws on the Sales of Livestock During Periods of Severe Drought

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ABSTRACT

Livestock producers were forced to sell their cattle and other livestock due to the drought conditions in 2008. These involuntary sales created significant income tax liabilities for the producers. However, in certain situations the producer can take advantage of relief provisions in the Internal Revenue Code which were enacted to reduce or eliminate these potential negative tax consequences. Some involuntary sales may qualify for tax deferral for at least one year and potentially a much longer period. Whether these tax provisions will provide any benefit to a producer depends on a detailed analysis of the specific circumstances of each case. This paper examines in detail the relevant tax laws including the Internal Revenue Code, Treasury Regulations, Internal Revenue Service rulings, and case law to determine whether there are ambiguities in the relevant laws that, when compared to other tax laws with similar purposes, could cause uncertainty in the application of these laws to common situations. The paper also sets forth the issues that should be considered when determining whether these tax laws will provide any benefit to a particular livestock producer. Based on a review of the existing laws and a comparison with other similar provisions, there are at least a few items that need clarification in order to provide the same level of clarity and certainty that exists with other provisions of tax law.

KEY WORDS: livestock, tax deferral, drought, gain, taxation

INTRODUCTION

The current drought throughout much of the United States has caused farmers and ranchers to liquidate their herds in record numbers. According to the United States Department of Agriculture (USDA), the 90.8 million head of cattle in the U.S. as of January 2012 was 2% less than the 92.7 million inventory in January 2011. The national inventory decreased even further to 89.3 million in January 2013. In total, the cattle inventory has decreased 4% since the beginning of 2011, and the inventory in January 2013 is the lowest it has been since 1952 (NASS 2013). Oklahoma State University's Division of Agricultural Sciences and Natural Resources reported that feeder cattle sales

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in Oklahoma during July 2011, were up more than 50% from the same period in 2010 and cow and bull sales were up more than 200% (Oklahoma State University 2011).

Texas has been especially hard hit by the drought (Jervis 2011; Goodwyn 2011). In June 2011, the USDA designated 213 of 254 counties in Texas as primary natural disaster areas that qualified for federal aid as a result of the drought (USDA 2011). During the 12-month period ending in February 2013, the U.S. Drought Monitor reported at least some period of exceptional, extreme, or severe drought for all 254 Texas counties (U.S. Drought Monitor 2013). The agricultural losses due to the 2011 drought in Texas were estimated to be $7.62 billion, with $3.23 billion of that from livestock losses (Fannin 2012). In addition, Texas' cattle inventory has decreased significantly. In 2011, the overall number of cattle in Texas dropped by nearly 1.5 million, with 660,000 of that reduction being from beef cattle (NASS 2012). The overall Texas cattle inventory decreased another 600,000 in 2012, with 550,000 of that being a reduction in beef cattle (NASS 2013). The national drought has also impacted many states across the country (University of Arkansas 2012; AgWeek 2011).

The duration and severity of the drought have created uncertainty regarding future farming and ranching operations. Long-term recovery from the diminished livestock herds is a primary issue. In particular, some farmers and ranchers will be faced with the decision of whether to re-enter the market, when to do so, and to what degree. Undoubtedly, these decisions will be based on fundamental economic principles regarding whether the potential profit from continuing or starting a new ranching operation will be sufficient to justify the costs and risks involved (Lacy 2011). If cattle prices rise over time - which they may, given the decreased supply that results from current liquidations and increasing slaughter rates (Galbraith 2011), rising inflation, and increased world demand for beef - then the buying power of today's sales proceeds will be diminished and the ability to restart a sustainable operation may be affected (Texas 2011).

One of many difficult issues producers face during this period of uncertainty involves difficult tax decisions surrounding the liquidation of livestock. This paper addresses some provisions of the Internal Revenue Code (the "Code") that affect a farmer or rancher's decisions regarding the sale of livestock and the purchase of new livestock if and when current drought conditions subside.

METHODS AND MATERIALS

This paper provides an overview of the federal income tax treatment of livestock sales under existing tax laws. After providing a general overview, the author gives a more detailed analysis of Internal Revenue Code Sections 1033 and 451, two provisions that may allow farmers and ranchers to defer all or a portion of the gain recognized on livestock sales due to recent drought conditions. When compared to other similar provisions of the Code, Sections 1033 and 451 leave some important questions unanswered and other sources of authority do not provide any additional guidance. This paper discusses a few significant aspects of Code Sections 1033 and 451 and the related rules that need clarification in order to provide guidance for different situations that may arise. The paper also offers suggestions for how to clarify those ambiguities by identifying specific changes that could be made to make these laws consistent with

similar provisions of the Code and discusses issues that farmers and ranchers must consider when determining whether it would be beneficial to utilize these tax deferral provisions.

RESULTS

Gain from the sale of livestock. Generally, when an asset is sold the difference between the amount realized on the sale and the seller's adjusted basis in the asset is recognized as gain (I.R.C. § 1001(a)). The amount realized on the sale is equal to the amount of money received plus the value of any property (other than money) received and the amount of any liabilities of the seller that are discharged as a result of the transaction (I.R.C. § 1001(b)). An owner's tax basis in property is generally equal to the cost of acquiring the property, subject to certain special rules and adjustments (I.R.C. 1012(a)). If a sale results in a realized gain, the gain is included in the owner's taxable income unless there is a specific exception that allows him to defer or eliminate the gain (I.R.C. § 1001(c)).

Most family livestock producers and farmers have a zero basis in their livestock because the costs of raising the animals are deducted and not added to the livestock's basis (Treas. Reg. § 1.162-12(a)). If the farmer is a corporation or partnership that is required to use the accrual method of accounting, then Code Section 263A requires capitalizing certain costs, which would increase the tax basis in the livestock. Code section 447 requires corporate farmers to use the accrual method of accounting, unless the corporation has not had gross receipts in excess of $25,000,000 in any taxable year after 1985 (I.R.C. § 447(a) and (d)). However, when livestock is purchased, the basis is equal to the amount paid for the livestock (I.R.C. § 1012(a)). For producers whose herds consist of raised livestock with zero basis, the sale of a significant portion of a herd could result in substantial taxable gains. Furthermore, even if a herd is made of purchased livestock, unless the livestock was bought within the previous two or three years the gain will likely be substantial because of depreciation deductions that have significantly reduced the tax basis in the livestock. Depending on the year the livestock was purchased, the entire basis could be depreciated in the first year under the bonus depreciation provisions of Code Section 179. In addition, the depreciation recovery period for most livestock is between three and seven years, so basis is reduced quickly by depreciation.

Taxable gain is subject to tax at either ordinary income rates or reduced capital gain rates, depending on the circumstances. Gains from the sales of capital assets held for more than one year are currently taxed at a maximum rate of 15% (I.R.C. § 1(h)(1)(C)). The capital gains rate was set to revert back to 20% at the end of 2010, but the lower rate was extended by the Tax Relief, Unemployment Insurance Reauthorization, and Job Creation Act of 2010. The maximum capital gain rate will increase to 20% if the "Bush tax cuts" are not extended again after 2012. The maximum individual tax rate on ordinary income and gains from the sale of capital assets held for less than one year is 35% (I.R.C. § 1(i)(2)). The maximum ordinary income rate will increase to 39.6% if not extended again after 2012.

The Code defines a capital asset by describing what it is not. Section 1221 of the Code states that a capital asset means property held by the taxpayer (whether or not connected with a trade or business), but does not include:

i. stock in trade;

ii. inventory;
iii. property held primarily for sale to customers;
iv. depreciable property used in a business;
v. real property used in a business;
vi. certain intellectual property;
vii. accounts receivable acquired in the ordinary course of business;
viii. certain publications of the United States government;
ix. hedging transactions; and
x. supplies used by the taxpayer in the ordinary course of business (I.R.C. § 1221(a)).

Assets that do not qualify as capital assets are generally not eligible for the preferential capital gains rate when sold. For farmers and ranchers, cattle and other livestock do not qualify as capital assets because they are either held primarily for sale to customers or are depreciable property used in a business.

However, there is an additional provision that may treat gains from the sale of livestock as capital gains. Code Section 1231 provides that if section 1231 gains for any taxable year exceed section 1231 losses for that year then such gains and losses are treated as long-term capital gains and losses (I.R.C. § 1231(a)(1)). However, any gain that results from depreciation recapture will be subject to tax at ordinary income rates (I.R.C. § 1245(a)(1)). If 1231 gains do not exceed 1231 losses, then they are treated as ordinary gains and losses (I.R.C. § 1231(a)(2)). "Section 1231 gains" are gains from the sale of property used in a trade or business or from the involuntary conversion of such property, and "section 1231 losses" are losses resulting from the sales of such property (I.R.C. § 1231(a)(3)). For purposes of these rules, only certain property qualifies as being used in a trade or business. That property includes any personal property that is (i) used in a trade or business, (ii) subject to depreciation under Code Section 167, and (iii) held for more than one year, but does not include property that is inventory or held primarily for sale to customers or certain intellectual property and government publications (I.R.C. § 1231(b)(1)). The term also includes real property used in a trade or business and held for more than one year. In addition, the term includes specific types of livestock. Cattle and horses held for more than two years for draft, breeding, dairy, or sporting purposes are covered as is other livestock held for more than one year for the same purpose (I.R.C. § 1231(b)(3)). However, poultry do not qualify for Section 1231 treatment.

Therefore, livestock held for draft, breeding, dairy, or sporting purposes may qualify for capital gain treatment under Section 1231, but livestock held primarily for sale to customers does not qualify for capital gain treatment under either the capital asset test or Section 1231. The determination of whether livestock is held for draft, breeding, dairy, or sporting purposes is based on the facts and circumstances of each case (Treas. Reg. § 1.1231-2(b)). This issue has been litigated many times and the cases illustrate the importance of keeping accurate records regarding farming and ranching operations (Bales 1989; A. Duda 1977).

Potential tax deferral. For many producers, additional taxable income will not create a tax liability because there are sufficient losses and deductions to offset the gain. However, for those faced with the possibility of a current income tax liability resulting from livestock sales, there are several options for deferring tax payments. The availability

of these deferral provisions and which options may be better suited for a particular farm or ranch operation depend on the circumstances in each case and the different.

Section 1033 of the Internal Revenue Code. This Code section provides that if property is involuntarily converted into money and subsequently reinvested in similar property within a specified time period then no gain or loss is recognized on the transaction (I.R.C. § 1033(a)(1)). Generally, this provision applies to condemnation proceedings where all or a portion of some real estate is condemned or seized by a government entity and the land owner is compensated for any land taken. In such a case, the landowner is not required to recognize taxable gain if the proceeds are invested in other real estate within a specified replacement period (I.R.C. § 1033(a)(2)).

This special deferral provision also applies to the sale of livestock as a result of drought, flooding, or other weather-related conditions (I.R.C. § 1033(e)). Only livestock (other than poultry) held for draft, breeding, or dairy purposes qualify for this treatment (I.R.C. § 1033(e)(1)). If a taxpayer sells more qualifying livestock solely due to drought or other weather conditions than he would have under normal business practices, then the gain from the sale of "extra" livestock can be deferred under Code Section 1033(e). It is not necessary that the livestock be held or sold in the affected area, but the sale must be solely on account of weather-related conditions that affected "the water, grazing, or other requirements of livestock so as to necessitate their sale" (Treas. Reg. § 1.1033(e)-1(b)).

In order to qualify for deferral, a taxpayer generally must purchase replacement livestock within the replacement period discussed below. Replacement livestock should be similar or related in service or use to the livestock that was sold (Treas. Reg. § 1.133(e)-1(d)). That is, the new livestock must be held for the same purpose as the old livestock. For example, dairy cows can be replaced with dairy cows, but they cannot be replaced with draft or breeding animals. The rules under Code Section 1033 do not specify whether replacement livestock must be the same sex as the livestock that was involuntarily converted. However, the like-kind exchange provisions of Code Section 1031 specifically provide that the exchange of one sex of livestock for the other sex is not an exchange of like-kind property (I.R.C. § 1031(e)). A lack of specificity in Section 1033 provisions suggests that the replacement livestock need not be the same sex as converted livestock as long as the use and purpose is the same.

In addition, if because of drought, flooding, or other weather-related conditions it is not feasible for the taxpayer to reinvest his proceeds from involuntarily converted livestock in new similar livestock, he may purchase other property used in farming (including real estate) and qualify for tax deferral (I.R.C. § 1033(f)). The taxpayer's basis in replacement property is the same as the basis in the involuntarily converted property, decreased by the amount of money that was not reinvested in replacement property and any loss recognized on the involuntary conversion (I.R.C. § 1033(b)(1)). Tax basis is increased by the amount of any gain recognized on the conversion. If replacement property cost exceeds the involuntary conversion proceeds, then the basis is increased by such excess cost (I.R.C. § 1012(a)).

If livestock sales meet the requirements set forth above, then the taxpayer must reinvest the proceeds in qualifying replacement property (either livestock or other farm property as discussed above) within a specified replacement period. Generally, the replacement period is two years from the end of the tax year in which the livestock were sold due to adverse weather conditions (I.R.C. § 1033(a)(2)(B)). If the area affected by

weather has been designated as eligible for disaster assistance from the Federal Government, then the replacement period for livestock is four years (I.R.C. § 1033(e)(2)(A)). However, when replacing livestock with other farm equipment the replacement period is always two years. In 2006, the IRS issued a notice that extends the replacement period for livestock sold on account of drought, flooding, or other weather-related conditions until the end of the first year ending after the "first drought-free year for the applicable region" (IRS Notice 2006). With respect to individual taxpayers, the first drought-free year for the applicable region is the first twelve-month period that (i) ends on August 31, (ii) ends in or after the last year of the taxpayer's standard four-year replacement period, and (iii) does not include any weekly period for which exceptional, extreme, or severe drought is reported for any location in the applicable region (IRS Notice 2006). In September of each year, the IRS publishes a notice that includes a list of counties in which extreme drought conditions were reported for any period during the previous twelve months. If the taxpayer's region is listed in the notice, then his replacement period is extended pursuant to the rule outlined in the notice (IRS Notice 2010). The 2010 IRS Notice included 31 states with at least one county that qualified for an extended replacement period.

The following hypothetical example helps illustrate the replacement period rules. Mr. Smith is a calendar year taxpayer and raises cattle for breeding in Kendall County, Texas. In July 2006, Mr. Smith sold 40 cows due to the drought (he would not have sold any if it were not for the drought). At the time of the sale, Kendall County qualified for assistance from the Federal government because of the drought conditions. As a result, Mr. Smith had until December 31, 2010, to purchase replacement cows. However, in September 2010, the IRS released a notice that listed Kendall County as one of the regions in which extreme drought was reported, thus extending the replacement period. In September of 2011 and 2012, the IRS notice did not list Kendall County as one of the severe drought regions. At that point, Mr. Smith has to purchase his replacement cows by December 31, 2012, in order to qualify for the relief of Section 1033.

A taxpayer who desires to take advantage of this deferral provision should report the details of the involuntary conversion on the return for the taxable year in which the conversion took place (i.e. the year the sales were finalized and the proceeds received) (Treas. Reg. § 1.1033(a)-2(c)(2)). The report should include the following:

i. Evidence of the drought conditions which forced livestock sales;
ii. A computation of the amount of gain realized on the sale;
iii. The number and kind of livestock sold; and
iv. The number of livestock of each kind that would have been sold under usual business practice in the absence of drought conditions (Treas. Reg. § 1.1033(e)-1(e)).

Deferring some or all of the gain. Only the amount of gain that the taxpayer desires to recognize currently as a result of the involuntary conversion should be included in the gross income on that return. The taxpayer reports the details of the purchase of replacement livestock on the return for the year in which replacement livestock is purchased (Treas. Reg. § 1.1033(a)-2(c)(2)). If the taxpayer fails to purchase replacement property or only uses a portion of the involuntary conversion proceeds for such property, then he must amend the return on which the original involuntary conversion of livestock was reported and pay any resulting taxes. In addition, if the taxpayer originally reported

gain from the involuntary conversion but later decides to apply Code Section 1033 to defer that gain, he should file a tax refund claim (Treas. Reg. § 1.1033(a)-2(c)(2)).

Another potential deferral provision is Code Section 451. This Code section allows farm and ranch taxpayers who use the cash method of accounting to defer to the following tax year the gain from the sale of certain livestock due to drought or other weather-related conditions (I.R.C. § 451(e)(1)). Once made, this election is irrevocable unless the taxpayer obtains IRS consent. This election may be made only by individuals whose principal trade or business is farming (I.R.C. § 451(e)(2)) and is not available with respect to livestock held for draft, dairy, breeding, or sporting purposes for more than twelve months (twenty-four months in the case of cattle and horses) (Treas. Reg. § 1.451-7(a)). Just as with the deferral under Code Section 1033, this elective provision is available only to the extent that the number of livestock sold exceeds the number that would have been sold under normal business practices. In addition, sales must be solely on account of drought or other weather-related conditions, and the affected area must have been designated as eligible for assistance by the Federal government (Treas. Reg. § 1.451-7(a)). If a sale occurs before the area is designated for Federal assistance, the sale can still qualify under Section 451 if the sale occurred as a result of weather conditions that caused the area to become eligible for Federal assistance. Again, it is not necessary that the livestock be raised or sold in the affected area, but the taxpayer must be able to show that the sales occurred because of weather conditions that affected the water, grazing, or other requirements of the livestock so as to necessitate a liquidation (Treas. Reg. § 1.451-7(c)(1)).

Generally, taxpayers must make the Section 451(e) deferral election prior to the due date for filing the income tax return for the taxable year in which the sales occurred (Treas. Reg. § 1.451-7(g)). However, if the sale would qualify for the extended replacement period under Code Section 1033(e)(2), then the Section 451 election is valid if made prior to the replacement period expiration (generally, two years from the end of the year in which the sale occurred, but possibly longer in severe drought situations) set forth in Section 1033(e)(2) (I.R.C. § 451(e)(3)). The practical effect of this rule is that the Section 451 election does not have to be made until the end of the extended replacement period under Section 1033(e)(2) because all sales that qualify for Section 451 treatment would also qualify for the extended replacement period under Section 1033(e)(2). Code Section 1033(e)(2) extends the replacement period from two to four years if the area has been designated as eligible for Federal assistance. Given that Section 451 applies only when the drought caused an area to be eligible for Federal assistance, any sale that qualifies for Section 451 deferral would satisfy the requirements of Section 1033(e)(2).

Need for clarification. The application of the rules outlined above is relatively straight-forward. However, there are several issues that are unclear and warrant additional guidance. First, given the anticipated severity and duration of the current drought that affects nearly the entire country, it is important to understand what qualifies as replacement livestock under the Code Section 1033(e) deferral rules. As currently drafted, the Code and Treasury Regulations require that replacement livestock be of similar or related use or service and be "functionally the same as" the livestock that was sold. That is, the new livestock must be held for the same purpose as the old livestock. Treasury Regulations indicate that livestock held for breeding cannot be replaced with

livestock held for draft or dairy purposes, but, presumably, a taxpayer could replace hogs held for breeding with cows held for breeding. However, the Code, IRS rulings and publications, and case law do not provide a clear answer to this question.

The like-kind exchange rules of Code Section 1031 could provide guidance in clarifying what qualifies as replacement livestock. Under Section 1031, property held for investment or use in a trade or business can be exchanged for "property of like kind which is to be held either for productive use in a trade or business or for investment" without recognizing gain or loss on the transaction. Whether two properties are like kind depends on the nature or character of the property, not their grade or quality (Treas. Reg. § 1031(a)-1(b)). As a result, real estate exchanges are a common use of the 1031 exchange rules because of the ability to exchange one type of real estate for another. For example, the following exchanges qualify as like-kind exchanges of real estate: city real estate exchanged for a farm (Treas. Reg. § 1031(a)-1(c)); gold mines exchanged for coal mines (Peabody 2006); perpetual water rights exchanged for farm land (PLR 2004); and a fee simple interest in real estate for a lease with more than a thirty-year remaining term (Treas. Reg. § 1031(a)-1(c)).

The types of real estate that can be exchanged in a 1031 transaction have little in common other than being real estate held for investment or business use. The real estate need not consist of the same rights, be held for the same purpose, or produce (or have the potential to produce) the same type of income. Nonetheless, those properties are considered like-kind for purposes of Section 1031 and can be exchanged without the recognition of gain or loss.

Applying a similarly broad characterization to livestock held for breeding, draft, or dairy purposes would provide comparable flexibility when replacing livestock that were sold due to drought or other weather-related conditions. The Code refers to livestock as the broad category to which the section applies, and then narrows the application by limiting it to livestock held for breeding, draft, or dairy. Therefore, because cows and goats are both livestock, a rancher should be permitted to exchange cows for goats, as long as the new livestock is held for the same purpose as the old livestock.

In addition, there are good policy reasons for allowing the exchange of different types of livestock. For example, due to the recent drought severity, certain forms of farming or ranching may not be sustainable in areas that have historically been used for that purpose. Therefore, good policy would permit farmers to change the kind of livestock they raise to a type better suited for the new conditions of drought-affected areas. This type of exchange satisfies the plain language of the Code and Regulations so long as the livestock are used for the same purpose, but it is not clear what position the IRS would take on this issue.

Assuming that different livestock cannot be used to replace the involuntarily converted livestock under Code Section 1033 provisions, it could also be argued that extreme drought conditions that make a different type of livestock more suitable for particular environments make it not feasible for the taxpayer to reinvest the proceeds in the same type of livestock that was sold due to the drought. In that case, the purchase of a different type of livestock may qualify as "other property used for farming purposes" so that Code Section 1033(f) would treat the other property as similar to the livestock that was sold. This could even apply to livestock used for other purposes so long as it is utilized for "farming purposes," which has not been defined. Attempting to fall within

Code Section 1033(f) provisions would require the taxpayer to show that the purchase of similar livestock was not feasible due to drought or other weather conditions, something that may be difficult or impossible, particularly if other farmers and ranchers in the area continue to raise the same type of livestock that the taxpayer sold due to drought.

The language of Code Section 1033 and the applicable Regulations also leave room for IRS interpretation. When given the opportunity to do so in the past, the IRS has made reasonable and rationale changes to the application of livestock exchange rules. For example, in 2006, the IRS realized that it did not make sense to require the purchase of replacement livestock if the drought or other weather conditions that caused the involuntary conversion still existed. As a result, the IRS issued a notice that extends the replacement period until after the extreme drought conditions have ended. This extension was made pursuant to specific statutory authority that allowed additional extensions when appropriate based on weather-related conditions I.R.C. § 1033(e)(2)(B)). There is no similar authority for determining the type of exchanges that qualify for deferral under Code Section 1033, but the IRS could reasonably interpret Section 1033 to provide that different types of livestock can qualify 1033 treatment under the circumstances described above.

Practical planning. Conventional wisdom suggests that tax deferral is the next best thing to tax elimination and a taxpayer should not recognize taxable income now in order to provide for future deductions. That thought process may lead many farmers and ranchers to take advantage of the deferral provisions discussed above when they are forced to sell livestock as a result of drought conditions. However, a careful analysis should be made to ensure that tax deferral is the best choice. In some cases, recognizing taxable gain now may be the preferred option.[1]

For example, a farmer might have significant net operating loss (NOL) carryovers that will expire at the end of the current tax year. In that case, recognizing gain now may allow him to use those expiring NOLs that would otherwise be wasted. As noted previously, it is not ideal to offset capital gains with ordinary losses and deductions, but it could be worth it to recognize the gain now in order to use the expiring NOLs.

It is also important to determine whether current and future sales will create ordinary income or capital gain. If a rancher sells twenty cows that he raised for breeding which have a zero tax basis, then the resulting gain will be a Section 1231 gain and could result in capital gain treatment if the Section 1231 gains exceed the Section 1231 losses for the year. If the rancher has sufficient deductions or losses to offset the current gain, then no tax will be due and he may decide it is not necessary to use the deferral provisions under Code Section 1033(e) or Section 451.

When a rancher buys cows to replenish his breeding program, he will get a tax basis equal to the cost of the replacement cows. He will be entitled to take depreciation deductions with respect to the cows (either by electing to expense the cost of the

[1] For a general discussion of situations in which tax-able transactions may be more efficient than tax-free transactions, *see* Timothy J. Devetski, *Avoiding a Tax-Free Transaction: When Taxable is Tax Efficient*, 5 HOUS. BUS. & TAX L.J. 90 (2005).

replacement cows pursuant to Code Section 179 or through the standard annual depreciation deductions determined under Code Sections 167 and 168), and if the new cows are sold in the future the rancher will likely have a zero basis at the time of sale due to depreciation deductions. This will result in all or a portion of the gain from the sale of his depreciated cattle being taxed as ordinary income due to the depreciation recapture rules. By choosing not to use the 1033(e) deferral provisions, the rancher will recognize capital gain now but may not have any current tax liability because of potential offsetting deductions. In the future, he will be entitled to depreciation deductions with respect to the replacement cows, but the resulting gain from the sale of the cows will be taxed as ordinary income.

In addition, the capital gains and ordinary income tax rates are set to increase after 2012. If a rancher believes that rate increases will occur, then he might recognize gain now and pay tax at potentially lower rates. A thorough analysis should be done to review current and anticipated income and other tax attributes to determine whether to elect the available deferral provisions. In any event, Code Section 1033 provides great flexibility in applying the provisions so that a taxpayer can effectively change its election at any time during the replacement period. For most sales that occur now, this means the taxpayer has at least four years to decide whether deferring the gain is the best choice, and in some cases the decision period may be extended even longer. However, the decision to defer gain under Code Section 451 may need to be made prior to the due date of the return for the taxable year in which the sales occurred.

CONCLUSION

Drought conditions continue to affect United States agriculture, forcing many farmers and ranchers to sell more livestock than is typical during a given time period. Some producers may face substantial federal income tax liabilities resulting from these "forced" sales. However, the Code contains two deferral provisions that allow for the deferral of resulting gains. Code section 451 allows taxpayers to defer the gain to the next taxable year. The other provision (Code section 1033) lets taxpayers defer the gain for more than one year (and possibly up to four years or more) and to invest the resulting proceeds in replacement livestock that are similar in use to the livestock sold. While the provisions are straight-forward, there are some issues that need clarification, including what type of livestock qualifies as replacement property. These issues will surely be tested in the coming years as severe drought conditions cause some ranchers to seek unconventional methods of applying the Code's deferral provisions, and additional guidance may be warranted to help these taxpayers. As these issues are presented to the IRS and courts for decision, additional research will be required to analyze the decisions and determine whether the existing deficiencies have been addressed and whether additional changes are necessary.

In addition, affected farmers and ranchers will need to determine whether the deferral provisions are the best option for them. In most cases, electing either provision will likely be beneficial. However, it is important to perform a detailed analysis of the facts and circumstances in each case.

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The Texas Journal of Agriculture and Natural Resources 25:46-56 (2012)

Effect of *Fusarium oxysporum* f. sp. *vasinfectum* Inoculum Density, *Meloidogyne incognita* and Cotton Cultivar on Fusarium Wilt Development

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ABSTRACT

A greenhouse experiment was conducted to evaluate the interactive effects of different *Fusarium oxysporum* f. sp. *vasinfectum* isolates at increasing inoculum densities with *Meloidogyne incognita* on partially resistant (Stoneville (ST) 4554B2RF) and susceptible (FiberMAX (FM) 9058F) cotton cultivars. Disease incidence and area under the disease progress curve (AUDPC) were significantly higher for FM 9058F compared to ST 4554B2F for all the *Fov* isolates, densities and *M. incognita* combinations. Differences in pathogenicity were observed among the *Fov* isolates tested, suggesting that variability in aggressiveness may exist in populations of the fungus, though all isolates were within the same Race grouping (Race 1). A total of four isolates showed significantly higher AUDPC in the absence of *M. incognita* at higher inoculum densities of *Fov*; while two isolates had significantly higher AUDPC in the presence of *M. incognita* at low inoculum densities. Plant growth differed between cultivars where FM 9058F plants were shorter, and had decreased root, shoot, and total plant weights compared to ST 4554B2RF. Plants inoculated with *M. incognita* had root galls and were stunted with reduced shoot weight and total plant weight. Management of Fusarium wilt can be substantially improved by using partially resistant cultivars or reducing the inoculum density of root-knot nematode.

KEY WORDS: *Gossypium hirsutum* L., root-knot nematode

INTRODUCTION

The Fusarium wilt – Root-knot nematode complex is an economically important disease of cotton (*Gossypium hirsutum* L.) in most cotton-growing regions of the world (Colyer et al., 1997). The disease complex is caused by a soil-borne fungus, *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *vasinfectum* (Atk.) W. C. Snyder and H. N. Hans (*Fov*), and the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood

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(DeVay et al., 1997). Since the first report of Fusarium wilt of cotton, in Alabama (Atkinson 1892), the disease has increased in importance (Davis et al., 2006) and is responsible for losses averaging $20 million each year across the cotton belt of the United States of America (Blasingame et al., 2008). Under conducive environmental conditions, high losses occur when susceptible cultivars are grown on heavily infested soil. Losses are greatest on sandy soils that are infested with *M. incognita* (DeVay et al., 1997). Losses due to Fusarium wilt of cotton vary depending upon the virulence of *Fov*, host resistance, environmental factors, soil type and fertility, and interactions with nematodes (Hao et al., 2009; Smith and Snyder 1975). Symptoms of Fusarium wilt appear earlier when densities of both *M. incognita* and *Fov* are increased (Garber et al., 1979).

There are eight races of *Fov* that have been described throughout the world, with Race 1 and Race 2 historically being the most prevalent in the United States (Kim et al., 2005). Recent studies have found that Races 1, 3, and 8 are mildly virulent and cause wilt symptoms in the presence of *M. incognita*. However, Race 4 of *Fov*, which was identified in California (Kim et al., 2005), is capable of causing severe wilt symptoms and economic loss in the absence of nematodes.

Fusarium oxysporum f. sp. *vasinfectum* density in soil and wilt incidence are correlated (DeVay et al., 1997; Starr et al., 1989). Development of varieties that have at least some ability to resist Fusarium wilt is important in managing this disease (Hillocks 1992). Understanding the virulence of local *Fov* isolates is critical in the development of a screening program. It is important to understand the effect of *Fov* inoculum density on incidence of wilt for cultivars that differ in their susceptibility to the disease. It is also important to determine how isolates of *Fov* may differ in aggressiveness to different genotypes of cotton, or more importantly, are some genotypes relatively resistant to a collection of isolates, or is there a cultivar × isolate interaction. Ideally, isolates should be selected for a breeding program that are fairly aggressive across both partially resistant and susceptible germplasm, but where the germplasm differences are still obvious. This may ultimately allow for separation between high levels of resistance (which have not been identified in commercial cultivars) and partial resistance in future screening efforts.

Initial symptoms of Fusarium wilt include chlorosis and necrosis of the leaf margins (Fig. 1A). Severely diseased plants can be killed as seedlings, or if they survive, may often remain stunted throughout the season (Fig. 1B). *Fov* invades the host through the taproots behind the root tip. The combined effect of fungal metabolites and the production of lipoidal substances by the host in response to infection may lead to the occlusion of the vascular tissues (Shi et al., 1992). The vascular system of plants exhibits discoloration due to systemic infection of the fungus. In most severely affected plants, leaves wilt and drop and the plants may die (Colyer 2001; Nelson 1981; Fig. 1). Plants that develop symptoms early usually die before producing any bolls, whereas plants that develop symptoms after the onset of flowering often survive but produce fewer bolls.

Chlamydospores are thick-walled specialized resting structures that remain dormant in the soil until exudates or leachates from plant roots stimulate their germination (Mai and Abawi 1987). The germinated chlamydospores produce hyphae that eventually form conidia and new chlamydospores if a suitable host is not found. Once a field is infested with *Fov*, the fungus usually persists indefinitely in the decaying plant tissues and soil as chlamydospores (Nelson 1981; Smith and Snyder 1975). The fungus is capable of surviving for over 10 years in the soil not planted to cotton (Smith et al., 2001). The ability of the pathogen to survive in soils for long periods has important consequences on disease management. Management options available for Fusarium wilt

are typically aimed at control of *M. incognita*, rather than *Fov*. These options include rotations with non-host crops for *M. incognita*, nematicides, and planting of nematode resistant cultivars (DeVay 1986). Use of rotations to reduce *Fov* has been of limited value because of its saprophytic ability (Smith and Snyder 1975). There are few economically viable crops for use by cotton producers in a rotation program due to the broad host range of *M. incognita*. Control of nematodes with nematicides has resulted in considerable decrease in Fusarium wilt and increased yield (Hyer et al., 1979; Jorgenson 1979; Smith 1948).

Figure 1a. Fusarium wilt symptoms. Chlorosis and necrosis of the leaf margin.

Figure 1b. Range of symptoms on field grown cotton.

Field observations indicate that use of a partially nematode resistant cultivar (Stoneville 5599BR) over a period of three years led to a substantial decrease in disease incidence (Wheeler, *personal communication*). A recent survey found that extensive genetic diversity exists in *Fov* populations in West Texas. However, all isolates collected to date were found to be characterized as Race 1 (Woodward, *unpublished*). The purpose of this study was to characterize the effects of *Fov* Race 1 isolates, their interaction with *M. incognita*, and cotton cultivars with varying levels of resistance to *Fov* on Fusarium wilt development.

MATERIALS AND METHODS

An experiment was conducted in the greenhouse during spring and fall of 2009. The experiment was designed as a split-split-split plot with four replications. Twelve isolates of *Fov* Race 1 served as the main plot, *Fov* inoculum densities (0, 2.5×10^3, 9.5×10^4, 6.5×10^5 colony forming units (cfu)/cm^3 of potting mix) served as sub-plots, two cultivars (Stoneville 4554B2RF (partially resistant), and FiberMax 9058F (susceptible)) served as sub-sub-plots and root-knot nematode densities (0 and 1,000 eggs/pot) served as sub-sub-subplots. Plastic containers (Stuewe & Sons, Tangent, OR) (30 cm height and 6 cm diameter at top) were filled with 700 cc soil mix/pot (70% sand, 25% top soil, and 5% peat moss). The number of eggs/pot represents the population of nematode eggs in a typical infested field soil. *Fov* inoculum was prepared from 3-week-old cultures maintained on Komada's Selective Medium (Komada 1975) and maintained at room temperature under continuous light. Petri dishes were flooded with water and conidia were scraped off the culture with a rubber spatula. The conidial suspension was then filtered through four layers of cheesecloth, quantified with the aid of a hemacytometer, and diluted with water to make the desired density for each *Fov* isolate. *Fov* inoculum was delivered using pipette inoculation technique (Latin and Snell 1986) into a potting mixture.

Root-knot nematodes were reared in a greenhouse on a susceptible tomato cultivar 'Homestead' and inoculum was extracted according to methods described by Hussey and Barker (1973). Soil was inoculated with *M. incognita* eggs (1,000/pot) to ensure root-knot nematode infestation and cotton (three seeds per cone) was planted. Plant densities were thinned to two plants per cone after three weeks.

Plant height and disease incidence were measured four weeks after planting. Disease incidence was observed every five days after the appearance of the first disease symptom. The percent disease incidence was rated on the following scale: 0% - no symptoms, 50% - chlorosis, necrosis, and wilting of one plant, and 100% - chlorosis, necrosis, and wilting of both plants. After 12 weeks of planting, plant growth was scored by measuring height, fresh root weight, and fresh shoot weight before termination of the experiment in each season. Root galling was also noted at this time to confirm the presence or absence *M. incognita*. Vascular discoloration was examined from cross and longitudinal section of the stem at the soil line and then positive samples were placed on to Petri dishes containing potato dextrose agar media to confirm *Fov* presence. Area under the disease progress curve (AUDPC) was calculated for quantitative disease assessment using repeated disease incidence as described by Shaner and Finey (1977).

Plant height, AUDPC, root weight, shoot weight, and total plant weight were analyzed using Proc MIXED (SAS Institute Inc., 2008, Ver. 9.2, Cary, NC, USA). The method used to adjust the degrees of freedom (df), to match adjustments in the sums of square, was the Satterthwaite option in the LSMEANS statement. Standard error and LSD values were determined from the PDIFF option.

RESULTS AND DISCUSSION

Interaction of *Fov* Race 1 isolates, their inoculum densities, *M. incognita* densities, and cotton cultivars was significant ($P \leq 0.0001$) for AUDPC. FM 9058F (susceptible cultivar) had significantly ($P \leq 0.05$) higher AUDPC than ST 4554B2RF (partially resistant cultivar) (Fig. 2) which indicated the importance of planting a partially resistant cultivar. *Fov* isolates 3, 4, 5, 6, 7, 10, 11, and 12 had significantly ($P \leq 0.05$) higher AUDPC with FM 9058F than isolates 1, 2, 8, and 9 (Fig. 2) suggesting that variability in aggressiveness occur among *Fov* isolates. Isolates 3, 4, 6, and 7 showed significantly higher AUDPC in the absence of *M. incognita* at higher inoculum densities of *Fov* (6.5×10^5 cfu/cm^3) with cultivar FM 9058F compared to other *Fov* isolates tested (Fig. 2) which implies that disease incidence may be higher even in the absence of *M. incognita* when *Fov* inoculum density in soil is high. Isolates 5 and 11 of *Fov* Race 1 had significantly ($P \leq 0.05$) higher AUDPC in the presence of *M. incognita* at lower inoculum density (2.5×10^3 cfu/cm^3) compared to other *Fov* isolates tested (Fig. 2) indicating the presence of varying levels of virulence within the same *Fov* Race 1 isolates. Isolates 4, 7, 10, and 11 showed significantly higher AUDPC than the rest of *Fov* isolates tested in the presence of *M. incognita* at *Fov* inoculum density of 9.5×10^4 cfu/cm^3. Isolates 2, 4, 6, 7, and 10 resulted in higher AUDPC at highest *Fov* inoculum density used for the study (6.5×10^5 cfu/cm^3) in the presence of *M. incognita* compared to other *Fov* isolates tested (Fig. 2). Isolate 3, for example, resulted in the highest level of symptoms in the absence and presence of *M. incognita* on FM 9058F, as well as ST 4554B2RF, but there was still clear separation between the two cultivars, both in the presence and absence of root-knot nematodes. Both the intermediate and highest density of the fungus resulted in clear differences between susceptible and partially resistant cultivars. This isolate would be a good choice to use in a germplasm screening program.

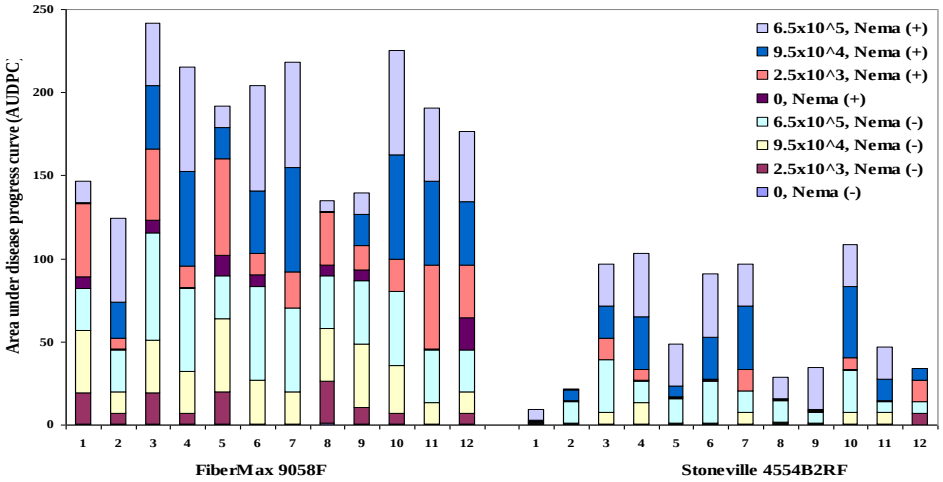

Figure 2. Effect of interaction of twelve *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) isolates, inoculum density, *Meloidogyne incognita* and cotton cultivar on area under the disease progress curve (AUDPC). Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) using mixed model analysis and df were determined using the Satterthwaite option. LSD for comparing means between *Fov* isolates was 20.76, for cultivars was 20.3, for nematode levels was 19.6, and for inoculum densities was 20.4 at $P \leq 0.05$ according to Fisher's Protected LSD. FiberMax 9058F was

susceptible and Stoneville 4554B2RF was partially resistant to *Fov*. Four inoculum densities of *Fov* used in the study were 0, 2.5×10^3, 9.5×10^4, and 6.5×10^5 colony forming units/cubic centimeter of soil. Nema (-) and Nema (+) represent *Meloidogyne incognita* levels 0 and 1,000 eggs/pot, respectively.

The interaction between inoculum densities of *Fov*, *M. incognita*, and cultivar was significant ($P \leq 0.0001$) for shoot weight. ST 4554B2RF had higher shoot weight than FM 9058F with or without root-knot nematode at all four *Fov* inoculum densities tested (Table 1). AUDPC was significantly higher ($P \leq 0.05$) for FM 9058F than ST 4554B2RF for all *Fov* isolates tested with or without *M. incognita* (Fig. 3). Total plant weight was significantly higher for plants not inoculated with *M. incognita* at all four *Fov* inoculum densities tested (Table 2) showing that root-knot nematode affects plant growth and development. The plants inoculated with *M. incognita* showed low total plant weight at higher *Fov* inoculum densities (6.5×10^5 cfu/cm^3 and 9.5×10^4 cfu/cm^3) than low *Fov* inoculum density (2.5×10^3 cfu/cm^3) or non-inoculated plants (Table 2). High *Fov* inoculum density (6.5×10^5 cfu/cm^3) resulted in significantly higher AUDPC with both the cultivars tested and AUDPC was significantly higher for FM 9058F than ST 4554B2RF (Table 3). Interaction between inoculum densities of *Fov* and cultivars tested was significant for plant height ($P \leq 0.05$) and total plant weight ($P \leq 0.0001$). ST 4554B2RF had higher plant height and total plant weight than FM 9058F. High inoculum density of *Fov* had higher plant heights and total plant weight than low inoculum densities (Table 4). Interaction between *Fov* Race 1 isolates and cultivars tested was significant for plant height ($P \leq 0.05$) root and shoot weight ($P \leq 0.0001$). All these parameters were significantly higher for ST 4554B2RF than FM 9058F (Table 5).

Table 1. Effect of interaction between inoculum density of *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) Race 1, *Meloidogyne incognita*, and cotton cultivar on shoot weight[a].

Fov inoculum density (cfu/cm^3)	Shoot weight (g)				
	M. incognita (0 eggs/pot)		*M. incognita* (1,000 eggs/pot)		
	FiberMax	Stoneville	FiberMax	Stoneville	
0	8.2 B[b], a[c]	8.9 A, b[c]	7.3 B[b], a	8.5 A, a[c]	
2.5×10^3	8.2 B, a	9.3 A, ab	7.3 B, a	8.2 A, a	LSD[e]
9.5×10^4	8.5 B, a	9.3 A, ab	6.7 B, b	8.2 A, a	0.6
6.5×10^5	8.0 B, a	9.5 A, a	5.6 B, c	8.5 A, a	
LSD[d]	0.6				

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [b]Upper case letters are for comparing means between cultivar FiberMax and Stoneville (row). [c]Lower case letters are for comparing means between inoculum densities (column). [d]LSD for comparing means between two cultivars. [e]LSD for comparing means between four *Fov* inoculum densities. FiberMax 9058F is susceptible and Stoneville 4554B2RF is partially resistant to *Fov*.

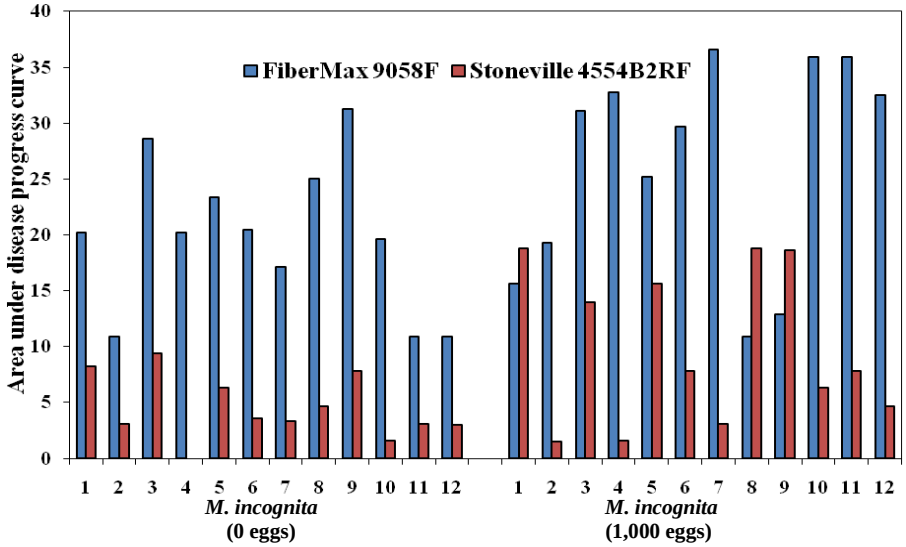

Figure 3. Effect of 12 *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) isolates, *Meloidogyne incogita*, and cotton cultivar on area under the disease progress curve (AUDPC). Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) using mixed model analysis and df were determined using the Satterthwaite option. Level of significance was determined at $P \leq 0.05$ according to Fisher's Protected LSD. LSD = 16.0 for comparing means between cultivars FiberMax and Stoneville at each nematode level for each *Fov* isolate. LSD = 17.7 for comparing means between *Fov* isolates of each cultivar at each nematode level. FiberMax 9058F was susceptible and Stoneville 4554B2RF was partially resistant to *Fov*. N = 31.

Table 2. Effect of interaction between inoculum density of *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) Race 1 and *Meloidogyne incognita* on total plant weight[a].

	Total plant weight (g)		
Fov inoculum density (cfu/cm^3)	*M. incognita* (0 eggs/pot)	*M. incognita* (1,000 eggs/pot)	
0	10.8 A[b], a[c]	10.2 B, a[c]	
2.5×10^3	11.1 A, a	10.1 B, a	LSD[e]
9.5×10^4	11.2 A, a	9.7 B, ab	0.6
6.5×10^5	11.1 A, a	9.3 B, b	
LSD[d]	0.50		

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [b]Upper case letters are for comparing means between nematode densities (row). [c]Lower case letters are for comparing means between inoculum densities (column). [d]LSD for comparing means between nematode levels. [e]LSD for comparing means between inoculum densities. N = 188.

Root galling was found to be associated with *M. incognita* presence and plants that exhibited galls were stunted, had reduced shoot and total plant weight; whereas, root weight was not affected by the presence of root galls (Table 6), because roots infested with *M. incognita* were smaller and less fibrous than roots without galls. Plant growth and symptoms expressions were affected by the susceptibility of cultivar. FM 9058F had significantly stunted plants, decreased root weight, shoot weight, and total plant weight

compared to ST 4554B2RF (Table 7) suggesting the importance of planting a resistant cultivar in infested fields.

Table 3. Effect of inoculum density of *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) and cotton cultivar on area under the disease progress curve (AUDPC)[a].

Fov inoculum density (cfu/cm^3)	AUDPC		
	FiberMax	Stoneville	
0	3.4 A[b], d[c]	0.3 A, c[c]	
2.5×10^3	20.7 A, c	2.3 B, c	LSD[e]
9.5×10^4	29.9 A, b	9.4, B, b	7.0
6.5×10^5	38.8 A, a	16.7 B, a	
LSD[d]	6.5		

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [b]Upper case letters are for comparing means between cultivar FiberMax and Stoneville(row). [c]Lower case letters are for comparing means between inoculum densities (column). FiberMax 9058F is susceptible and Stoneville 4554B2RF is partially resistant to *Fov*. N = 188.

Table 4. Effect of interaction between inoculum density of *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) and cultivar on plant height (35 DAP) and total plant weight[a].

Fov inoculum density (cfu/cm^3)	Plant height (cm)		Total plant weight (g)	
	FiberMax	Stoneville	FiberMax	Stoneville
0	15.9 A[b], ab[c]	16.2 A[b], ab[c]	10.0 B[b], a[c]	11.0 A[b], a[c]
2.5×10^3	15.7 B, b	16.8 A, a	10.0 B, a	11.2 A, a
9.5×10^4	16.2 B, a	16.8 A, a	9.7 B, a	11.1 A, a
6.5×10^5	16.0 A, ab	16.4 A, ab	8.9 B, b	11.4 A, a
LSD[d]	0.4		0.5	
LSD[e]	0.4		0.6	

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [b]Upper case letters are for comparing means between cultivars FiberMax and Stoneville for plant height and total plant weight (row). [c]Lower case letters are for comparing means between *Fov* inoculum densities (column). [d]LSD for comparing means between cultivars for plant height and total plant weight. [e]LSD for comparing means between *Fov* inoculum densities. FiberMax 9058F is susceptible and Stoneville 4554B2RF is partially resistant to *Fov*. N = 189.

There is a positive correlation between *Fov* inoculum density and disease incidence (DeVay et al., 1997; Hao et al., 2009; Starr et al., 1989). *Fov* Race 1, which resulted in significant damage when plants were co-infected with *M. incognita*, caused significant symptom expression with isolates 3, 4, 6, and 7 in the absence of nematodes on FiberMax 9058F at 6.5×10^5 cfu/cm^3 inoculum density (data not shown). This suggests that variability exists in aggressiveness among *Fov* Race 1 isolates at high inoculum density especially with planting a susceptible cultivar. Garber et al. (1979) found that Fusarium wilt occurs with *Fov* alone at high spore numbers (≥ 77,000 propagules per g soil) or in combination with root-knot nematodes at much lower densities (< 650 propagules per g soil with 50 second-stage juveniles) on a susceptible cultivar (Acala SJ-2). Kim et al. (2005) found that virulent Australian *Fov* isolates in controlled experiments, which, like *Fov* Race 4, do not require damage from nematodes

to cause disease, and caused increasingly severe symptoms at higher aqueous suspensions of conidia. However, the effect of *Fov* inoculum density in soil varied with the resistance of the cotton cultivar.

Table 5. Effect of interaction between *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) isolates and cultivars on plant growth parameters[a].

Fov isolate	Plant height (cm)		Root weight (g)		Shoot weight (g)	
	FM	ST	FM	ST	FM	ST
1	22.6 B[b],bc[c]	24.1 A,ab[c]	2.0 B[b],b[c]	2.3 A,b[c]	6.7 B[b],b[c]	8.6 A,ab[c]
2	24.8 A,a	25.2 A,a	2.2 B,ab	2.5 A,ab	7.1 B,ab	8.9 A,ab
3	21.9 B,c	24.6 A,ab	2.1 B,ab	2.7 A,a	6.8 B,b	9.6 A,a
4	22.8 B,bc	24.6 A,ab	2.2 B,ab	2.5 A,ab	7.6 B,ab	9.1 A,ab
5	22.9 B,bc	24.5 A,ab	2.2 A,ab	2.4 A,ab	7.4 B,ab	8.8 A,ab
6	22.4 B,bc	23.4 A,b	2.4 A,a	2.1 B,b	7.6 A,ab	8.2 A,b
7	22.9 A,bc	23.5 A,b	2.3 A,ab	2.3 A,b	8.0 B,a	8.7 A,ab
8	22.6 B,bc	24.2 A,ab	2.1 A,ab	2.3 A,b	7.6 A,ab	8.2 A,b
9	23.3 B,b	25.3 A,a	2.0 B,b	2.6 A,ab	7.2 B,ab	9.6 A,a
10	22.4 B,bc	23.7 A,b	2.1 A,ab	2.3 A,b	8.0 B,a	9.5 A,a
11	23.4 A,ab	24.0 A,ab	2.2 A,ab	2.3 A,b	7.8 A,ab	8.2 A,b
12	23.8 A,ab	24.6 A,ab	2.2 A,ab	2.3 A,b	8.1 A,a	8.3 A,b
LSD[d]	0.9		0.6		0.7	
LSD[e]	1.4		0.4		1.1	

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [b]Upper case letters are for comparing means between cultivar FM (FiberMax 9058F) and ST (Stoneville 4554B2RF) (row). [c]Lower case letters are for comparing means between isolates (column). [d]LSD for comparing means between cultivar. [e]LSD for comparing means between *Fov* isolates. N = 63.

Indeed, results of this greenhouse study may not reflect the field responses of cotton cultivars because field grown plants are frequently under environmental or biotic stresses not present in the greenhouse. Another inherent difference between the conditions of this greenhouse study and those of the field is the inoculum itself. *Fov* overwinters in field soil primarily as chlamydospores (Nelson 1981), but micro- and macro-conidia were used in this study to infest the potting mixture. Conidia are not as well suited for long term survival in the soil as are the thick-walled chlamydospores, but may germinate in pot cultures (Nelson 1981). Despite the differences between this experiment and field conditions, disease development in the field likely follows the general trends observed here. In the present study, four isolates of Race 1 showed significantly higher AUDPC in the absence of *M. incognita* at higher inoculum densities of *Fov*; while two isolates of Race 1 showed significantly higher AUDPC in the presence of *M. incognita* at low inoculum densities, indicating the presence of varying levels of *Fov* virulence. Further research is necessary in order to determine the soil inoculum threshold for disease development in the field with varying levels of *Fov* virulence. The relationship between *Fov* inoculum density, *M. incognita*, and severity of Fusarium wilt-Root-knot nematode complex is important for the development of management strategies because populations of *Fov* in the soil may be affected by the cotton cultivar selection.

Table 6. Effect of *Meloidogyne incognita* density on plant growth parameters[a].

M. incognita density (eggs/pot)	Plant height (cm)		Root weight (g)	Shoot weight (g)	Total plant weight (g)
	35 DAP	90 DAP			
0	17.2 a[b]	25.4 a[b]	2.3 a[b]	8.7 a[b]	11.0 a[b]
1,000	15.3 b	21.9 b	2.3 a	7.5 b	9.8 b
LSD[c]	0.2	0.3	ns	0.5	0.5

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. [b]Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [c]LSD for comparing *M. incognita* densities. N = 754.

Table 7. Effect of cultivar selection on plant growth parameters[a].

Cultivar	Plant height (cm)		Root weight (g)	Shoot weight (g)	Total plant weight (g)
	35 DAP	90 DAP			
FiberMax 9058F	15.9 b[b]	23.0 b[b]	2.2 b[b]	7.5 b[b]	9.6 b[b]
Stoneville 4554B2RF	16.5 a	24.3 a	2.4 a	8.8 a	11.2 a
LSD[c]	0.4	0.4	0.1	0.5	0.7

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. [b]Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [c]LSD for comparing cotton cultivar. FiberMax 9058F is susceptible and Stoneville 4554B2RF is partially resistant to *Fusarium oxysporum* f. sp. *vasinfectum*. N = 75.

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