INTEGRATED PEST MANAGEMENT OF NOCTUIDS IN KANSAS SORGHUM: A BIOECONOMIC APPROACH TO AGRICULTURAL PEST MANAGEMENT

by

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Abstract

Several lepidopteran species infest developing panicles. Larval identification is challenging and time intensive, so current recommendations are often simplified by treating all larvae equally across species. Consequently, the yield-loss model developed for corn earworm (*Helicoverpa zea*) by Buckley and Burkhardt (1962) has been the foundation for management recommendations in modern sorghum Integrated Pest Management (IPM) programs for the last 49 years. Additionally, although pest populations primarily include both fall armyworm (*Spodoptera frugiperda*) and corn earworm, only a single species damage estimate is used in economic threshold (ET) and economic injury level (EIL) calculations despite multi-species infestations. This research demonstrates both the validation of current management recommendations for corn earworm and the verification of previously assumed damage potentials for fall armyworm feeding in developing sorghum panicles. These results have important implications for sorghum producers faced with making a management decision for multi-species infestations.

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Chapter 1 - Literature review of a noctuid pest complex in sorghum production systems

INTRODUCTION

Sorghum, *Sorghum bicolor* (L.) Moench, is a grain of global importance (Anderson and Martin 1949) because it is a rich source for food, feed, and fuel (Wang et al. 2008, KGSPA 2011, USCP 2010). In the US, advancements in sorghum breeding programs have provided improvements to yields and tolerance of environmental stressors (Rosenow et al. 1983, Ougham and Stoddart 1986, Howarth 1989, Craufurd et al. 1993, Tuinstra et al. 1997, Kebede et al. 2001, Dillon et al. 2007, USCP 2010). Despite these advancements, sorghum yields are still vulnerable to insect pests (Teetes 1982, Wilde 2006). For example, southern US sorghum production is frequently subject to damage from the sorghum headworm complex that is primarily comprised of two larval noctuid pests: corn earworm, *Helicoverpa zea*, and fall armyworm, *Spodoptera frugiperda* (Young and Teetes 1979, Teetes and Pendleton 2000, Wilde 2006). This complex can cause significant yield loss if left untreated (Teetes and Wiseman 1979); however, current treatment recommendations are based on a single species model, where little is known about the contribution of each competing species to total yield loss (Buckley and Burkhardt 1962, Martin et al. 1980, Knutson and Cronholm 2007). Due to potential differences in larval feeding behaviors, host phenological preference, and damage potential in the field, the role of each species in relation to yield must be determined. Further yield losses in sorghum may be avoided if mixed-species recommendations are improved by incorporating species-specific considerations, which may allow producers to make well informed and timely management decisions. The following review will provide a synopsis of sorghum production, headworm natural history, and the current headworm management strategies that are the foundation to the subsequent research investigations of the afore mentioned issues.

IMPORTANCE OF SORGHUM

Sorghum is the fifth most produced cereal crop in the world, representing a critical source of food and feed for many countries. Following only wheat, rice, maize, and barley, global sorghum production exceeds 66.8 million metric tons (FAOSTAT 2011). In addition, the US ranks second in world production at just below 12 million metric tons valued at over $872 million (FAOSTAT, 2011). Sorghum was first domesticated in northeastern Africa around 4000-3000 BC, and has become an important global source of dietary protein, carbohydrates and other nutrients required for both human and livestock health (Smith and Frederiksen 2000, Dillon et al. 2007). Sorghum is cultivated on nearly 40 million ha of land throughout the arid and semi-arid regions of the globe (FAOSTAT, 2011) due to its excellent heat and drought tolerance (Rosenow et al. 1983, Dillon et al. 2007). In the US, grain sorghum is grown throughout the southern and central Great Plains. As the leading producer of grain sorghum in the US, Kansas produced approximately 44% of the nation's sorghum in 2010 (KGSPA). Sorghum also represents the second largest commodity in food aid, accounting for 10% of US agricultural exports (Sorghum Checkoff 2011).

GRAIN SORGHUM FOR FOOD AND FUEL

Several markets comprise the US sorghum industry. For animal production, sorghum grain, stalks, and leaves are processed into feed-based products for dairy and beef cattle, swine, and poultry consumption; over 80% of the sorghum seed produced in Kansas is used for livestock feed. Ethanol production accounts for 24.9% of harvested sorghum. Sorghum is currently the number two crop used in grain-based ethanol production in the US and represents a sustainable alternative to corn. For example, one bushel of sorghum produces the same amount of ethanol as a bushel of corn but uses a third less water during the growing process, and usually requires less fertilizer, herbicides, and insecticides (Wang et al. 2008). In Kansas, about 329 million gallons of ethanol are produced per year, creating a market for an extra 117 million bushels of Kansas grain (KGSPA 2009).

Sorghum Growth and Development

There are approximately 25 domesticated species of sorghum in four major groups including, sweet sorghum, grass sorghum, broomcorn sorghum, and grain sorghum (Dillon et al. 2007). Nearly all grain sorghum varieties grown in the US are dwarf hybrids of endemic cultivars found in Africa and Asia. Kansas farmers typically cultivate the shorter varieties, which only reach 0.5 to 1.0 m and are easily harvested with modern combines. In the early vegetative stages, sorghum plants have similar phenotypic characters to that of corn, but during the reproductive periods plants produce colorful seed heads. The average sorghum head or panicle will produce between 1500 and 2500 seeds, which may vary in color (white, yellow, red, brown) depending on the variety (Cothren et al. 2000). Because sorghum seed is relatively small and has limited energy and mineral reserves, germination can be highly variable depending on environmental conditions. Typically, about 75 to 80% of planted seed will germinate when soil temperatures are around 13°C (Stichler et al., 1997). While most hybrids follow the same growth pattern, the specific timing between growth stages can vary somewhat among hybrids, seasons, or locations (Vanderlip 1993). Seedling development to the 5-leaf stage from germination takes approximately 20 d. After 30 d, growing point differentiation occurs, nutrient uptake is rapid, and the panicle begins to develop. During this period, the plant has taken up 70% of its nitrogen, 60% of its phosphorus and 80% of its potassium (Stichler et al., 1997). By the time the flag leaf is visible in the whorl (40 d), 80% of the total leaf area is capturing sunlight and converting it into energy. Around 50 d after germination the boot stage occurs, which is when the seed head is enclosed by the flag leaf sheath. By this time seed number is determined. When 50% of the field is in bloom it is known as the half-bloom stage, typically occurring after 60 d. Between 60 to 70 d, grain fill begins, transitioning from milky, liquid filled seed to soft-dough. Hard-dough stage occurs when approximately 75% of the total dry grain weight has accumulated (85 days). Finally, maximum dry weight has been achieved by physiological maturity (100 days) and is marked by a dark spot on the tip of each kernel, opposite the embryo.

Factors Affecting Yield Potential

Although sorghum is highly tolerant to extreme heat and drought (Rosenow et al. 1983, Ougham and Stoddart 1986, Craufurd et al. 1993), environmental stresses can negatively influence both seed number and size (Rosenow and Clark 1995, Kebede et at. 2000). Stress prior to flowering (pre-anthesis) has been shown to delay leaf appearance and panicle development (Craufurd et al. 1992). Post-anthesis occurs during seed fill and can contribute to significant yield losses due to reduced seed size, susceptibility to disease, and premature plant death (Rosenow and Clark 1995, Dillon et al. 2007). Post-flowering drought tolerance, referred to as "stay-green", is bred into some varieties to maintain photosynthetic capabilities and overall yield under severe moisture stress (Tuinstra et al. 1997, Dillon et al. 2007).

Insect pests also contribute to sorghum yield losses globally. At least 150 insect species have been reported as pests of sorghum worldwide (Harris 1995). Major pests include the sorghum midge (*Contarinia sorghuicola*), green bug (*Schizaphis graminum* Rondani), shootfly (*Atherigona soccata* Rondani), and stem borer (*Chilo partellus* Swinhoe) (Young and Teetes 1977, Sharma 1993, Teetes and Pendleton 2000, Wilde 2006). Spider mites (*Oligonychus* spp.)

can also become problems when cultural practices or crop varieties change, or following injudicious use of insecticides applied for key pests.

SORGHUM HEADWORM COMPLEX

In the US, yield losses due to insect damage are estimated to cost sorghum producers over $80 million annually (Teetes 1982). A number of Lepidopteran species infest sorghum panicles and feed directly on developing seed including the sorghum webworm (*Celama sorghiella)*, Old World webworm (*Stenachroia elongella)*, and American bollworm (*Heliothis armigera)*. In the southern Great Plains, the predominating and economically damaging pests are members of the sorghum headworm complex that is primarily dominated by corn earworm and fall armyworm (Wilde 2007).

Corn Earworm

Biology and development. In 1965, *Heliothis zea* was placed into the newly described genus *Helicoverpa* due to its lack of morphological similarity in the genitalia of Linnaeus' *Heliothis* type species, *dipsacae* (Hardwick 1965). The complete life cycle of the corn earworm consists of four life stages: egg, larva, pupa, and adult. Development through the entire life cycle will usually take 3 to 4 wk. Eggs are flatten, ribbed spheres 1.2 mm in diameter and are laid singly on pre-flowering and flowering host plants. Moths begin to mate and oviposit about 3 d after emergence. An adult female moth will live about 12 d and is capable of depositing anywhere from 350 to 3000 eggs (Teetes and Pendleton 2000). Although corn earworm is multivoltine, the total number of generations produced per year decreases as populations reach northern latitudes and cooler temperatures. For example, this species may have up to seven generations in southern

Texas but only three in Kansas, two in the northeastern US, and one in Minnesota and Canada (Capinera 2007, Sandstrom et al. 2007, Molina-Ochoa 2010).

After emergence from the egg, corn earworm larvae will go through six instars; larval development typically occurs in 15 to 22 d. Degree-day (DD) models show that larval development takes 185 ± 54 (SD) DD at a lower threshold of 12.5°C, but the minimum requirements for early and late instars range from 81.7 to 120.6 DD, respectively (Hartstack et al. 1976, Coop et al. 1993). The first three instars are not aggressive and will not attack other members of its cohort. The last three instars are highly aggressive and cannibalistic, which plays an important role in reducing corn earworm populations when larval density per panicle is high (Barber 1936). Late sixth instars will cease feeding and burrow into the soil to create pupal chambers 5 to 10 cm below the soil surface. The pupa is about 17-22 mm in length, 5.5 mm in width, and is mahogany-brown in color. An individual will remain in the pupal stage for 10 to 25 d during the summer months. With the onset of winter (i.e. reduced temperatures, shortened photoperiod, etc.), pupae will remain in the soil and enter diapause. Only after early summer temperatures begin to warm the soils in the following year will the papa complete their development and emerge as adults.

Larvae vary widely in color. In general, the head is light brown with net-like reticulations but the body can be anything from green, yellow, and pink to brown or mostly black. A pair of dark dorsal stripes runs the length of the body. A broad lateral band also runs just above the spiracles along with a whitish band below them. Black microspines on the body along with a light-colored head help distinguish this species from the fall armyworm, which lack spines and have black heads.

The corn earworm moth varies as much in color as the larvae. Some distinguishing characters include a dark spot near the center of each front wing. The forewings also bear a dark, broad transverse band near the distal margin. The hind wings are light basally, becoming dark distally. The total wingspan is approximately 38 mm. Flight capabilities of the corn earworm, a facultative migrant, in addition to favorable weather patterns contribute significantly to its overall distribution and pest status (Sandstrom et al. 2007, Westbrook 2008).

Distribution and dispersal. Corn earworm is active throughout the year in tropical and subtropical regions but is annually found across much of the US and southern Canada during the summer months (Young and Teetes 1979, Capinera 2007). This species is highly mobile, spreading northward from source populations in southern Texas and Mexico each spring (Sandstrom et al. 2007, Westbrook 2008). Overwintering is typical below about 40 degrees north latitude except in the Pacific Northwest where corn earworm can survive as far north as southern Washington (Capinera 2007, Sandstrom et al. 2007). Local overwintering corn earworm in Kansas has not been confirmed, however, recent changes in production practices such as reduced- and no-till may reduce soil disturbances and provide adequate soil temperatures to sustain diapausing pupae (Matocha 1990).

Host range and economic impact. Due to its wide host range, corn earworm has several common names including as sorghum headworm, tomato fruitworm, vetchworm, and cotton bollworm. In addition to the Poaceae (grasses like corn and sorghum), corn earworm also attacks crops in the Brassicaceae, Solanaceae, Fabraceae, Asteraceae, and Cucurbitaceae families (Capinera 2007). Economic crops that often injured by corn earworm infestation include: alfalfa, clover, cotton, flax, oat, millet, rice, soybean, sugarcane, sunflower, tobacco, vetch, and wheat. Over 15 fruits and ornamentals are also attacked. Sorghum is particularly favored among field crops by corn earworm and has been utilized as a trap crop for other production systems like cotton (Tillman and Mullinix 2004).

Corn earworm larvae can cause foliar damage in the whorls of young sorghum plants but are more commonly found infesting the seed heads (Young and Teetes 1977, Chamberlain and Al 1991, Teetes and Pendleton 2000). Panicle infestations are more serious as they result in direct yield loss when larvae feed on developing seeds. Individual larvae are capable of causing approximately 6% seed loss (Buckley and Burkhardt 1962) and are estimated to cause an average grain loss of about 1.5% of the total sorghum grain produced in the US annually (USDA-APHIS 1978).

Fall Armyworm

Biology and development. During the warm summer months, of the southeastern US and throughout the Gulf Coast states, the fall armyworm completes its life cycle in about 30 d, or as much as 60 d in the spring and autumn and 80-90 d during the winter. Fall armyworm will complete several more generations in the Gulf region than farther north. It has been reported that only a single generation will occur in Minnesota and New York. Two generations are typical in Kansas (Capinera, 2005).

Female fall armyworm moths can produce on average 1500 to 2000 eggs in its life (Capinera 2005). Each dome-shaped egg measures about 0.4 mm in diameter and is laid in a cluster of 100-200. Each cluster of pearl-gray eggs is spread over a single layer, attached to the foliage of a host plant, and covered with a dense layer of wing scales. After 2-3 d the eggs will hatch and larvae move to adjacent plants.

Early instars are positively phototactic and tend to move upwards on a plant in response to light. As a result, 1-3rd instars will feed on the upper portions of the plant canopy, consuming less than 2% of the total foliage they will need to complete development. When larvae reach the 4th-6th instar they become negatively phototactic and retreat into the whorls, under leaves, or into the seed head or ears of the plant (Morrill and Greene 1973). The larval stage will last about 15 d during the summer months but can lengthen to nearly 30 days when temperatures drop. The typical rearing temperature for fall armyworm in the laboratory is 25°C, which corresponds to mean development times of approximately 3.3, 1.7, 1.5, 1.5, 2.0, and 3.7 d for instars 1 to 6, respectively.

Different from corn earworm, the larvae of fall armyworm have dark heads with a distinctive white suture shaped like an inverted "Y." The body is also somewhat dark but varies between light tan or green to almost black. Finally, three narrow, light-colored stripes run down the dorsal side and four distinct tubercles (black spots) appear in a square on the dorsal portion of the 8th abdominal segment.

After six larval molts have been completed and feeding has ceased, the larvae will drop to the ground and pupate 2-8 cm beneath the soil surface within a loosely constructed cocoon of debris. The pupa measures 14-18 mm in length, 4.5 mm in width, and is reddish brown in color. The duration of the pupal stage can range from 7-37 d when mean soil temperatures are 15-29°C, respectively (Sparks 1979). For example, in Florida, the pupal stage may last only 8-9 d during the summer months but extend to 20-30 d during the winters (Capinera 2005). Winter temperatures in Kansas will drop below freezing long enough to prevent pupal fall armyworm, which lacks a diapause mechanism, from overwintering so far north (Spark 1979, Sandstrom et al. 2007).

Adult moths emerge from their pupal tunnels during the evening hours just after sunset (Sparks 1979). Once the wings have expanded the total wingspan is 32-40 mm. Females are not distinctively marked; the forewings are typically a uniform grayish brown or a finely mottled. The forewings of the male are slightly more distinctive and have gray to brown coloring but with triangular white spots on the center of the wing near the distal tips. In both sexes the hind wings are iridescent silver with a dark border. During the first four to five days of life the female will deposit most of her eggs, but the oviposition period can last up the 3 wk. The average adult will live for about 10 d but may live as long as 21 d.

Distribution and dispersal. Fall armyworm, much like corn earworm spreads from its subtropical origins along the Gulf Coast and migrates across the eastern and central regions of the US and into southern Canada. Annual dispersal of adults from source ranges in the southern portions of Texas and Florida can be as much as 482 km per night and is often aided by weather fronts associated with favorable vertical profiles of temperature and wind speed (Sparks 1979, Sandstrom et al. 2007, Westbrook 2008). Unlike corn earworm, the fall armyworm is an obligate migrant, due to its inability to diapause, and must therefore return south to overwinter (Capinera 2005).

Host range and economic impact. Similar to the corn earworm, this species as a wide host range including over 80 recorded species, the majority of which are grasses (Sparks 1979, Capinera 2005). The fall armyworm frequently attacks field crops like corn and sorghum but many more economically important crops are affected including alfalfa, barley, Bermuda grass, buckwheat, cotton, clover, oat, millet, peanut, rice, ryegrass, sugarbeat, sudangrass, soybean, sugarcane, timothy, tobacco, and wheat. As for vegetable crops, only sweet corn is regularly damaged. Several varieties of weed serve as sufficient host plants to fall armyworm as well, including crabgrass (*Digitaria* spp.), bentgrass (*Agrostis* sp.), Johnson grass (*Sorghum* *halepense*), morning glory (*Ipomoea* spp.), nutsedge (*Cyperus* spp.), pigweed (*Amaranthus* spp.) and sandspur (*Cenchrus tribuloides*).

Fall armyworm larvae cause characteristic damage to the foliage of their host plants either by making holes in leaves or creating a pattern known as shot-holing in the whorls of corn or sorghum. When larval abundance is high, cannibalistic behavior will reduce densities to 1-2 larvae per plant (Chapman et al. 1999, Raffa 1987). Older larvae are capable of causing defoliation that can leave the plant looking torn and ragged. The late whorl stage (approx. 30-49 days after planting) is most sensitive to damage by fall armyworm and can result in yield losses of 5-44% in sorghum (Starks and Burton 1979, Martin et al. 1980). In Kansas, this species often infests sorghum panicles late in the growing season but the direct damage it causes to developing seeds has not been quantified (Martin et al. 1980, Chamberlain and Al 1991).

IPM IN SORGHUM

The goal of any pest management program is to manage pest populations below economic injury levels in order to achieve maximum yields while minimizing input costs. Integrated pest management (IPM) is a strategy by which sampling methods and control tactics for a given pest are cost effective, efficient, and conserve environmental quality (Pedigo and Rice 2006). Often, pest management programs incorporate tactics associated with reducing host-plant susceptibility to injury by developing insect tolerant cultivars, reducing pest numbers via the use of pesticides or biological control agents, or by reducing pest resistance to chemical controls. The latter, known as insecticide resistance management (IRM) practices are designed to reduce the risk of insect resistance to commonly employed insecticides. Over the past decade, IRM of corn earworm has been a growing concern among corn and sorghum producers trying to control populations that are increasingly resistant to commonly employed pyrethroid insecticides

(Hutchison et al. 2007, Jacobson et al. 2009). Host-plant resistance screenings of sorghum have resulted in the identification of several lines with acceptable levels of resistance to sorghum midge, stem borer, and shoot fly (Sharma et al. 2005). However, only sorghum midge resistant varieties have been developed for commercial production in Australia due to limited success in maintaining sufficiently high yields (Jordan et al. 1998, Tao et al. 2003, Dillon 2007).

Bioeconomics and Treatment Decisions

IPM programs are best implemented when pest managers understand the ecology and behavior of the pest and use that knowledge to optimize the impact of the chemical, cultural, and/or biological control tactics in their management program. This is accomplished through the development and implementation of decision rules based on the bioeconomics, the relationship between pest densities, host responses to injury, and economic losses of the system (Pedigo and Rice 2006). The most fundamental decision rules incorporated into IPM programs are the economic injury level (EIL) and economic threshold (ET) that were first described by Stern et al. (1959). The EIL represents the lowest population density that will cause economic damage; it is applied in situations requiring the implementation of responsive management tactics. Economic thresholds, on the other hand require preventative management tactics at a point when pest densities must be suppressed in order to avoid reaching the EIL. Maintaining and updating these decision tools allow land managers to make accurate and effective decisions that are essential for mitigating costs associated with applying unnecessary control tactics (Poston et al. 1983, Pedigo et al 1986).

Current Sorghum Headworm Management Recommendations

The control of sorghum headworm in Kansas requires timely scouting during the late flowering through soft-dough stages of seed head development. Headworm sampling involves a “beat-bucket” method in which randomly selected seed heads are vigorously shaken over a bucket (Merchant and Teetes 1992). This technique makes it possible to detect very small larvae that are otherwise unnoticed. Typically, a minimum of 30 heads are sampled for every 32 ha of sorghum. The economic threshold for corn earworm and fall armyworm is the same; 1-2 larvae per sorghum head (Teetes and Wiseman 1979). Decisions to treat an infestation, however, are not only influenced by the number of larvae per head but also the size of the larvae. Approximately 80% of potential damage caused by either species is done by the last two larval instars (Kinzer and Henderson 1968, Martin et al. 1980). Therefore, treatment of late instars is usually unjustified because yield loss has already occurred. A good treatment decision is made when the expected yield and market value of the crop is weighed against the cost of the treatment and the amount of damage that treatment can prevent.

The above management recommendations for sorghum headworm have been in place for over 40 years. Studies conducted by Buckley and Burkhardt (1962) and Kinzer and Henderson (1968) provide crop managers with essential information about the rate of yield reduction per larva present in a sorghum head and their potential impact on different seed head developmental stages. For nearly five decades the data provided by these studies have not only influenced treatment recommendations for headworm management in sorghum but also the (EIL) calculations on which those recommendations are based (Teetes and Wiseman 1979, Knutson and Cronholm 2007). Accurate calculations of the EIL are derived in part from the rate of injury inflicted by the pest (Stern et al. 1959, Pedigo et al. 1986). By combining the essential bioeconomic parameters associated with pest densities, host response to injury, and economic loss, the EIL of a given pest population becomes an important tool and integral part of any IPM program. Unfortunately, the pest injury rates determined in the 1960's was only completed for corn earworm. Due to the similarities in their life histories, and in lieu of quantitative data for the fall armyworm, the known injury rate for corn earworm has been applied to both species of the headworm complex (Martin et al. 1980, Teetes and Pendleton 2000).

PROJECT GOALS AND OBJECTIVES

In order to minimize the costs associated with implementing a control tactic, particularly when considering insecticide applications, accurate sampling and treatment recommendations are needed to allow producers to make an appropriate management decision. For this reason, I conducted the following research to answer questions related to whether corn earworm and fall armyworm damage capabilities are truly the same and whether our current management recommendations are not only still accurate for corn earworm, but also appropriate for use in the control of fall armyworm. The major objectives of this research were to 1) identify species differences in terms of growth on three stages of sorghum seed head development, 2) model species-specific yield loss rates for corn earworm and fall armyworm at different densities, and 3) validate the use of those models under mixed-species infestations. A brief synopsis of my major findings and their potential management impacts will be provided in the final chapter.

Chapter 2 - Effects of sorghum seed maturity on the weight and feeding duration of corn earworm (*Helicoverpa zea*) and fall armyworm (*Spodoptera frugiperda*) larvae

Introduction

Sorghum, *Sorghum bicolor* (L.) Moench, is the fifth most produced cereal crop in the world and the third most important cultivated grain in the US. Despite its environmental tolerances to drought (Rosenow and Clark 1995, Kebede et at. 2000) and stressful temperatures (Ougham and Stoddart 1986, Howarth 1989), sorghum remains susceptible to a wide array of insect pests. Yield losses caused by insects in sorghum costs US producers approximately $80 million annually (Wilde 2006). Pest management programs in US sorghum production systems typically focus on the sorghum midge, *Contarinia sorghicola* (Coquillett), greenbug, *Schizaphis graminum* (Rondani), and several panicle-feeding caterpillars including sorghum webworm, *Celama sorghiella* (Riley), corn earworm, *Helicoverpa zea* (Boddie), and fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Young and Teetes 1977, Wilde 2006). Under favorable conditions, corn earworm and fall armyworm populations can cause significant yield reduction by feeding directly on developing sorghum (Young and Teetes 1977). Corn earworm and fall armyworm are the most frequently observed lepidopterous (Noctuidae) pests that feed on whorl-stage sorghum as well as on developing seeds (Chamberlain and All 1991).

Mixed-species infestations of corn earworm and fall armyworm in panicles occur throughout US sorghum producing regions (Teetes and Pendletom 2000). Colonization of these species is significantly aided by adult flight capabilities and favorable weather fronts that drive moths northward from source populations in southern Texas or Mexico (Sandstrom et al. 2007, Westbrook 2008). Although both species are polyphagous, early-season migrants prefer to lay eggs in silking corn (Capinera 2005, Capinera 2007). Larvae of both species are often associated with plant blossoms, buds, and fruits. Consequently, as silking corn begins to senesce in Kansas, adjacent, late-planted sorghum fields in the half-bloom through hard-dough stages are readily infested when moths disperse away from maturing corn (Stichler et al. 1997, Vanderlip 1993, Sloderbeck et al. 2008). Planting early to avoid damaging larval infestations is not always feasible or effective depending on the timing of moth immigration events.

Interactions between timing of larval infestations and sorghum growth stage (Vanderlip 1993, Stichler et al. 1997) can further complicate treatment decisions for sorghum producers. For example, both corn earworm and fall armyworm must complete 6 larval stages before burrowing into the soil to pupate (Capinera 2005, Capinera 2007). Host availability becomes essential to successive generations. During peak moth activity and subsequent larval development, sorghum panicles are also progressing through three reproductive stages, which include the flowering, soft-dough, and hard-dough stages (Vanderlip 1993, Stichler et al. 1997). Soft-dough stage sorghum is most vulnerable to third to sixth instar corn earworm feeding, which accounts for 95% of the damage observed in the field (Kinzer and Henderson 1968). Kinzer and Henderson (1968) also found that first and second corn earworm instars preferred flowering sorghum. Consequently, sorghum phenology plays a key role in determining yield loss relationships as in other systems such as tobacco and tomato hornworms (Lepidoptera: Sphingidae) in tobacco, western corn rootworm (Coleoptera: Chrysomelidae) in corn, and false chinch bug (Hemiptera: Lygeaidae) in spring canola (Kolodny-Hirsch and Harrison 1986, Spike and Tollefson 1989, Demirel and Cranshaw 2006). In addition, late-planted fields are at greater risk to infestation and insecticide treatments are often warranted, especially when natural enemies are in low abundance

(Wiseman 1985, Sloderbeck et al. 2008). However, the effect of sorghum phenology on fall armyworm growth and feeding habits is not known.

In Kansas sorghum, current management guidelines for headworm infestations urge growers to balance market values with treatment costs prior to making a treatment decision (Michaud et al. 2010), which is based on previous bioeconomic models developed by Buckley and Burkhardt (1962) and Kinzer and Henderson (1968). Consequently, individual corn earworm larvae cause approximately 6% grain damage per larva (Buckley and Burkhardt 1962, Kinzer and Henderson 1968) while 1 to 2 larvae per panicle constitutes an economic threshold (Teetes and Pendleton 2000). Despite the frequent occurrence of mixed-species infestations, quantitative data necessary for developing yield loss relationships are not available for fall armyworm (Buntin 1986, Chamberlain and All 1991), yet the same economic injury level (EIL) established for corn earworm (2 to 3 larvae per panicle; Knutson and Cronholm 2007) is applied to fall armyworm infestations (Martin et al. 1980, Teetes and Pendleton 2000, Michaud et al. 2010). As a result, independent management guidelines do not currently exist for fall armyworm in developing sorghum panicles. While it is generally known that both species feed directly on developing sorghum grain (Buntin 1986, Teetes and Pendleton 2000), quantitative data showing the impact of panicle feeding exists only for corn earworm (Buckley and Burkhardt 1962, Kinzer and Henderson 1968, Teetes and Wiseman 1979). A major assumption in these guidelines is the damage capacity for the two species and sorghum stage preferences are equal. While both species share similar biological characteristics such as developmental times, reproductive capacity, and dispersal rates (Capinera 2005, Capinera 2007, Westbrook 2008, Sparks 1979, Chamberlain and All 1991), differences in larval growth and development at critical sorghum development stages is not known.

Assumptions made in the development of pest management strategies can greatly influence the ability of growers and managers to make correct decisions. Consequently, validating such assumptions leads to improved decision-making, which can lead to increased yields and high-value integrated pest management (IPM) programs. In this regard, it is important to learn how these two species respond to host developmental stages and whether they represent an equivalent threat to maturing sorghum grain. Therefore, the objectives of this study were to: 1) compare larval weights and feeding duration between corn earworm and fall armyworm feeding on key sorghum reproductive stages, 2) determine differences in larval growth between field and laboratory reared populations, and 3) identify the applicability of using lab-reared larvae as experimental proxy for estimating sorghum yield loss due to field infestations.

Materials and Methods

To control for other factors affecting the feeding behavior of larvae, such as species competition or food preference, laboratory experiments were conducted on individual larvae of corn earworm and fall armyworm using no-choice feeding assays containing a single sorghum spikelet. Experimental units or the no-choice feeding arenas were made of a thin-walled, clear-plastic tube, 17 cm × 5.6 cm diameter (Cleartec Packaging, Park Hills, MO), with a tight-fitting end-cap at the base. The top of each arena was covered with white, no-see-um mesh (Quest Outfitters, Sarasota, FL) that allowed for air passage, and secured with a rubber band. All larvae were weighed (g) every 24 hr using an analytical balance (Denver Instrument, Pinnacle Series P-114, Bohemia, NY; error ± 0.001). Change in larval weight was used as an indirect measure of sorghum consumption and larval growth. Feeding arenas were arranged in a completely randomized design on a laboratory bench and kept at room temperature (~22°C) with a photoperiod of 16:8 (light:dark) hr.

Corn earworm and fall armyworm larvae were obtained from 20-yr old laboratory colonies, neither of which had received field material for at least 11 yr (Benzon Research, Inc. Carlisle, PA). Individuals remained in the same feeding arena for the duration of the experiment but received a newly excised sorghum spikelet of the same developmental stage every 24 hr. Dead larvae were replaced with new individuals from the same cohort, which were concurrently maintained on an artificial corn-based diet (Benzon Research Inc., Carlisle, PA); new larvae were recorded as separate replicates in the analysis (Kinzer and Henderson 1968).

Sorghum spikelets were cut from field-collected panicles during a two-week period from early to mid August, 2010. Every 3 to 4 d, panicles of the appropriate developmental stage (flowering, soft-dough, or hard-dough; Vanderlip et al. 1993) were collected from production fields in Geary, McPherson, Riley, and Washington Counties, KS. Varied planting dates created differences in sorghum developmental stages. Specifically, hard-dough panicles were collected from early-planted fields while flowering and soft-dough stages were readily found in later-planted fields; commercially available varieties (i.e. Pioneer 84G62, 84P74, 85G03, 85Y40) were used in all studies. Extra panicles were collected and stored in a refrigerator (3°C ± 1°C) up to 3 d prior to larval exposure.

Larval weight and feeding duration. A 2 × 3 factorial design was used to assess larval growth on sorghum spikelets at different phenological stages. Each species-sorghum treatment combination was confined using individual feeding arenas (*n* = 180). Specifically, main effects consisted of species (corn earworm and fall armyworm) and sorghum growth stage (flowering, soft-dough, and hard-dough) with duration of exposure (d) to each sorghum stage as a repeated measure. Due to the feeding capacity and therefore damage potential of late-instars (third through 6th; Kinzer and Henderson 1968) feeding assays were initiated using third instars.

Source colony validation. To identify whether laboratory findings could be applied to larval populations in the field, differences in larval weights were tested between laboratory-reared and field-collected source populations. Specifically, two populations of corn earworm (field-collected and lab-reared CEW, hereafter referred to as "field CEW" and "lab CEW", respectively) and a lab-reared population of fall armyworm (hereafter "lab FAW") were compared. Field-collected fall armyworm larvae were not available for this experiment. For the wild population, 30 third-instar corn earworms were collected from a production sorghum field in the soft-dough stage (var. Pioneer 84G62) at the Ashland Bottoms Research Farm near Manhattan, KS on 6 August 2010. No-choice feeding assays were conducted for each of the three source populations tested (*n* = 30 per treatment) and larvae were allowed to feed for 5 d. Based on results from the previous experiment (see results; Fig.1, 2), third instars were only fed field-collected sorghum spikelets in the soft-dough stage.

In a concurrent study, the differences in source colonies were examined in the field using exclusion cages (*n* = 10 per source colony type), which enclosed a single sorghum panicle (var. Pioneer 84G62). Enclosure of the panicle prevented any seed damage by other arthropods and vertebrates (e.g., birds), while protecting experimental larvae from natural enemies. Exclusion cages consisted of white, no-see-um mesh (Quest Outfitters, Sarasota, FL) with zippered tops (23 cm diameter and 71 cm long). Zippers provided access to the panicle after cage installation. The base of each exclusion cage was secured using 15.2 cm zip-ties (Gardner Bender, Butler, WI) just below the peduncle. To allow free-movement of larvae within the cage, cylindrical supports made of 14-gauge, galvanized steel wire rope (Impex Systems Group, Inc. Miami, FL) were added, keeping the mesh from resting on the panicle. All panicles were sampled prior to cage installation using the beat-bucket method (Merchant and Teetes 1992) to avoid selection of naturally infested panicles. Exclusion cages were infested with 10 third-instars from the lab and field CEW source colonies. Larvae were placed on panicles using fine, camel-hair paint brushes (#1). All cages were left in the field for the duration of seed head maturity and remained on each sorghum panicle through harvest in late September 2010. Following harvest, damaged seeds on individual sorghum panicles were counted and used as a measure of larval feeding. Control panicles, caged at the time of infestation, were used to determine the level of environmental damage (seed counts described below) experienced by treatment panicles over the course of the experiment.

Larval growth or survivorship was not directly measured in the field, so yield loss was used as an indirect measure to differentiate population performance. To accomplish this, damaged seeds were categorized and counted as undeveloped seed, fungus-infected seed, or larva-consumed seed, as previously described by Buckley and Burkhardt (1962). Undeveloped seeds can be the result of larval feeding on and clipping the palea and lemma structures during the early flowering stage, which ultimately prevents embryo development. Consequently, environmental factors like water stress (Rosenow and Clark, 1995) can also prevent seeds from forming or filling properly. Saprophytic “field fungi” and some *Fusarium* spp. will often invade exposed germplasm after larval feeding and cause the fungus-infected seeds to appear dark and moldy (Cunfer 2008). Finally, feeding damage includes seeds with exposed white germplasm, which is a direct result of larval consumption. Once damaged seeds had been counted, the entire seed head was threshed. Undamaged seed was easily separated during the threshing process and weighed (g). Proportion yield loss was calculated as;

[1]

$$Y = \frac{S_D}{S_D + \left(\frac{S_W}{S_S}\right)}$$

where Y equals the proportion yield loss for an individual sorghum panicle; S_D represents the total number of damaged seeds across all damage categories; S_W is the total weight (g) of threshed seed per panicle; S_S equals the mean seed size (g/seed), which was estimated using mean 100-count seed weights (3 per head); and S_W/S_S represents the estimated number of undamaged seeds in the panicle.

Statistical analysis. To standardize the larval growth response, daily proportion weight change was calculated for all individuals used in the study. Specifically, the end weight (g) of larva for each 24 hr period was divided by the initial larval weight (g) at the time of first exposure to a treatment. To account for within-subject, time-dependent correlations associated with taking multiple measurements on the same individuals (Wang and Goonewardene 2004, Littell et al. 2006) a mixed model approach was implemented using a repeated measures analysis to test differences in larval weight change (PROC MIXED, SAS Institute 2002). The fixed main effects in the model included species (corn earworm, fall armyworm), stage (flowering, soft-dough, and hard-dough sorghum), and exposure (total number of d a larva was exposed to treatment). Starting weight (g) was a covariate to account for the influence of larval size on growth rates (Abrams et al. 1996). The PROC MIXED procedure uses residual maximum likelihood (REML) estimation procedure and the final model included an autoregressive covariance structure that was chosen based on the smallest Akaike information criterion or AIC (Littell et al. 2006). The model also used the model-based calculation of standard error and the between-within method for calculating degrees of freedom. *F*-ratios were used to test various model parameters at a significance level of α = 0.05 and assumptions of normality were met according to the Shapiro-Wilk test statistic (PROC UNIVARIATE, SAS Institute 2002). Simple effects tests were explored for significant interactions by slicing main effects (Littell et al. 2006). Differences in treatment groups were determined using generalized least squares with a Tukey-Kramer multiple comparisons adjustment. Because the response variable for feeding duration was the number of d larvae survived and fed on a given sorghum reproductive stage, a generalized linear model was used to test for differences in the main effects of species and sorghum stage (PROC GLM, SAS Institute 2002).

For the source colony experiment, weight response for each of the three colonies examined (field CEW, lab CEW, and lab FAW) was calculated and analyzed in a second repeated measures model. With the exclusion of sorghum stage, all explanatory variables were the same, but the final model had an unstructured covariance structure.

In the field study, pre-existing seed damage was corrected for in the exclusion cages by subtracting the proportion yield loss observed in control cages from the damage calculated in treatment panicles. Differences in the mean proportion yield loss between corn earworm colonies (field CEW versus lab CEW) were estimated using a two-sample t-test (PROC TTEST, SAS Institute 2002). The *F*-ratio was used to test fit at a significance level of α = 0.05.

Results

Larval weight and feeding duration. The effect of sorghum reproductive stage on changes in larval weight and feeding duration was consistent between both species tested (Table 1). Corn earworm and fall armyworm weight gains remained unchanged when averaged across all sorghum reproductive stages (Fig.1a). Contrastingly, larval feeding duration was different between species, such that corn earworm larvae fed and survived for approximately 2 d longer than fall armyworm (Fig. 2a). In general, changes in larval weight was influenced by the length of exposure (d) to a given sorghum reproductive stage regardless of species (Fig. 3). Larval starting weights (g) did not significantly influence larval growth (Table 1); therefore we excluded this explanatory variable as a covariate in the final model. There was no significant interaction between sorghum reproductive stage and species in terms of larval weight change or feeding duration (Table 1). Although the proportion weight change for corn earworm and fall armyworm was the same at each sorghum stage, weight gain was 1.6 times greater in soft-dough than either the flowering or hard-dough stages (Fig.1b). Similarly, larvae of both species fed for nearly 2 d longer when exposed to soft-dough sorghum rather than flowering or hard-dough stages (Fig. 2b). Changes in larval weights were significantly affected by the interaction between sorghum reproductive stage and exposure time (d). Slicing for sorghum stage showed that this main effect did not significantly influence larval weight change until 2 or more d after initial exposure (*P*s < 0.0001). Conversely, sorghum stage significantly influenced larval weight change at flowering ($F = 2.64$; df = 7, 1559; $P = 0.0104$), soft-dough ($F = 23.91$; df = 7, 1559; $P < 0.0001$), and hard-dough stages ($F = 6.99$; df = 7, 1559; $P < 0.0001$) when sliced across exposure time. The proportion weight change of larvae tested was influenced by the interaction between species and exposure time (d). Slicing for effects showed that corn earworm ($F = 5.80$; df = 10, 1559; $P <0.0001$) and fall armyworm ($F = 7.34$; df = 7, 1559; $P <0.0001$) significantly affected the proportion weight change of larvae tested. Exposure time was an inconsistent effect on larval weight change. A three-way interaction between stage, species, and exposure time was also observed. When sliced across stage and species, the effect of exposure time to treatment was not different for corn earworm feeding on either the flowering or hard-dough stages but was significant for both species feeding on soft-dough (*P*s <0.0001). Exposure was also significantly reduced in all fall armyworm treatments (*P*s <0.008). Keeping the effects of stage and exposure time fixed, species response did not change over time for any sorghum stage (*P*s >0.05). Slicing the interaction by species and exposure time showed that the effect of sorghum stage did not become significant until 2-3 d following initial exposure for fall armyworm and corn earworm, respectively (*P*s < 0.007).

Source colony validation. When larvae were exposed to soft-dough sorghum, proportion weight change was significantly different between larval sources; lab CEW colony gained 35 to 40% more weight than either the field CEW or lab FAW colonies, respectively (Table 1; Fig. 4). Feeding duration was not significantly different between colonies ($F = 1.27$; df = 2; $P = 0.285$). However, exposure time to soft-dough sorghum had a significant effect on the proportion larval weight change when averaged across source colony, increasing by 2.6 over 5 d. Again, larval starting weights (g) did not significantly influence larval growth and was not included as a covariate in the final model (Table 1). There was a significant interaction between source colony and exposure time on larval weights. Slicing this interaction showed that both colony and exposure time after 1 d made a positive difference to larval weight change (Ps <0.006).

Environmental damage observed in control panicles in the field was 28%. Artificial infestation of field CEW and lab CEW colonies in field cages showed no significant differences in mean proportion yield loss due to larval feeding damage (t = -0.13, df = 18; $P = 0.8996$).

Discussion

This study provides insight into the effects of host phenology on the success of noctuid pest larvae in developing sorghum panicles. Specifically, this research demonstrates that both corn earworm and fall armyworm respond positively to soft-dough stage sorghum in terms of weight gain and feeding duration. This result not only supports previous work conducted by Kinzer and

Henderson (1968), which showed that corn earworm of the third through sixth instars preferred to feed on soft-dough sorghum in the laboratory, but provides evidence that fall armyworm responds similarly to developing sorghum seed. Additionally, fall armyworm did respond in equal magnitude to corn earworm in terms of weight gain when averaged across sorghum stage. These results provide the first quantitative evidence that corn earworm and fall armyworm may be equivalent threats to sorghum seed yields, which has important implications for assessing field infestations under current management guidelines.

Although fall armyworm did not survive as long as corn earworm under laboratory conditions they were still capable of gaining 10% more weight than corn earworm on the flowering stage and nearly 30% more on the hard-dough stage. This data, in light of that gathered by Kinzer and Henderson (1968) that showed that 1st-2nd instar corn earworm preferred flowering stage sorghum, demonstrates that insect feeding behavior may change in response to host crop phenology. For example, larvae were able to subsist on both the flowering and hard-dough stages in the laboratory for a short period of time. Therefore, infestations of corn earworm or fall armyworm in flowering sorghum fields may be able to subsist long enough for seed fill to begin (soft-dough), at which time their growth and survival is optimized. There were no interactions between sorghum stage and species, suggesting that larval weight change and feeding duration are additive effects; changing equally in response to each sorghum stage (Perring et al. 1983; Wiseman et al. 1986). When averaged across sorghum stage, there was no difference in larval weight change between species. Slicing of the three-way interaction between stage, species, and exposure time, suggests that despite the reduction in time surviving and feeding by fall armyworm, the cumulative exposure time each species was the same, and may explain the lack of species effect in terms of larval weight change. This result in addition to the species differences observed in terms of weight change and feeding duration when larvae were fed soft-dough sorghum provides evidence that corn earworm and fall armyworm are not equal in their abilities to consume soft-dough seed over an extended period of time. This condition would not occur in the field, however; where larvae are exposed to the continuous physiological development of sorghum seed. Our results show that when proportion weight change is average across all three sorghum developmental stages, the species are equal.

The relationship between the affect of spikelet excision from sorghum panicles is unknown. Kinzer and Henderson (1968) utilized individual spikelets from field-collected sorghum panicles to examine larval feeding preference under laboratory conditions. A number of environmental factors such as temperature, water availability, and light may dramatically influence the nutrient content and potential quality of developing seed in the field (Fenner 1993). For example, high temperatures and drought can increase seed protein content and may alter the balance of fatty acids. While it has been shown that tannin content within various developmental stages of sorghum seed does not affect fall armyworm growth on meridic diet (Wiseman et al. 1986), the effect of nutrient content in developing sorghum seeds on either corn earworm or fall armyworm development has not been investigated and may be a focus for future work.

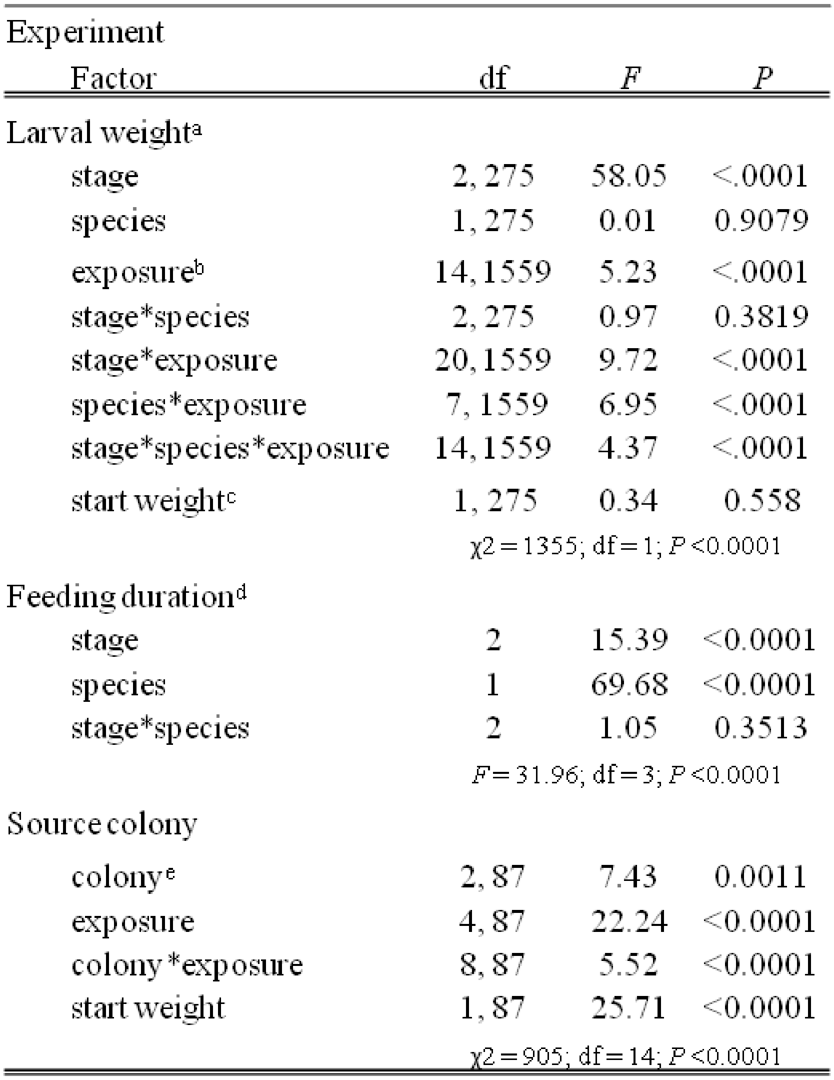
Laboratory feeding assays containing only soft-dough sorghum spikelets showed that the lab CEW colony grew significantly more in terms of weight gain than either the lab FAW or field CEW colonies. The difference in the lab-reared colonies confirmed the results we saw in the larval weight and feeding duration study. A similar growth differential occurred between the corn earworm populations; lab CEW growing significantly more than field CEW. The lack in field CEW response could have been an artifact of a wild population being ill-adapted to a transition to laboratory conditions or to moderate handling.

While proportion yield loss in the field study cannot directly be compared to the weight response of larvae in the laboratory, both response variables can be interpreted as indirect measures of larval consumption or damage potential for CEW populations in sorghum. In the field, no differences were apparent between the two CEW colonies in terms of damage potential. Although these colony studies were conducted using larvae maintained in a colony for over 20 yr, this data suggests that use of the lab-reared CEW colony was valid in the field and could be used to generate conservative estimates in the lab.

Sorghum is an increasingly important field crop in the US and with ongoing advancements in sorghum breeding programs this cereal is well placed for shaping the future of food in many parts of the world (KGSPA 2011). In order for sorghum IPM programs to be successful, we must be able to provide growers with updated and accurate management recommendations. Although guidelines have been in place for the management of corn earworm populations in sorghum, this research has confirmed that sorghum reproductive phenology plays an important role in determining corn earworm survival and damage potential and provides the first documentation for it in fall armyworm. Although this research demonstrates that the assumption of equivalence of corn earworm and fall armyworm larvae feeding in sorghum panicles may be correct, it also confirms that sorghum is most vulnerable to yield loss by corn earworm and fall armyworm during the soft-dough stage. Scouting during the early seed fill will be critical to making an accurate management decision for either species independently or in combination. This study also confirmed the applicability of these results to corn earworm populations in the field and provides insights into the biological responses fall armyworm feeding has on developing sorghum seed in the laboratory. Future field studies could investigate the use of currently implemented management recommendations, which are based on 49 yr estimates for corn earworm yield loss potentials (Buckley and Burkhardt 1962), and to test for species differences with fall armyworm in the field.

Tables and Figures

Table 2.1 Analysis of variance results for the effects of sorghum reproductive stage (flowering = F, soft-dough = SD, and hard-dough = HD) on the mean proportion weight change and feeding duration (d) of corn earworm (*Helicoverpa zea*) and fall armyworm (*Spodoptera frugiperda*) larvae in the laboratory.



Experiment Factor	df	*F*	*P*
Larval weight[a]			
stage	2, 275	58.05	<.0001
species	1, 275	0.01	0.9079
exposure[b]	14, 1559	5.23	<.0001
stage*species	2, 275	0.97	0.3819
stage*exposure	20, 1559	9.72	<.0001
species*exposure	7, 1559	6.95	<.0001
stage*species*exposure	14, 1559	4.37	<.0001
start weight[c]	1, 275	0.34	0.558
	$\chi 2 = 1355$; df = 1; $P < 0.0001$		
Feeding duration[d]			
stage	2	15.39	<0.0001
species	1	69.68	<0.0001
stage*species	2	1.05	0.3513
	$F = 31.96$; df = 3; $P < 0.0001$		
Source colony			
colony[e]	2, 87	7.43	0.0011
exposure	4, 87	22.24	<0.0001
colony*exposure	8, 87	5.52	<0.0001
start weight	1, 87	25.71	<0.0001
	$\chi 2 = 905$; df = 14; $P < 0.0001$		

[a] Change in larval weight was calculated as a proportion by dividing daily end weight (g) by the initial starting weight (g) for each individual larva.

[b] Exposure time (d) is defined as the number of days a larva was exposed to feeding treatments containing sorghum of a given reproductive stage .

[c] The starting weight (g) of individual larvae upon introduction to the study was used as a covariate.

[d] Feeding duration (d) was the length of time larvae survived and fed during the study.

[e] Three source colonies (lab CEW, lab FAW, and field CEW) were compared. Laboratory source colonies of corn earworm and fall armyworm were reared by Benzon Research, Inc. (Carlisle, PA). A field collected corn earworm colony was obtained at Ashland Bottoms Research Farm KSU, near Manhattan, KS.

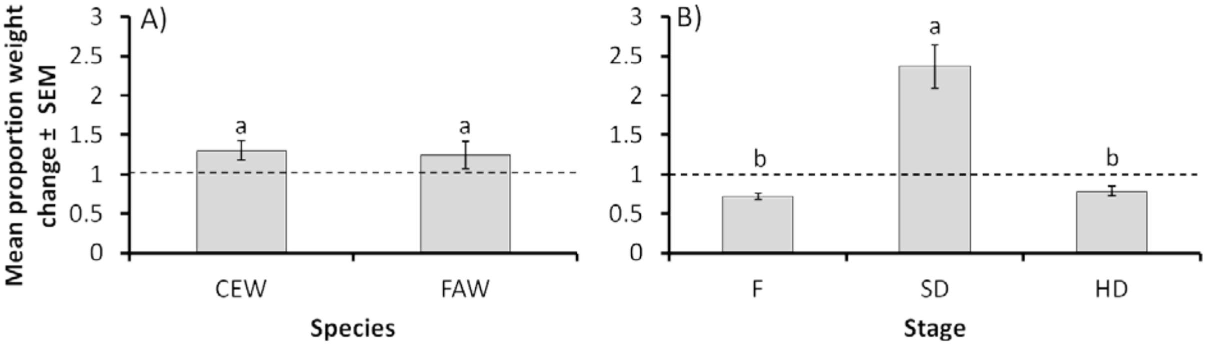


Figure 2.1 The A) species and B) sorghum stage (flowering = F, soft-dough = SD, and hard-dough = HD stages; Vanderlip et al. 1993) effects on the mean proportion weight change ± SEM of corn earworm (CEW), *Helicoverpa zea*, (*n* = 172) and fall armyworm (FAW), *Spodoptera frugiper*da, (*n* = 110) larvae used in a repeated measures laboratory study. Weight measured in g (± 0.0001). Mean proportion weight change values above or below 1.0 represent weight gains or losses, respectively. Bars with the same letter are not significantly different at α = 0.05.

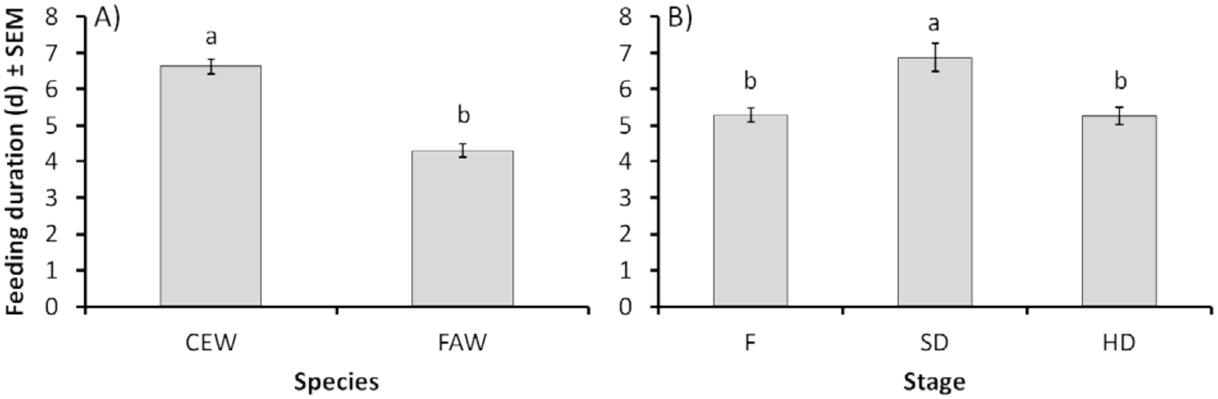


Figure 2.2 Mean exposure time (d) ± SEM for corn earworm (CEW) and fall armyworm (FAW) larvae feeding on sorghum at three reproductive stages: flowering = F, soft-dough = SD, and hard-dough = HD.

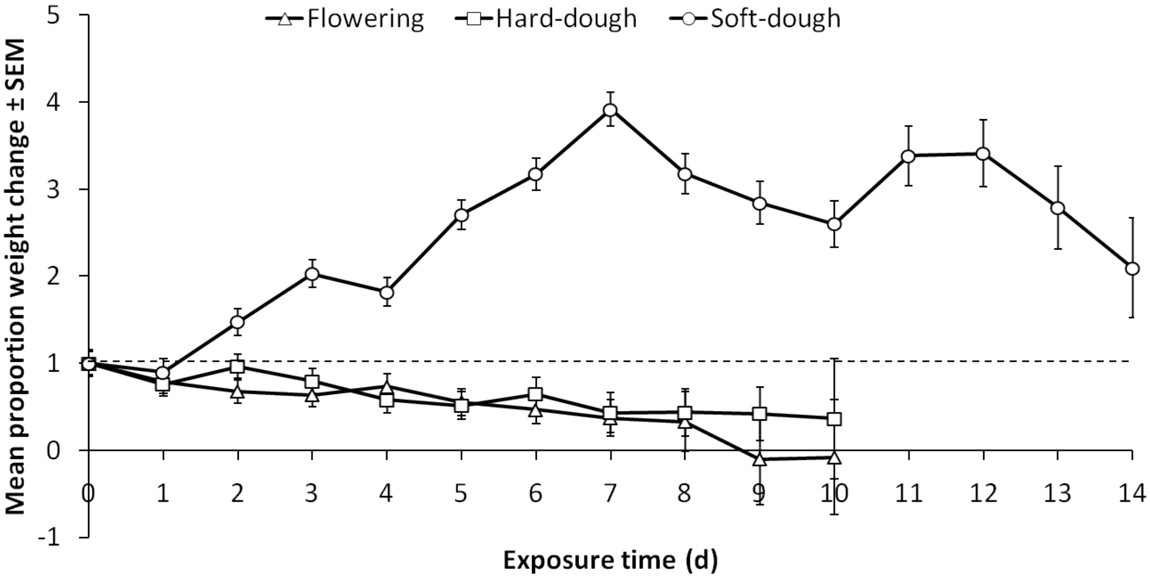


Figure 2.3 Mean proportion weight change ± SEM representing larval growth of the pooled response of corn earworm and fall armyworm over time (d) when feeding on three reproductive stages of sorghum (flowering, soft-dough, and hard-dough) in a repeated measures laboratory study. Mean proportion weight change values above or below 1.0 represent weight gains or losses, respectively.

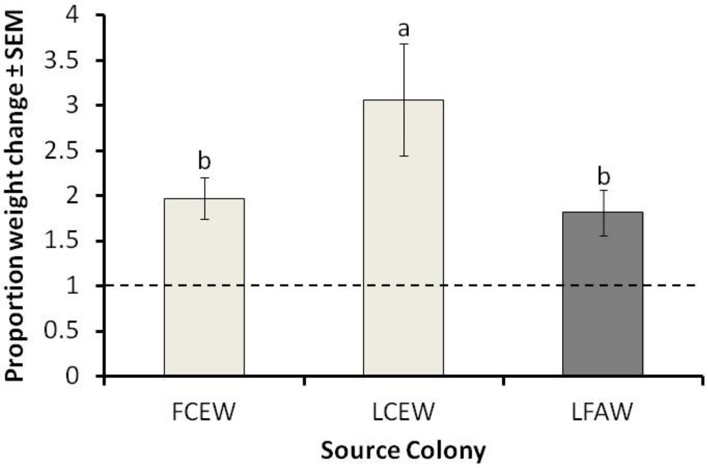


Figure 2.4 Mean proportion weight change ± SEM for larvae from three source colonies: “FCEW” = field-collected corn earworm, *Helicoverpa zea*, (*n* = 29), “LCEW” = laboratory-reared corn earworm, (n = 30), and “LFAW” = laboratory-reared fall armyworm, *Spodoptera frugiperda*, (*n* = 32) feeding on soft-dough stage sorghum for 5 d in a repeated measures laboratory study. Mean proportion weight change values above or below 1.0 represent weight gains or losses, respectively. Bars with the same letter are not significantly different at α = 0.05.

Chapter 3 - Multiple species threshold recommendations in sorghum—is a “one-model-fits-all” strategy the best approach?

Introduction

Insect pests and environmental stressors represent key factors that negatively affect crop yields worldwide. In grain sorghum, *Sorghum bicolor* (L.) Moench, an array of insect herbivores contributes to yield reductions that cost the US sorghum producer $80 million annually (Wilde 2006). Although sorghum is grown predominantly for its drought and heat tolerance (Ougham and Stoddart 1986, Howarth 1989, Kebede et at. 2000) in the arid to semi-arid regions of the Great Plains, severe lack of moisture during panicle differentiation prior to flowering (pre-anthesis) or grain fill (post-anthesis) can cause significant reductions in total seed number or grain size (Rosenow and Clark, 1995).

Corn earworm (CEW), *Helicoverpa zea* Boddie, and fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith, are the primary lepidopteran (Noctuidae) pests of sorghum in the US (Young and Teetes 1977, Wilde 2006). These insects are annual, late-season pests of grain sorghum panicles and infest fields during the early reproductive stages when sorghum is the most vulnerable (Buntin 1980, Chamberlain and All 1991, Teetes and Pendleton 2000). By feeding directly on developing seeds, CEW may cause significant reductions in sorghum yield and a justified treatment at 1 to 2 larvae per panicle is often needed to avoid incurring economic losses (Teetes and Wiseman 1979).

Analysis of yield reductions caused by CEW larvae have consisted of whole-panicle damage assessments based on three damage types originally described by Buckley and Burkhardt (1962) and includes herbivore-consumed, fungus-infected, and undeveloped seeds. Herbivore-consumed seeds included those with exposed white germplasm as a direct result of larval feeding. When

CEW larval infestations are high, molds can occasionally develop in response to the accumulation of frass in the panicle (Kirk 1959) but saprophytic “field fungi” will often invade exposed germplasm after larval feeding, resulting in fungus-infected seeds (Cunfer, 2008). Conversely, undeveloped seeds are those that failed to develop and have been considered the result of larval feeding on or within florets, which ultimately prevents the embryo from developing into a mature, harvestable seed. Although Buckley and Burkhardt (1962) attributed all categories of damage to the direct and indirect results of larval feeding on developing panicles, a number of environmental factors may also affect seed development that results in undeveloped seeds, and includes water and temperature stresses (Fenner 1993; Rosenow and Clark 1995). The degree by which different feeding types affects yield loss models and corresponding management recommendations is not known.

In general, most current management recommendations for CEW in sorghum are based on several studies that model the yield impact of panicle-feeding CEW at varying densities. In natural (Burkhardt and Breithaupt 1955, Burkhardt 1957a & b, DePew 1957) and artificial infestations (Buckley and Burkhardt 1962; Kinzer and Henderson 1968), CEW is shown to reduce grain yields by 10 to 80% at densities of 1 to 16 larvae per panicle, respectively. Despite the frequent occurrence of both CEW and FAW feeding in sorghum panicles during grain development, FAW is more often associated with the vegetative stages of sorghum (Sparks 1979; Buntin 1986; Teetes and Pendleton 2000) yield reductions due to this species have not been determined. Larvae are often difficult to correctly identify by growers in the field. For simplicity in making treatment decisions, equivalent economic threshold (ET) recommendations based on economic injury level (EIL) calculations (Knutson and Cronholm 2007) exist for these two headworm species even in lieu of quantitative data documenting yield loss potential for FAW or mixed species infestations (Martin et al. 1980).

Understanding the biology of economically important pests is an essential step in developing integrated pest management (IPM) programs that effectively mitigate yield losses and costs associated with unwarranted insecticide applications. Changes in commercially available sorghum varieties in conjunction with nearly 50 yr for potential genetic changes in corn earworm populations are substantial reasons for reevaluating current host-pest interactions within this production system.

Using a range of larval densities on commercially available varieties, the objectives of this study were to: 1) determine whether existing yield loss models are still valid for natural populations of CEW infesting sorghum, 2) develop independent yield loss models for CEW and FAW and test the assumption that damage potentials are equal between species, and 3) characterize the contribution of larval feeding to the number of undeveloped seeds in infested sorghum panicles.

Materials and Methods

Two separate field exclusion cage experiments were used to test the above hypotheses. These experiments were located in east-central Kansas and sorghum panicles were infested with CEW and FAW at varying larval densities. Exclusion cages were constructed of white, no-see-um mesh (Quest Outfitters, Sarasota, FL) with zippered tops; cage dimensions were 23 x 71 cm (diameter x length). Each exclusion cage was supported with a 14-gauge, galvanized steel wire (Impex Systems Group, Inc. Miami, FL) cylinder and secured below the peduncle using 15.2 cm zip-ties (Gardner Bender, Butler, WI). All caged panicles were left in the field for the duration of seed development and harvested on 22 September 2010.

Corn earworm yield-loss potential. An exclusion cage experiment was conducted in sorghum (var. Pioneer 84G62) plots at the Ashland Bottoms Research Farm near Manhattan, KS. Field infestations of CEW were identified in early-planted (26 May 2010) and late-planted (11 June 2010) fields at the Ashland Bottoms Research Farm and caged on 29 July and 8 August 2010, respectively. Larval densities were not determined *a priori* but infestation levels were observed in experimental units approximately 7 d following cage installation (flowering stage). Naturally occurring densities ranged from 0 to 16 and 0 to 21 larvae per panicle in the early- and late-planted fields, respectively. Exclusion cages (*n* = 58 experimental units per location) were arranged in a completely randomized design and cage installation coincided with panicle emergence from the boot stage (Vanderlip 1993; Stichler et al. 1997), which provided a protective enclosure against potential CEW predators and reduced losses caused by other seed-feeding insects. Larvae were allowed to feed on developing sorghum seeds until panicles were harvested. At physiological seed maturity, caged panicles were collected and seed damage was assessed in the laboratory (22 September 2010). Larval survival within cages was not assessed during the study but the number of pupae in all experimental units was noted when panicles were harvested and evaluated for damage; these data were not included in our yield-loss analysis.

Species dependent yield-loss models. In a companion study at two different locations, populations of laboratory-reared CEW and FAW (Benzon Research, Inc. Carlisle, PA) were independently introduced into exclusion cages (*n* = 56 per location) at the Ashland Bottoms Research Farm on 29 July 2010 and in a production sorghum field near Palmer, KS on 20 August 2010. Sorghum panicles were infested with third instar CEW or FAW when sorghum seed reached the soft-dough stage. We used fixed-level, single-species infestation densities of 0, 1, 3, 6, 9, 12, and 16 larvae per caged panicle. For meaningful model comparisons, we chose infestation levels based on the studies by Buckley and Burkhardt (1962) and Kinzer and Henderson (1968). Larvae were transferred to developing sorghum seeds using a fine, camel-hair paint brush (#1) and experimental units were arranged in a randomized complete block design.

Seed damage assessment. Seed damage assessments were made for each sorghum panicle using criteria outlined by Buckley and Burkhardt (1962). Specifically, we recorded total number of undeveloped seed, fungus-infected seed, or herbivore-consumed seed for each panicle. After damaged seeds were counted, entire seed heads were individually threshed using a small-grain thresher (Almaco Inc.). Undamaged seed was readily separated during the threshing process and weighed (g). Estimated seed size (g/seed) was determined using mean 100-count seed weights (3 independent samples per head). Proportion yield loss was calculated as,

$$Y = \frac{S_D}{S_D + \left(\frac{S_W}{S_S}\right)} \tag{1}$$

where Y equals the proportion yield loss for an individual sorghum panicle, which was the response variable in all analyses; S_D represents the number of damaged seeds summed for all damage categories or feeding damage only (fungus-infected + larva-consumed); S_w is the weight (g) of threshed seed; S_s equals the mean seed size (g/seed); S_w/S_s represents the estimated number of undamaged seeds in the panicle.

Statistical analysis. Yield loss relationships for naturally occurring and laboratory-reared larvae were analyzed using a mixed effects model (PROC MIXED, SAS Institute 2002) that accounted for fixed and random effects in the experimental design. This approach allowed the coefficients of the regression (slope and intercept) to account for the random effects associated with sorghum panicle selection in the natural (experimental unit) and artificial infestations (block) as well as fixed main effects (species and density). To ensure that estimates represented the true effect of the density treatment we tested slope homogeneity for first, second, and third order polynomials (Robson and Atkinson 1960). Comparisons were made using *F*-ratios at a significance level of α = 0.05. Slopes were homogeneous (within-treatment regression coefficients varied only with respect to intercept) when the quadratic coefficient was incorporated into the model statement. Residual maximum likelihood (REML) was used to estimate model parameters and standard errors. The Satterthwaite method was used for calculating degrees of freedom. Final model selection, using backward elimination, was based on the smallest Akaike information criterion (AIC) (Littell et al. 2006). Again, *F*-ratios were used to test model parameters at a significance level of α = 0.05. Assumptions of normality for variance residuals were met according to the Shapiro-Wilk test statistic (PROC UNIVARIATE, SAS Institute, 2002). Differences in treatment groups were determined using the ESTIMATE statement with contrasts. Differences in damage types were determined using the 95% confidence interval or CI.

Results

CEW yield-loss validation. Increasing larval densities of CEW on sorghum panicles significantly reduced undamaged sorghum seed counts at both locations (Table 1). A polynomial model best explained the yield loss relationship. Larval density, as explained by both first and second order estimates, influenced sorghum yield loss regardless of damage type (all types versus feeding damage only). Specifically, the rate of yield loss for individual panicles was 8 ± 4% for every CEW larva regardless of field location (βX ± 95% confidence interval or CI). Yield losses increased with additional CEW larvae per panicle but reached a plateau before causing reductions in loss at larval densities exceeding 10 to 12 larvae per panicle (Table 2; Fig.1).

Interestingly, control panicles containing no larvae (α or y-intercept) showed yield loss predictions of 54 and 26% in Fields 1 and 2, respectively (Table 2). In other words, yield losses in the control cages were 14-28% higher in the early-planted sorghum field (Field 1) than in the late-planted despite infestation at the same physiological stage (flowering). When undeveloped seed was removed as a damage type, ambient damage levels fell below 8% in Field 2. Additionally, damage was reduced nearly 33% in Field 1 control panicles, but differences were not significant as 95% CI estimates were overlapping (Table 2). When yield loss estimates from both models were compared, removing undeveloped seeds from yield loss calculations did not significantly affect the rate of yield loss for larvae confined to panicles in the field (Table 2). In general, the rate of yield loss for each additional larva ranged from 7 to 10% with overlapping 95% CIs ranging from 2 to 3% for both response variables (all damage types versus feeding only) across locations.

Species dependent yield-loss models. The overall model effects showed that the main effect of species was significant at the Ashland and Palmer fields when all damage types were used to determine yield loss under artificial infestation densities (Table 1). For feeding damage, the species effect was also significant at Palmer but was not significant at Ashland. Despite CEW consistently causing more yield loss than FAW at each location, species were equal based on the 95% confidence intervals CEW and FAW (Table 3; Fig. 2). Additionally, the feeding damage and all damage models were significantly different for CEW at Ashland only. Regardless of damage type, first and second order effects of larval density were significant factors affecting sorghum yield loss (Table 1). At both field replications, the linear relationship between larval density (βX ± 95% confidence interval) and proportion yield loss was such that one larva of either corn earworm or fall armyworm caused approximately 5 ± 2% seed loss (Table 3). As with the natural infestations of CEW, regression coefficients for the quadratic density term (βX^2 ± 95% confidence interval) showed that as the number of larvae per panicle increased, yield loss was reduced at Ashland and Palmer (Table 1; Fig.2)

Discussion

This research demonstrates that CEW and FAW cause equivalent levels of damage and that estimating potential yield loss due to either species in the field is highly dependent on larval density within the panicle. Regardless of the damage types assessed, the rates as well as the pattern of yield loss observed for CEW and FAW were similar. Based on the currently employed ETs and EILs that were originally developed for CEW, our results demonstrate that the assumption that these pests will inflict the same levels of yield loss in the field is correct. This study provides the first quantitative yield loss estimates for FAW feeding in sorghum panicles and provides evidence that treating CEW and FAW as equal threats to grain loss may remain appropriate for sorghum producers needing to manage these species.

Under CEW infestation scenarios, this research supports previous sorghum yield loss estimates generated for natural infestations in developing panicles. Although the observed yield loss was highly variable in control cages, total damage per insect remained fairly consistent and ranged from 4 to 12% regardless of the damage types (with or without undeveloped seeds) assessed. Buckley and Burkhardt (1962) found similar responses ranging from 5 to 12% loss per larva. Increases in larval densities were non-linear with respect to yield loss, however. Across damage types assessed yield losses were typically maximized at larval densities over 10 to 12 larvae per panicle. Not surprisingly, this observed trend suggests that larval density is a significant factor determining overall seed development in infested sorghum panicles despite ambient environmental influences.

Given the frequency of simultaneous infestations of CEW and FAW, this research offers the first species-specific yield loss models for FAW in sorghum panicles. Providing quantitative data, it demonstrates that FAW causes the same level of yield loss as CEW when infesting developing sorghum panicles at equivalent densities in the field. Although species differences were not significant in replicates infested at the flowering and soft-dough stages, CEW did consistently out-perform FAW in terms of seed damage at various larval densities. This suggests that under mixed-species infestations, current treatment recommendations may be over estimating 'headworm' damage, which could result in unnecessary and costly chemical applications. If mixed-species infestations do not cause the yield losses expected under CEW infestations alone, development of a multi-species EIL may be necessary and would require field identification of larvae. Future studies might focus on quantifying the mixed-species impacts on sorghum yield loss in order to determine the necessity of developing a multi-species EIL for headworm infestations.

In this study, yield loss models indicated that regardless of species, assessments of seed damage were not different at a given larval density. Despite the potential for environmental influence on seed development during panicle differentiation prior to flowering (pre-anthesis), these data suggest that larval presence in the panicles is an additional factor affecting seed development. The effects of caging alone on seed development were not determined. It was observed, however, that while undeveloped seeds occurred throughout a given sorghum panicle, they were often concentrated near the base of the panicle (A.M.S. personal observation). Seed development within a panicle occurs from the top-down (Teetes and Pendleton 2000). Therefore, under high infestation densities and high summer temperatures, seeds near the bottom of the panicle may not have developed due to plant stress or the accumulation of frass. Additionally, reductions in yield loss were observed at very high larval densities. Competition for limited resources within a sorghum panicle along with cannibalism could result in increased mortality of larvae and have resulted in the observed decreases in yield loss caused by each species (Wiseman and McMillian 1969, Teetes and Pendleton 2000).

This research provides the first estimates of yield loss for FAW feeding within sorghum panicles. Nearly 50 years after the development of the current CEW recommendations we have also provided a validation of the estimates used in modern CEW IPM programs. Furthermore, this research has confirmed the hereto assumed equivalency of CEW and FAW in terms of yield loss in the field, demonstrating that the application of the CEW EIL to populations of FAW infesting sorghum panicles has been appropriate. Natural infestations of headworm typically consist of both CEW and FAW, however. As a consequence, future studies might focus on quantifying the mixed-species impacts on sorghum yield loss in order to determine the necessity of developing a multi-species EIL for headworm infestations.

Tables and Figures

Table 3.1 Mixed effects models for yield loss in sorghum panicles infested by corn earworm or fall armyworm larvae at varying densities in research fields at Ashland Bottoms Research Farm near Manhattan, KS and a commercial production field in Palmer, KS in 2010.

Experiment Location Effect	All damage [a] df	All damage [a] *F*	All damage [a] *P*	Feeding damage df	Feeding damage *F*	Feeding damage *P*
CEW validation [b]						
Field 1						
Density	1, 141	29.83	<0.0001	1, 141	20.45	<0.0001
Density^{2} [c]	1, 141	10.05	0.0019	1, 141	10.37	0.0016
Field 2						
Density	1, 113	76.38	<0.0001	1, 113	31.5	<0.0001
Density^{2}	1, 113	38.04	<0.0001	1, 113	16.33	<0.0001
Species yield-loss						
Ashland						
Species [d]	1, 49	18.45	<0.0001	1, 49	2.61	0.1129
Density	1, 49	22.81	<0.0001	1, 49	25.97	<0.0001
Density^{2}	1, 49	6.98	0.011	1, 49	17.2	0.0001
Palmer						
Species	1, 49	22.23	<0.0001	1, 52	20.44	<0.0001
Density	1, 49	18.15	<0.0001	1, 52	25.18	<0.0001
Density^{2}	1, 49	7.13	0.0102	1, 52	10.95	0.0017

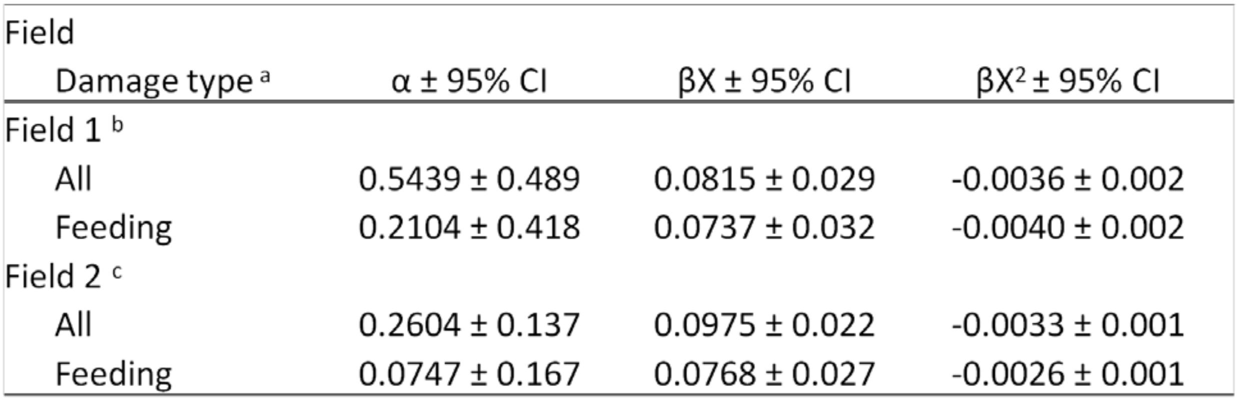
[a] All damage type refers to three seed damage categories (undeveloped, fungus-infected, and larva-consumed seed) used to calculate the proportion yield loss within treatments. Feeding damage type excludes undeveloped seed.

[b] Natural infestations of corn earworm (CEW) occurring at densities ranging from 0-16 in Field 1 and 0-21 in Field 2 at Ashland Bottoms Research Farm, Manhattan, KS.

[c] Second order polynomial describing the relationship between larval density and sorghum yield loss.

[d] Corn earworm, *Helicoverpa zea*, and fall armyworm, *Spodoptera frugiperda*.

Table 3.2 Intercept and slope coefficients (± 95% confidence interval or CI) for a mixed effects model on sorghum yield loss, based on two damage types, for field infestations of corn earworm larvae in early- and late-planted fields at Ashland Bottoms Research Farm near Manhattan, KS in 2010.



Field Damage type [a]	α ± 95% CI	βX ± 95% CI	βX^2 ± 95% CI
Field 1 [b]			
All	0.5439 ± 0.489	0.0815 ± 0.029	-0.0036 ± 0.002
Feeding	0.2104 ± 0.418	0.0737 ± 0.032	-0.0040 ± 0.002
Field 2 [c]			
All	0.2604 ± 0.137	0.0975 ± 0.022	-0.0033 ± 0.001
Feeding	0.0747 ± 0.167	0.0768 ± 0.027	-0.0026 ± 0.001

[*] Intercepts with non-overlapping 95% confidence intervals are different.

[a] All damage types includes undeveloped, fungus-infected, and larva-consumed seeds; Feeding damage excluded undeveloped seed.

[b] The early-planted field (Field 1) was established on 26 May 2010.

[c] The late-planted field (Field 2) was established on 11 June 2010.

Table 3.3 Intercept and slope coefficients (± 95% confidence interval or CI) for mixed effects model on sorghum yield loss based on two damage types for artificial infestations of two species of noctuid larvae in two locations in KS, 2010.

Location [a], Species [b] Damage type [c]	α ± 95% CI	βX ± 95% CI	βX^2 ± 95% CI
Ashland, CEW			
All *	0.3757 ± 0.088	0.0503 ± 0.021	-0.0017 ± 0.001
Feeding	0.1211 ± 0.083	0.0505 ± 0.019	-0.0026 ± 0.001
Ashland, FAW			
All	0.2360 ± 0.088	0.0503 ± 0.021	-0.0017 ± 0.001
Feeding	0.0716 ± 0.083	0.0505 ± 0.019	-0.0026 ± 0.001
Palmer, CEW			
All	0.2224 ± 0.088	0.0477 ± 0.022	-0.0019 ± 0.001
Feeding	0.0745 ± 0.083	0.0509 ± 0.020	-0.0021 ± 0.001
Palmer, FAW			
All	0.0593 ± 0.088	0.0477 ± 0.022	-0.0019 ± 0.001
Feeding	-0.0675 ± 0.083	0.0509 ± 0.020	-0.0021 ± 0.001

[*] Intercepts with non-overlapping 95% confidence intervals are different.

[a] Fields located at Ashland Bottoms Research Farm, Manhattan, KS and in Palmer, KS.

[b] Corn earworm (CEW), *Helicoverpa zea*, and fall armyworm (FAW), *Spodoptera frugiperda*.

[c] All damage type includes undeveloped, fungus-infected, and larva-consumed seeds; Feeding damage type excludes undeveloped seed.

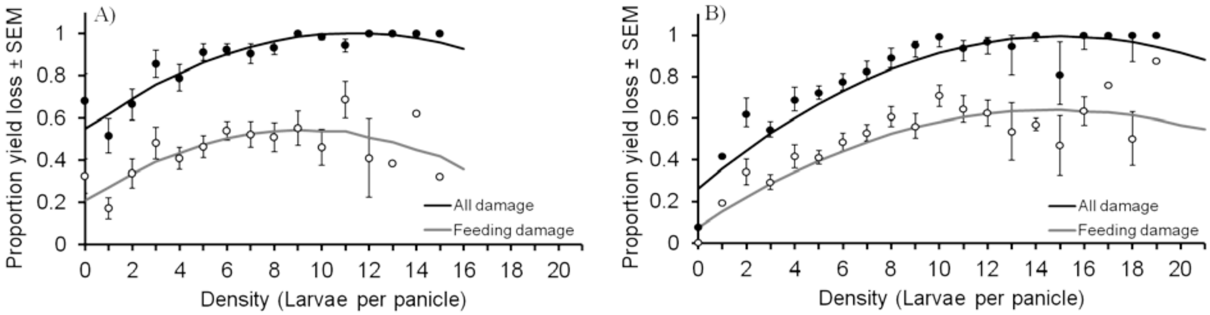


Figure 3.1 Models of the best linear unbiased predictors for the proportion yield loss based on all damage types or feeding damage only in caged sorghum panicles when infested with field populations of corn earworm, *Helicoverpa zea*, at varying densities in A) early-planted and B) late-planted fields infested at the soft-dough stage at the Ashland Bottoms Research Farm near Manhattan, KS in 2010.

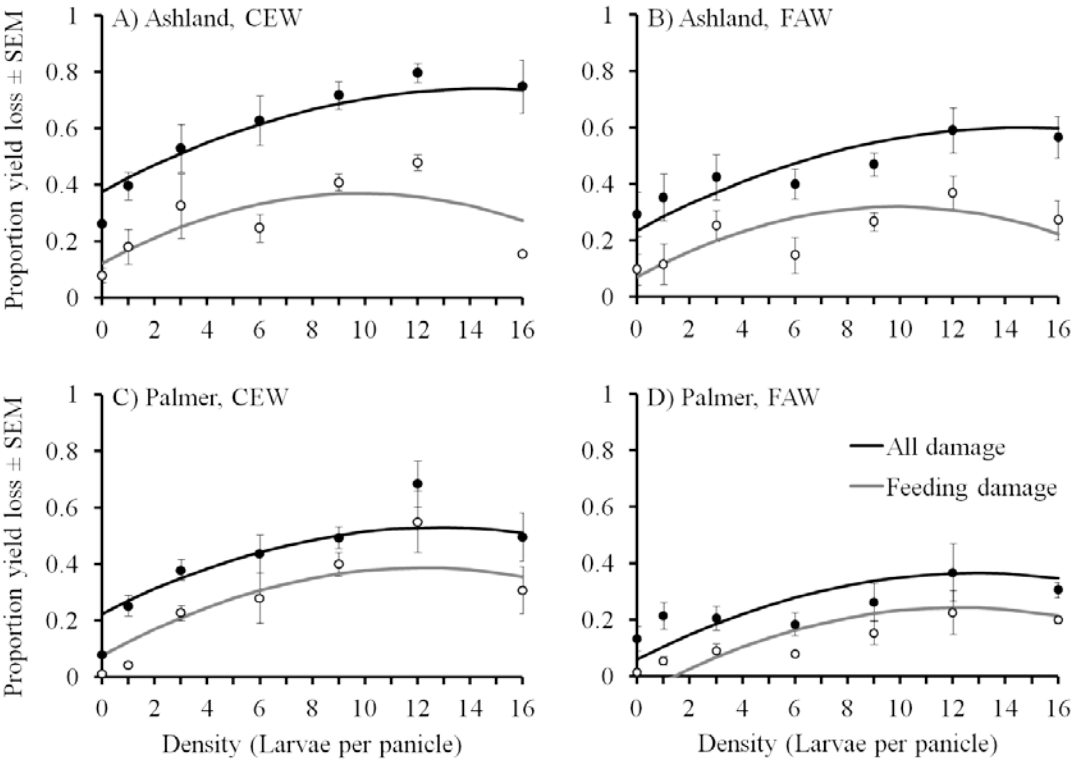


Figure 3.2 Models of the best linear unbiased predictors for proportion yield loss based on all damage types or feeding damage only in caged sorghum panicles when artificially infested at the soft-dough stage with lab-reared populations of corn earworm (CEW), *Helicoverpa zea*, and fall armyworm (FAW), *Spodoptera frugiperda*, at varying densities at the Ashland Bottoms Research Farm near Manhattan, KS and Palmer, KS in 2010.

Chapter 4 - Quantifying impacts of two headworm species on yield loss in sorghum production fields

Introduction

Sorghum headworm is a species complex primarily composed of corn earworm (CEW), *Helicoverpa zea* Boddie, and fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith. These insects are annual, late-season pests of grain sorghum, *Sorghum bicolor* (L.) Moench, panicles and are often found in mixed species infestations throughout the southern Great Plains (Chamberlain and All 1991, Teetes and Pendleton 2000). Although FAW is most often reported as a concern in sorghum during the vegetative stages, it also feeds directly on seeds within the panicle (Henderson et al. 1966, Sparks 1979, Buntin 1986). Conversely, CEW is well documented as the dominant seed consumer in developing sorghum panicles in the field (Buckley and Burkhardt 1962, Kinzer and Henderson 1968, Teetes and Wiseman 1979). As a result, CEW research has been the foundation for the bioeconomic models and management guidelines currently employed for headworm control in sorghum (Teetes and Wiseman 1979, Martin et al. 1980, Knutson and Cronholm 2007).

The current assumption that CEW and FAW larvae cause equivalent levels of seed damage in sorghum (Teetes and Pendleton 2000, Martin et al. 1980) was recently confirmed in field studies demonstrating that the yield loss due to FAW infestations was not different from that due to CEW (Chapter 3). While the implementation of independent species models for estimating yield loss in the field were shown to be unnecessary in Chapter 3, the effects of combined species infestations on yield loss are unknown. For example, the current headworm economic threshold (ET) of 1 to 2 larvae per panicle (Teetes and Wiseman 1979) and economic injury level (EIL) of

2 to 3 larvae per panicle (Knutson and Cronholm 2007) were determined for CEW infestations only.

Often, interactions between pests can make the development of multiple species recommendations and EILs challenging if not unsuitable for use in many integrated pest management (IPM) programs (Pedigo et al. 1986). For headworm species, cannibalism and inter-specific competition (Wiseman and McMillian 1969, Raffa 1987, Dial and Adler 1990, Chapman et al. 1999) may affect yield losses when the two species are mixed under field conditions. Multi-species EIL and ET decision rules may be applicable, however, when pest injuries produce the same host response, such as fruit destruction, or when injuries are additive rather than interactive for the species involved (Hutchins et al. 1988).

Timely and effective agricultural decision making tools for crop producers are essential elements in any successful integrated pest management (IPM) program. Providing such tools to growers can be particularly challenging when multiple species infestations threaten yields. The objectives of this study were to 1) determine sorghum yield losses under mixed-species scenarios, 2) establish whether yield loss models for CEW accurately estimate the observed sorghum damage under mixed-species infestations, and 3) to discuss the potential for the development of a multiple-species threshold and EIL for sorghum headworm.

Materials and Methods

Experimental design. A field exclusion cage experiment in sorghum (var. Pioneer 84G62) was used to test the hypothesis that mixed ratio populations consume disproportionate amounts of sorghum compared to single-species population. The experiment was conducted at two locations in east-central Kansas. Artificial infestation of laboratory-reared CEW and FAW (Benzon Research, Inc. Carlisle, PA) were infested into cages (*n* = 60 per location) at the

Ashland Bottoms Research Farm on 29 July 2010 and in a production sorghum field near Palmer, KS on 20 August 2010. This study was concurrent with experiments outlined in Chapter 3. Cages were constructed of white, no-see-um mesh (Quest Outfitters, Sarasota, FL) with zippered tops; where cage dimensions were 23 x 71 cm (diameter x length). A galvanized wire (14-guage) cylinder was used to support the cages (Impex Systems Group, Inc. Miami, FL) and zip ties (Gardner Bender, Butler, WI) were secured below the peduncle to prevent larvae from escaping the panicles. Experimental units were arranged in a randomized complete block design. Third instars were infested onto developing sorghum panicles using a fine, camel-hair paint brush (#1). We used fixed-level, mixed-species infestation densities where the total number of larvae per cage was determined *a priori*. At both locations, density levels of 4, 10, and 16 larvae were infested into caged panicles at 5 fixed species ratios (CEW:FAW) within each density level at 0:1, 1:3, 1:1, 3:1, and 1:0 (Table 1); such that populations comprised of 0, 25, 50, 75, or 100% FAW, respectively. Total densities were chosen based on the range in artificial infestation levels that were observed in Chapter 3. All caged panicles were harvested on 22 September 2010 and larvae were allowed to feed for the duration of seed development.

Yield loss per panicle was determined according to the methods described in Chapter 3 and included whole-panicle damage assessments with counts of herbivore-consumed, fungus-infected, and undeveloped seeds (Buckley and Burkhardt 1962). Mean 100-count seed weights (3 independent samples per head) were used to estimate seed size (g/seed) and yield loss was calculated as follows;

$$Y = \frac{S_D}{S_D + \left(\frac{S_W}{S_S}\right)} \tag{1}$$

Y is equal to the proportion yield loss for an individual sorghum panicle; the number of damaged seeds summed for all damage categories is represented by S_D; the weight (g) of undamaged seed is S_W; S_S is the calculated mean seed size (g/seed); S_W/S_S represents the estimated number of undamaged seeds in the panicle; the summation of the denominator is equal to the total number of seeds within a given panicle.

Statistical analysis. The response variable measured for both observed and predicted data was proportion yield loss. Predicted yield loss values were determined for each larval density level observed from the CEW model developed in Chapter 3. For example, at a CEW:FAW ratio of 3:1, the predicted yield loss caused by 4 CEW was used, which should be equivalent under the assumption that both species causes the same amount of yield loss per larvae. The observed values for proportion yield were calculated as described above.

The observed and predicted yield loss relationships were analyzed using a mixed effects model (PROC MIXED, SAS Institute 2002) that accounted for fixed (ratio and density) and random effects (block) in our experimental design. Assuming that the non-linear relationship (quadratic) between larval density and yield loss accurately represents yield loss as a function of density regardless of species (Chapter 3), we employed models that included a second order density term. Although this term was non-significant (see results; Table 2) it was used to construct a general estimation of a mixed-species model but was removed in order to determine the true effects of species ratio on yield loss. Mean-squared error (MSE) was calculated for residuals between observed values of yield loss and the predicted values determined by each model in order to compare model fit (Steiger 1998). Residual maximum likelihood (REML) was used to estimate model parameters and standard errors. The Satterthwaite method was used for calculating degrees of freedom (Littell et al. 2006). Assumptions of normality for variance residuals were met according to the Shapiro-Wilk test statistic (PROC UNIVARIATE; SAS Institute, 2002). Differences in treatment groups were determined using the ESTIMATE statement with contrasts.

Results

Overall model effects for CEW yield loss predictions were provided from the Chapter 3 showing the main effects of larval density were significant, at Ashland and Palmer (Table 2). The quadratic term for density was also significant, where reductions in yield loss occurred with increasing numbers of larvae per panicle (Table 3, Fig. 1).

The analysis conducted on the observed values of yield loss showed that the main effect of mixed-species ratio was important at Ashland and Palmer, but the opposite was true for the effects of density and the quadratic density estimate (Table 2). Although there were no significant differences between the predicted and observed models according to 95% confidence interval (CI), observed mean values were 20% and 12% lower than the predicted at Ashland and Palmer, respectively (Table 3). Similarly, predicted levels of yield loss were approximately 5 ± 2% per larvae (βX ± 95% CI), whereas observed models predicted 2 ± 3% per larvae, regardless of species ratio. Mean-squared error values were lower in the observed models for both locations, showing a better model fit.

Removing the quadratic density term to determine the true effect of species ratio and larval density revealed that both were influential factors affecting proportion yield loss at either location (Table 2). Species differences were significant at Palmer and Ashland. FAW consistently caused the lowest levels of yield loss at both locations (Table 4). A significant difference was observed for one mixed-species combination at each location but varied by ratio and magnitude yield loss; 25% FAW ratios were significantly higher than any other species combination at Ashland while 50% FAW was significantly lower at Palmer. The highest observed mean yield loss estimates were found in mixed infestations consisting of 25% FAW. Additionally, infestations consisting of 75% FAW caused significantly higher levels of yield loss than panicles with 100% FAW. The 1:1 species ratio was not significantly different from the CEW controls at Ashland but was significantly lower at Palmer.

Discussion

This study demonstrates that the yield reduction potential for the combination of CEW and FAW in field infestations cannot adequately be predicted from CEW model estimates of yield loss and indicates that species damage potentials change under mixed infestation scenarios. Although the density-squared yield loss response to larval density was not significant in the observed model, as we had expected given the single-species trends determined in Chapter 3, it did provide a better yield loss model than the predicted model for mixed species infestations. Overall, these results begin to refine the insect-host relationships for headworm in sorghum with regard to yield loss and could have important implications for the future development of a multiple-species ET and EIL in this system.

Observed levels of yield loss caused by mixed headworm infestations are lower than those of the predicted model. Although there was no statistical difference between models, the predicted yield loss values were over double observed levels, which could have serious consequences for growers looking to reduce unnecessary insecticide applications. Additionally, the low MSE of the observed yield loss model suggests that this would be the most appropriate model to estimate mixed-species damage at varying densities in the field.

Damage estimates by ratio showed that CEW causes significantly more yield loss in the field than does FAW; an inconstant result with those found in Chapters 2 and 3. Although two of the three mixed-species ratios showed no differences when compared to the yield loss produced by CEW, the one that did varied by ratio, magnitude, and location. While the results for mixed-species ratio might be generalized as equivalent to CEW damage, the variability observed suggests that other confounding factors, such as larval behavior, may be influential in determining yield loss estimates under field conditions (Wiseman and McMillian 1969, Raffa 1987, Dial and Adler 1990, Chapman et al. 1999). Therefore the future development and implementation of a generalized mixed species model may be warranted.

Current management recommendations for headworm in sorghum panicles are based off of estimations of approximately 6% yield loss per larva (Buckley and Burkhardt 1962). In Chapter 3, it was demonstrated that one larva of either CEW or FAW could cause 5 ± 2% yield loss. This study confirms these results; despite the increased variance that was likely due to having only 3 density levels, an estimation of 2 ± 3% yield loss per larvae was determined. The observed species differences in terms of mean yield loss for CEW and FAW across densities suggest that estimates of yield loss per larva and the established ET and EIL (Teetes and Wiseman 1979, Knutson and Cronholm 2007) currently employed for headworm in sorghum may need further investigation and refinement.

Overall, these results indicate that the use of the CEW yield loss model under multiple-species infestations may not only overestimate levels of damage observed in the field but could result in costly and unnecessary chemical applications if such a strategy were implanted by sorghum producers. Additionally, the observed variability in yield loss by species or per larva suggests the need to further investigate how larval behaviors associated with competition or cannibalism may play a role in reducing sorghum yields (Raffa 1987, Dial and Adler 1990).

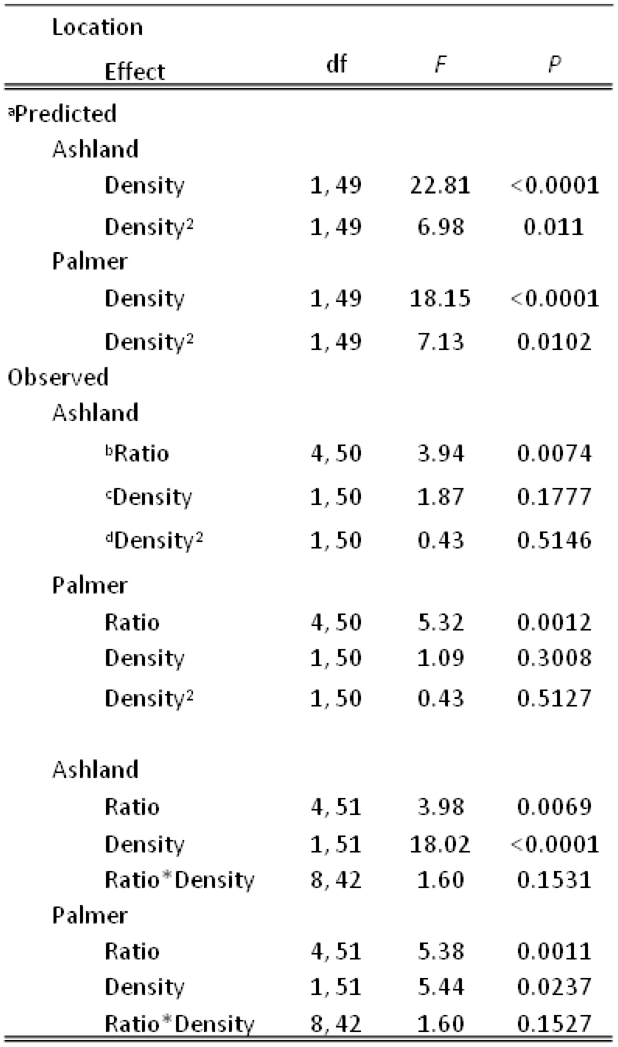
Multiple-species ETs and EILs, while challenging to develop, can be implemented successfully (Hutchins et al. 1988, Zi-Qiang et al. 1991, Hunt et al. 2003). As sorghum production systems continue to change (Matocha 1990) and with CEW insecticide resistance on the rise (Hutchison et al. 2007), the development of multiple-species decision rules may be of increased importance for future research.

Table and Figures

Table 4.1 Fixed, mixed-species and control infestation levels of corn earworm (CEW), *Helicoverpa zea*, and fall armyworm (FAW), *Spodoptera frugiperda*, on caged sorghum panicles consisting of three densities (larvae per panicle) at five CEW:FAW ratios equivalent to approximately 0:1, 1:3, 1:1, 3:1, and 1:0.

CEW	FAW	Density
0	4	4
1	3	
2	2	
3	1	
4	0	
0	10	10
2	8	
5	5	
8	2	
10	0	
0	16	16
4	12	
8	8	
12	4	
16	0	

Table 4.2 Mixed effects models for yield loss in sorghum panicles infested with corn earworm and fall armyworm larvae at varying ratios and densities in research fields at Ashland Bottoms Research Farm near Manhattan, KS and a commercial production field in Palmer, KS in 2010.



Location Effect	df	*F*	*P*
[a]Predicted			
Ashland			
Density	1, 49	22.81	<0.0001
Density^2	1, 49	6.98	0.011
Palmer			
Density	1, 49	18.15	<0.0001
Density^2	1, 49	7.13	0.0102
Observed			
Ashland			
[b]Ratio	4, 50	3.94	0.0074
[c]Density	1, 50	1.87	0.1777
[d]Density^2	1, 50	0.43	0.5146
Palmer			
Ratio	4, 50	5.32	0.0012
Density	1, 50	1.09	0.3008
Density^2	1, 50	0.43	0.5127
Ashland			
Ratio	4, 51	3.98	0.0069
Density	1, 51	18.02	<0.0001
Ratio*Density	8, 42	1.60	0.1531
Palmer			
Ratio	4, 51	5.38	0.0011
Density	1, 51	5.44	0.0237
Ratio*Density	8, 42	1.60	0.1527

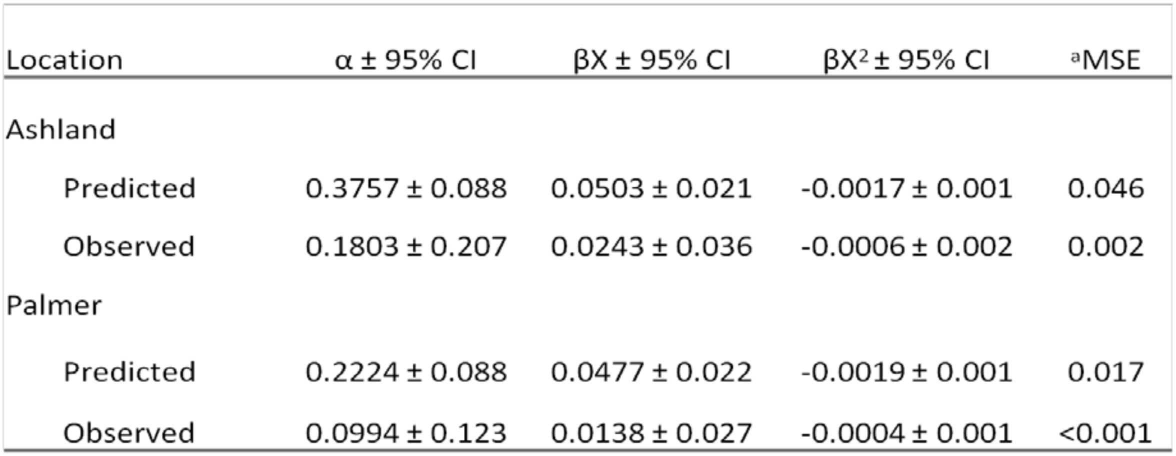
[a] Predicted model results for CEW yield loss from Chapter 3.

[b] Ratios of corn earworm (CEW), *Helicoverpa zea*, and fall armyworm (FAW), *Spodoptera frugiperda*, at 1:0, 3:1, 1:1, 1:3, and 0:1.

[c] Larval densities of 4, 10, and 16 larvae per panicle.

[d] Second order polynomial describing the relationship between larval density and sorghum yield loss.

Table 4.3 Intercept and slope coefficients (± 95% confidence interval or CI) and the mean-squared error (MSE) for the predicted or observed estimates of sorghum yield loss for corn earworm or mixed-species infestations of corn earworm and fall armyworm larvae, respectively, in fields at Ashland Bottoms Research Farm near Manhattan, KS and Palmer, KS in 2010.

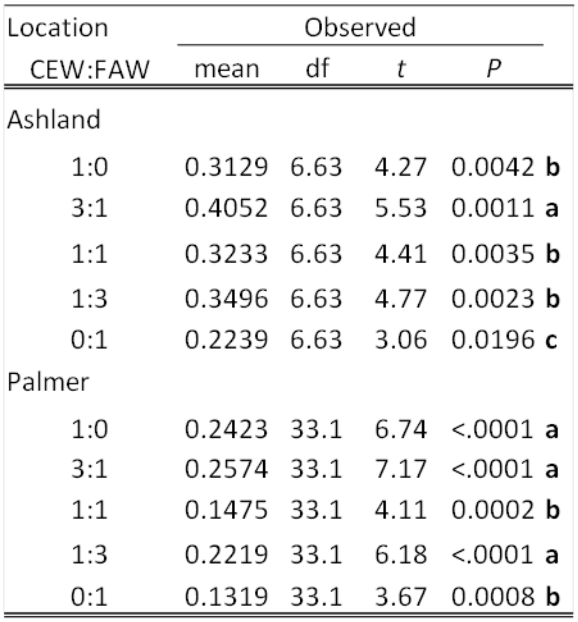


Location	α ± 95% CI	βX ± 95% CI	βX^2 ± 95% CI	[a]MSE
Ashland				
Predicted	0.3757 ± 0.088	0.0503 ± 0.021	-0.0017 ± 0.001	0.046
Observed	0.1803 ± 0.207	0.0243 ± 0.036	-0.0006 ± 0.002	0.002
Palmer				
Predicted	0.2224 ± 0.088	0.0477 ± 0.022	-0.0019 ± 0.001	0.017
Observed	0.0994 ± 0.123	0.0138 ± 0.027	-0.0004 ± 0.001	<0.001

[*] Intercepts with non-overlapping 95% confidence intervals are different.

[a] Mean-squared error (MSE) of the difference between observed and predicted estimates of yield loss.

Table 4.4 Observed yield loss estimates for five corn earworm: fall armyworm infestation ratios in sorghum panicles at Ashland Bottoms Research Farm nearly Manhattan, KS and Palmer, KS in 2010.



Location	Observed			
CEW:FAW	mean	df	*t*	*P*
Ashland				
1:0	0.3129	6.63	4.27	0.0042 **b**
3:1	0.4052	6.63	5.53	0.0011 **a**
1:1	0.3233	6.63	4.41	0.0035 **b**
1:3	0.3496	6.63	4.77	0.0023 **b**
0:1	0.2239	6.63	3.06	0.0196 **c**
Palmer				
1:0	0.2423	33.1	6.74	<.0001 **a**
3:1	0.2574	33.1	7.17	<.0001 **a**
1:1	0.1475	33.1	4.11	0.0002 **b**
1:3	0.2219	33.1	6.18	<.0001 **a**
0:1	0.1319	33.1	3.67	0.0008 **b**

*Different letters are different at a significance level of 0.05.

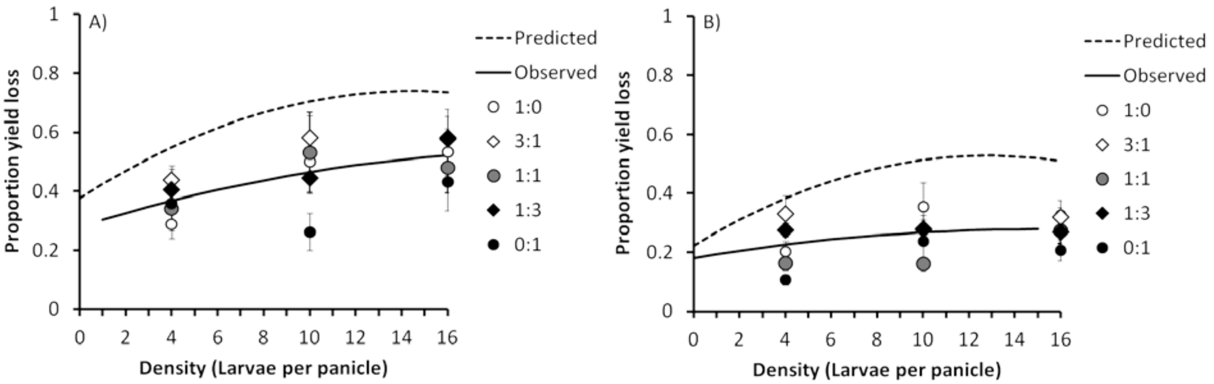


Figure 4.1 Observed models for the proportion yield loss of five corn earworm: fall armyworm ratios in caged sorghum panicles when infested at three densities (4, 10, and 16 larvae per panicle) in comparison to corn earworm prediction models for A) the Ashland Bottoms Research Farm near Manhattan, KS and B) a commercial production field near Palmer, KS in 2010.

Chapter 5 - Conclusions

Through my thesis research I have worked toward providing the Kansas grain sorghum producer with updated and relevant management recommendations for headworm in the field. My goals were to ensure that current treatment guidelines remain appropriate for CEW and mixed-species infestation management while also establishing the legitimacy of the assumption that FAW behaved as CEW does when feeding on sorghum seed in the field. As a result of my master's work, I have validated current recommendations, provided evidence for previously untested assumptions, and identified several areas of need for future research.

In summary of my key findings, I provided evidence in support of the feeding preference originally determined by Kinzer and Henderson (1968) by demonstrating in Chapter 2 that CEW responded positively in terms of growth and feeding duration to feeding assays containing soft-dough stage sorghum. The same was discovered for FAW, although my work provides the first documentation for this species. In Chapter 3, my research not only confirmed the CEW yield loss estimates that have been the foundation of management guidelines for nearly half a century but also the assumption that CEW and FAW are equal threats to sorghum seed loss in the field. Finally, through the research presented in Chapter 4, I found that while CEW and FAW may cause equal levels of yield loss under single-species conditions, the combination of both species results in significant differences.

Overall, my thesis research supports current headworm management in Kansas grain sorghum. After nearly 50 years of the current CEW recommendations I have validated the yield loss estimates used in modern CEW IPM programs. Furthermore, I have presented the first estimates of yield loss for FAW feeding within grain sorghum panicles and confirmed the previously assumed equivalency in pest status between CEW and FAW in the field;

demonstrating that the application of the CEW EIL to populations of FAW infesting sorghum panicles is appropriate. Under natural infestation scenarios that consist of both CEW and FAW, however, yield losses are not predictable based on the assumption of species equality, suggesting the necessity of developing a multi-species EIL for headworm infestations.

In order to minimize the costs associated with implementing a control tactic, particularly when considering insecticide applications, accurate sampling and treatment recommendations are needed to allow producers to make an appropriate management decision. The above research was essential to our current understanding of the yield loss dynamics within sorghum production systems faced with the need to control headworm. While establishing the applicability of existing treatment recommendations for the management of CEW and FAW populations independently, I have also determined that those same guidelines may provide growers with a conservative strategy to headworm control under mixed-species conditions. Inter-specific competition and cannibalistic behaviors could contribute to the observed deviation in expected yield loss by multi-species infestations and might be an important area for future work. Finally, pest sampling tactics within-field and in surrounding crops could be critical to determining the timing and phenological implications of larval infestations in grain sorghum. Together, with updated yield loss estimates and sampling strategies, Kansas sorghum producers would be able save both time and money that might otherwise be associated with unwarranted insecticide applications.

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