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EFFECTS OF DEFOLIATION IN SOYBEANS AND SUSCEPTIBILITY OF SOYBEAN LOOPERS TO REDUCED RISK INSECTICIDES

By

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EFFECTS OF DEFOLIATION IN SOYBEANS AND SUSCEPTIBILITY OF SOYBEAN LOOPERS TO REDUCED RISK INSECTICIDES

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Insect defoliation thresholds were reevaluated to determine their effectiveness during vegetative and reproductive stages of soybean. Field experiments were planted with maturity group IV soybeans and subjected 17, 33, 66, and 100% defoliation during R3, R5, and R6 growth stages. In addition to different amounts of defoliation for each growth stage, defoliation occurring within different portions of the plant canopy was also evaluated. Results of this experiment confirmed that soybeans during R3 and R5 stages are more susceptible to yield loss at high levels (>57%) of defoliation than R6 growth stage. However, yield loss was not significantly different below 57% defoliation. No significant yield difference was observed from defoliation occurring in the top or bottom part of the canopy. Yield loss from various levels of defoliation during the vegetative stages was significant at V6. No yield loss was observed from defoliation during the V3 growth stage. Both maturity group IV and V soybeans behaved similarly to each level of defoliation. Results from these experiments were used to determine dynamic economic injury levels for each growth stage based on yield loss equations, value of the crop, and cost of control and can be used to make insecticide application recommendations based on the amount of defoliation at a particular reproductive growth stage. Soybean looper, *Chrysodeixis includens* (Walker), is an economic pest of soybeans that has developed resistance to several insecticide classes. New insecticides have recently been labeled for control of lepidopteran pests in soybeans, including soybean loopers. Field reference strains were collected in 2010 and 2011 from soybean fields in Mississippi and Louisiana and subjected to insecticide incorporated diet treated with flubendiamide, chlorantraniliprole, and methoxyfenozide. Susceptibility of soybean loopers to flubendiamide and chlorantraniliprole did not differ. However the overall susceptibility to methoxyfenozide was greater than chlorantraniliprole. Diet incorporated assays determined a 9.4 fold variation in susceptibility to flubendiamide among the seven soybean looper populations tested. Variation to chlorantraniliprole was 6.25 fold and variation for methoxyfenozide was 5.37 fold. These data can be used as a benchmark for referencing future soybean looper populations in Mississippi and Lousiana.

DEDICATION

I would like to dedicate this dissertation to my grandfather, Johnnie B. Baumgardner (Bebo). Thank you for the guidance and life lessons you taught me. You are the reason I chose a career in agriculture.

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CHAPTER I

LITERATURE REVIEW

Soybean

Soybean (Glycine max) is a legume crop of great economic importance. Millions of hectares are grown in many countries around the world. Soybean is one of the oldest crops still grown. The first record of the soybean plant dates back to 2000 B.C. in China where it was one of the sacred plants that the Chinese relied on for existence. The first record of soybean in the United States was 1804 and it is a major crop that we still rely on today. Soybean was first introduced into the United States during the early 1700s, and productions spread into the Midwest during the early 1800s. During the 1920s, soybean was grown mainly as a forage crop. It wasn't until the 1940s when production really expanded throughout the United States due to the discovery of the oil content of soybean (Gibson and Benson 2005).

Soybean is a legume crop with agronomic requirements similar to those of corn and cotton (Martin et al. 2006). The United States produces an average of 200 billion kg soybean grain on approximately 188 million hectares each year (USDA 2010). The leading soybean producing states are Illinois, Iowa, Minnesota, Indiana, and Nebraska. Mississippi's soybean hectares have increased from 640,000 hectares in 2005 to 880,000 hectares in 2009 (USDA 2010). Therefore, soybean is a crop of great importance to Mississippi's economy. Soybeans are short day plants; therefore they have been adapted to grow in many regions of the United States. Reproduction in short-day plants is initiated by the shortening of daylength or longer dark periods. There are ten different maturity groups (00-VIII), all separated by the daylight requirements to initiate flowering. Typically the lower maturity groups (00-III) are adapted to the northern states and the higher maturity groups (IV-VIII) are adapted to the southern states. Northern maturity groups are different from southern maturity groups in that they require longer days to initiate reproduction. Photoperiod length during summer increases as latitude increases. Planting a variety that is adapted to the north in the south will result in minimal vegetative growth and low yields. Planting a southern variety in the north would result in excess vegetative growth and the increased risk of frost due to delayed flowering. Soybean can be either determinate, where all vegetative growth stops when flowering begins (maturity groups V-VIII), or indeterminate where vegetative growth continues after flowering. Maturity group IV and V are most commonly grown in the Mississippi.

Soybean planting in the southern United States begins in April and will often continue until June. Seeding rates vary from 250,000 to 500,000 seeds per hectare and can be planted on narrow (19 cm) or wide rows (97 cm). Seeding rate will vary with the row spacing; as row spacing decreases, seeding population will increase. Optimum seeding depth for soybean is 2.5-5 centimeters. Soybean, like any other plant, will undergo a series of physiological changes crucial for production upon emergence. However, much of the early documented literature that discusses the development of soybean is confusing (Dunphy et al. 1979). Many citations in the literature state that treatments were presented or effects were observed “one week prior to flowering” or “six weeks after flowering”. This leaves the reader unclear as to when events took place during the stages of soybean development. It is difficult to compare one study in the literature to another due to the differences in soybean development description. Kalton

(1945) first published a system that described the development of soybean. Hanway and Thompson (1967) later devised another system that was represented with colored charts of soybean development stages. However, this system still had it flaws, which left the stages of development open to interpretation. It was some years later when a discrete system was developed by Fehr and Caviness (1977) that was universal for all soybean maturity groups and varieties. This system outlined both vegetative and reproductive stages separately and is exclusively used in the literature from the time of publication to present. Vegetative stages are designated at emergence (VE) and cotyledons (VC). The remaining vegetative stages are designated by the number of trifoliate leaves to the nth degree (Vn). Reproductive stages are represented by R1-R8 designating two stages to flowering (R1 and R2), two stages for pod setting (R3 and R4), two stages for pod filling (R5 and R6), and two stages for physiological maturity (R7 and R8). Soybean is considered reproductive stage one (R1) when at least one flower is present at any node on the plant. Following R1, reproductive stage 2 (R2) occurs when there are >1 flower at multiple nodes on the plant. Reproductive stage three (R3) begins when there is a pod 0.5 cm present at one of the upper four nodes of the plant. Reproductive stage four (R4) follows when one full size pod is present at one of the uppermost four nodes of the plant. Next is reproductive stage five (R5); at R5 seed development begins and there should be a seed present 0.5 cm within a pod in the upper four nodes. Reproductive stage six (R6) is reached when the seed is full size within the pods in the upper four nodes of the plant. Reproductive stage seven (R7) occurs when one mature pod is present on the plant. Reproductive stage eight (R8) occurs just prior to full senescence when 95% of the pods are mature. During R8 (95% brown pods), leaves will turn yellow and begin to fall from the plant. Moisture levels within the pods will decrease as the plant completely senesces.

When soybeans reach this point they are mature enough to be harvested. However, harvesting is commonly delayed until seed moisture is 13-15% to minimize drying costs.

Yields typically range from 30-50 bushels per acre in Mississippi (USDA 2010). A bushel of soybeans weighs 27 kg. A 27 kg bushel of soybean produces 22 kg of meal and 5 kg of oil. Soybean oil is used primarily for shortening, margarine, and salad oil. The lecithin in the oil is used for baked goods, candies, chocolate, cocoa, and margarine (Martin et al. 2006). Soybean oil quality can be measured by the drying property represented by an iodine number. The iodine number for soybean oil ranges from 118-141, which will vary among varieties (Lloyd and Burlison 1939). The oil content of soybean seed ranges from 14-24% or more and protein content ranges from 30-50% or more (Beeson and Probst 1961).

Defoliating Insect Complex

Soybeans can harbor a rich fauna of phytophagous insects. However, most commonly encountered in Mississippi soybean fields are bean leaf beetle, Ceratoma trifurcata (Foster), green cloverworm, Hypena scabra (F.), velvetbean caterpillar, Anticarsia gemmatalis (Hübner), cabbage looper, Trichoplusia ni (Hübner), and soybean looper, Chrysodeixis includens (Walker). These insects are all considered part of a complex that feed on soybean foliage. Although these insects utilize the same resource, they have been shown to feed on different parts of the plant. Some defoliating caterpillars feed in the upper part of the canopy on young tender vegetation, and others feed in the bottom of the canopy on the older leaves (Higley 1994).

The bean leaf beetle, Ceratoma trifurcata (Foster), is native to the eastern United States. They are phytophagous beetles in the family Chrysomelidae. Adults are small (5 mm), often with distinct spots present on the elytra. However, spots are not always present. Color is often tawny, although a crimson color is not uncommon. A key character that is always present is a black triangle present behind the prothorax.

Bean leaf beetles are mostly a pest in soybeans, and can be especially damaging on seedling soybeans. Adults invade soybean fields in the spring as they leave their overwintering site. Bean leaf beetles will often overwinter in wooded areas, grass, and leaf liter. Once they become active in the spring, they will move to the host, feed, and lay eggs. Each female can lay between 130 and 200 eggs, usually in the upper 4 cm of the soil near the base of the plant host (Higley 1994). Eggs usually hatch within about 5-7 days and the larvae will begin feeding on roots and in many cases soybean nodules, which can be an economically important. Larvae will under go three larval stages or molts over 15-30 days before pupation. Duration of the larval stage is dependent on temperature. The pupal stage requires about 7 days for adult emergence. The number of generations throughout most of the United States is usually 1-2, however 3 generations are not uncommon in the southern United States (Higley 1994).

Sampling for bean leaf beetles is most commonly achieved with a sweep net. In pre flowering soybeans in Mississippi, the threshold for bean leaf beetles is when defoliation reaches 35% and beetles are present. In flowering soybeans, insecticides should be applied when defoliation reaches 20% or 50% of the plants show pod injury or two beetles are caught per sweep after pod set (Catchot et al. 2010). Organophosphate and pyrethroid insecticides have commonly been used to control bean leaf beetles. However, tolerance to the pyrethroid insecticides has been documented in Mississippi. Musser et al. (2012) found that resistance ratios were as high as 63 fold for bean leaf beetles collected in the delta region versus a susceptible bean leaf beetle population collected in the hill region of Mississippi. However, control of bean leaf beetles can still be achieved using many organophoshate insecticides.

Green cloverworm, Hypena scabra (F.), is native to the United States and parts of Canada. Green cloverworm is a member of the Noctuidae family within the order Lepidoptera. Adults are triangular at rest with an overall charcoal background with brown patches (Pedigo 1994). Males are generally 15-20% larger than females. Larvae are green and about 25 mm in length at the last larval instar. Larvae usually have two pale white stripes present along their side running the length of the body. Larvae also have three abdominal prolegs, in contrast to many noctuid species that generally have four abdominal prolegs. Eggs, like most noctuid eggs, are pearly white when they are freshly laid and become golden brown right before they hatch. Pupae of the green cloverworm are brown and are often found within a cocoon.

Green cloverworms overwinter south of 41o N latitude (Pedigo 1994). In the southernmost regions of the United States, near the Gulf coast, feeding and reproduction activity occur year round. Population distributions expand each year by migration of adult moths to the northern parts of the United States. Female green cloverworms lay eggs singly on the underside of foliage. Eggs hatch in 3-4 days and undergo 6-7 molts over 14 days. The pupal stage lasts 7-10 days before adult moths emerge. The entire lifecycle lasts for approximately one month. In the southern part of the United States there are 3-4 generations per year.However in the north there are generally only two generations annually.

Sampling for green cloverworm in Mississippi is most commonly achieved with a sweep net. However, other sampling methods can be used. The damage caused by green cloverworms is interveinal feeding of foliage. In addition to the defoliation threshold, a sweep net threshold of 75 larvae per 25 sweeps in pre-blooming soybeans and 39 larvae in blooming soybeans (Catchot et al. 2010). Green cloverworms are considered an occasional pest of soybeans, and many insecticides are still effective against green cloverworms in Mississippi (Pedigo 1994).

Velvetbean caterpillar, Anticarsia gemmatalis (Hübner), is another native foliage feeding pest of soybeans in the Noctuidae family widely distributed throughout North America. Larvae typically pass through six instars and are usually pale to dark green in color. Often they will have white longitudinal stripes present on each side running the length of their body. They can be distuinguished from green cloverworms by the presence of four abdominal prolegs. However, in very small 1st instar larvae these are small and difficult to see. They can also be identified by the violent wiggling motion that they make when they are disturbed. This will help distinguish them from other caterpillar pests found in soybeans that have four abdominal prolegs such as the corn earworm, Helicoverpa zea (Boddie), tobacco budworm, Heliothis virescens (Boddie), and armyworm species, Spodoptera spp. Similar to other Lepidoptera pests in the insect defoliation complex discussed already, the velvetbean caterpillar overwinters in the southern regions of the United States and migrates north each year during the warm summer months. Outbreaks are in Mississippi are sporadic, however in high numbers larvae can defoliate large amounts of foliage in a very short period of time. Adult female moths lay eggs singly or occasionally in clusters of 2-3 eggs in the upper portion of the canopy. Life history and duration of life stage are similar to green cloverworm (Funderburk 1994).

Sampling methods for velvetbean caterpillar are also the same as for green cloverworm, and all insecticides that are recommended for control of green cloverworm in Mississippi will be effective against velvetbean caterpillars (Higley 1994).

Soybean looper, Chrysodeixis includens (Walker) and the cabbage looper, Trichoplusia ni (Hübner) are both within the subfamily Plusiinae, family Noctuidae, and order Lepidoptera. Both species are native to North, South, and Central America and widely distributed. Although, the two species are hard to distinguish from one another during the larval stage, they are distinguished from other non-Plusiinines by the presence of only two pairs of abdominal prolegs plus one pair of anal prolegs. Adult soybean loopers are distinguished from cabbage loopers by a dark spot present on the outside margin of the forewing of soybean loopers as described by Lafontaine and Poole (1991). Soybean looper larvae have sometimes been identified by dark thoracic leg coloration. However, studies have shown that pigmentation of the soybean looper is inconsistent and should not be used for identification (Canerday and Arant 1967, Pitre 1998). Larvae of soybean and cabbage loopers can be distinguished however, as ridges 2 and 3 of the soybean looper mandible will not completely extend to the outside end of the mandible (Stehr 1987). Cabbage looper larvae mandibles do not exhibit this characteristic. Canerday and Arant (1967) also documented the pupae of the soybean looper are light green in color in contrast to dark brown colored pupae of the cabbage looper. Soybean loopers pass through 6 instars before pupation, whereas the cabbage looper only completes five instars prior to pupation. Identification of these two species is important because the cabbage looper can still be controlled with numerous insecticides labeled in soybeans. Soybean looper has developed resistance to most major classes of insecticides (carbamates, cyclodienes, organophosphates, and pyrethroids) (Boethel et al.1992).The soybean looper is the most abundant plusiinae insect attacking soybean in North America (Sullivan and Boethel 1994). However, mixed populations are often found within fields in Mississippi (Sullivan and Boethel 1994). Generally, cabbage loopers are found frequently at low numbers throughout the growing season. Soybean looper populations peak in the southern United States during mid-August to September (Carner et al. 1974). Sampling for soybean looper as well as cabbage looper can be achieved with the sweep net technique. The action threshold for loopers is 39 loopers per 25 sweeps in pre-blooming soybeans and 19 per 25 sweeps in blooming soybeans (Catchot et al. 2010). The reason that this threshold is lower than for other foliage feeding lepidopteran pests in soybeans is because loopers tend to be aggregated in the lower part of the plant canopy, so the sweep net captures a lower percentage of the larvae feeding in soybeans. This threshold should be used in conjuction with the defoliation threshold discussed previously.

Soybean looper has become one of the most costly pests to control in soybeans (Mascarenhas and Boethel 1997). Soybean looper control failures, along with their ability to defoliate massive amounts of foliage, has caused great concern among growers (Mascarenhas and Boethel 1997). During the mid 1980s, control failures with pyrethroids were reported even when properly applied and at recommended labeled use rates (Felland et al. 1990). Soybean looper pyrethroid susceptibility levels were measured in 1995 and the LC50 for permethrin ranged from 1.59 to 60.87 (Mascarenhas and Boethel 1997). The authors observed that all populations collected in the study had significantly higher LC50s than the susceptible USDA strain. It is documented that increased pyrethroid resistance of soybean looper is present where soybean and cotton are grown in the same area (Felland et al. 1990, Leonard et al. 1990, and Mink and Boethel 1992).

Soybean looper has developed resistance to most major classes of insecticides (carbamates, cyclodienes, organophosphates, and pyrethroids) (Boethel et al.1992). In addition to the difficulty of controlling soybean loopers, they have the ability to cause great defoliation to soybean. Mascarenhas and Boethel (1997) and Bergman et al. (1985) reported >10% annual losses to crop yield, and crop damage plus control costs due to soybean looper.

Mascarenhas and Boethel (1997) also reported that some of the field strains collected were 4.8 times more resistant to thiodicarb (Larvin) than the USDA reference strain. Also, they make reference to unpublished data from a field strain collected in Puerto Rico that was 15 times more resistant than the USDA reference strain. This is a concern because of the migratory nature of the soybean looper. This may result in poor efficacy with thiodicarb in the future (Mascarenhas and Boethel 1997). Results from the spinosad (Tracer) bioassay showed no significant difference between any field strains collected and the USDA reference colony.

Defoliation

Soybeans are attacked by several species of insects each year. Insect damage to soybeans has become more frequent due to the increased number of hectares (Todd and Morgan 1972). The interest in soybean production has grown with the production area and inflated commodity prices. Since the crop is more valuable, growers are less hesitant to spend money on crop protection. Foliage feeding insects cause defoliation that can result in an overall decrease in the productivity of soybean. Defoliation is defined as the removal of leaves or the loss of foliage from a plant and has an indirect effect on soybean yield.The soybean defoliation threshold for Mississippi and various other states is 35%

defoliation during vegetative stages and 20% defoliation during reproductive stages. These thresholds were based on studies conducted by Nettles et al. (1968) where he suggested that insecticide applications should be made when 35% defoliation is reached through the blooming stage and then the threshold should be reduced to 20% for the remaining growth stages. Older thresholds, such as this defoliation threshold, should be reevaluated, because most of the previous research was conducted on varieties that are no longer commercially available using a production system that is rarely used today.

Defoliation injury may affect transpiration, net photosynthesis, nutrient deficiencies, water loss, and any other abiotic factor that could influence soybean yield. Fehr et al. (1985) reports that defoliation to soybean, especially when grown on calcareous soils, can reduce yield in three ways: (1) defoliation can be detrimental to yield (Fehr et al. 1983), (2) the increased iron chlorosis from defoliation can reduce production (Froelich and Fehr 1981), and (3) the effects of both defoliation and iron chlorosis can be additive. Ostlie and Pedigo (1984) found that water loss of soybean increased as the amount of defoliation increased, which was in agreement with previous results found by Hammond and Pedigo (1981). Past research indicates that both simulated and actual insect defoliation do not reduce photosynthesis from the remaining foliage; however photosynthesis was reduced on the whole plant level by the reduction of total leaf area (Boote 1981, Higley 1992, Peterson and Higley 1996). A variety of insects, diseases, and environmental conditions (hail) can cause defoliation. Defoliation thresholds can be used to initiate treatment for a particular defoliating pest feeding at a specific growth stage. Both determinate and indeterminate soybeans are sensitive to defoliation from the beginning of pod (Dungun 1939) formation to the filling of pods (Fehr et al. 1981). Simulated insect defoliation methods provide a reliable and feasible method for determining damage-loss relationships; and levels of damage, placement within a plant canopy, and distribution through time can be precisely measured (Ostlie and Pedigo 1984). Begum and Eden (1964) conducted a simulated defoliation study to determine the influence it had on yield and seed quality. The varieties used in their experiment were 'Lee', a late Group VI, and 'Jackson', a Group VII. They evaluated four levels of hand defoliation (0, 33, 67, and 100%) at three growth stages. The growth stages when the levels of defoliation were initiated were at bloom, seeds half grown in pods, and when beans were fully grown in the pods. Many researchers in the past have reported that yield reductions are more significant from defoliation when pods are forming (Dungun 1939, Fuellman 1944, Kalton et al. 1945, McAlister and Krober 1958, Begum and Eden 1964, and Turnipseed 1972). Researchers have also reported that defoliation after pod filling has no significant impact on soybean yield, even at very high levels (Kalton et al. 1945 and Turnipseed 1972). Weber (1955) observed that there was a significant loss in seed quality of soybeans with extremely high levels of defoliation. Begum and Eden (1964) found that at any level of defoliation above 33% when beans were half grown in the pods, there was a reduction in yield. The authors also reported that when the beans were fully grown in the pods, the effects of defoliation at any level were not significant. They reported that the greatest yield loss from each defoliation level occurred when the beans were half grown in the pods. However, the varieties that they used were determinate, so all vegetative growth ceased after flowering. Previous research by Fehr et al. (1977) showed that indeterminate and determinate varieties responded differently to 100% defoliation, with determinate varieties yielding much less than indeterminate varieties. Past research may also indicate that early (prior to R3) and late (after R6) defoliation does not result in dramatic yield losses, and defoliation at certain levels may have different impacts on yield at different growth stages. Weber (1955) found that defoliation at early vegetative growth stages gave the plants more time to recover from the loss of foliage and resulted in smaller yield losses. This is likely due to the ability of soybean to compensate from the loss of leaf tissue during the vegetative growth stages. The ability of soybean to avoid substantial damage from different levels of defoliation depends on the amount of defoliation, when the defoliation occurs, the ability if the variety to compensate for the level of defoliation, and certain environmental factors such as soil fertility and precipitation (Pedigo et al. 1986, Haile et al. 1998). Yield loss from defoliation has previously been referred to as a function of development stage and amount of foliage loss (Fehr et al. 1981). Many simulated defoliation studies have been conducted over the years. However, when pertaining to soybean defoliation, much of the work has been conducted using single day applications. Seemingly, this simulates the effect of a hail storm stripping off leaves, but does not truly mimic a building insect population that will continually remove leaves over an extended period of time. Ostlie and Pedigo (1984) reported that the effects from single-day and sequential defoliation differed in reproductive soybeans. In order to correctly mimic the effects of an insect population, simulated defoliation techniques must be conducted both spatially and temporally (Pedigo et al. 1986). According to Hunt et al. (1994), sequential defoliation achieved defoliation levels closer to the desired level than the single-day defoliation. Many insects will feed on the foliage of soybean such as bean leaf beetle and the soybean looper. However, location and rate at which they feed may vary from one pest to another. The area within the plant where feeding takes place will often differ. Soybean defoliation can have different impacts on yield depending upon when and where the foliage is removed. Yield losses due to defoliation are not the only consideration that should be made when determining economic injury levels (EIL). Seed size, number of pods per plant at harvest, oil content, protein content and seed germination can also be affected (Thomas et al. 1976). Kalton et al. (1945) reported that seed size at harvest was reduced by 25-30% and oil content was decreased with 100% defoliation at Hanaway and Thompson's stages 7 and 9. Turnipseed (1972) conducted a simulated defoliation study where he removed 17, 50, and 67% of the foliage at from mid bloom to pod set. He reported that yield reductions were present in conjunction with seed weight and protein content reductions. However, he did not witness any differences in seed germination. Todd and Morgan (1972) determined differences in seed size and oil content following single applications of 33, 67, and 100% defoliation at several stages of soybean growth. Thomas et al. (1976) reported that leaf and pod loss affects seed size, number of pods per plant, oil content, protein content, and germination. Weber (1955) found that seed weight was reduced by high levels of defoliation, however seed quality was not affected. Caviness and Thomas (1980) found that reduction in seed size alone cannot account for yield reductions. The authors further explained that with 100% defoliation at R4, yield was reduced by 50%, however seed size was only reduced by 8%. They also found that defoliation had no effect on seeds per pod and that yield reductions from defoliation were largely a result of fewer pods per plant.

It is unknown where, within the plant canopy, defoliation causes the most yield loss. Most defoliation estimates are determined by looking at the top of the soybean canopy once the row middles have lapsed. Defoliation within the bottom portion of the plants is often overlooked. Also, we don't know if current defoliation thresholds during reproductive and vegetative growth stages are valid. New insecticides have been labeled in soybeans that are effective against the soybean looper, and baseline data are lacking for these insecticides that will assist resistance monitoring in the future. Therefore, the focus of this dissertation is to address these issues and increase our understanding of the effects that defoliation has on soybeans grown in MS. The following research objectives were proposed:

I. Determine the effects of defoliation on MG IV soybeans from simulated insect defoliation techniques at various levels across three growth stages and different portions of the plant.

II. Determine effects from simulated insect defoliation at various levels during vegetative growth stages on determinate and indeterminate soybeans.

III. Evaluate the susceptibility of soybean looper Chrysodeixis includens (Walker) to novel insecticides.

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CHAPTER II

IMPACT OF DEFOLIATION ON YIELD OF GROUP IV SOYBEANS IN MISSISSIPPI

Abstract

Field experiments were conducted during 2009 and 2010 to evaluate the effects of defoliation on maturity group IV soybeans grown in Mississippi. During each year of the experiment two locations were planted with maturity group IV soybeans that were subjected to various levels of defoliation during R3, R5, and R6 growth stages. In addition to different amounts of defoliation within the three growth stages, defoliation occurring within different portions of the plant canopy was also evaluated. Soybeans were subjected to various levels of defoliation within the upper 50% of the plant canopy, lower 50% of the plant canopy, and whole plant canopy. No differences were observed in yields from defoliation occurring in the bottom of the plant canopy compared to the top of the plant canopy. Yield loss from whole plant defoliation was greater because defoliation was twice as much. Therefore, economic injury levels and thresholds are supported by defoliation on a whole plant basis. Results also confirmed that soybeans during R3 and R5 stages are more susceptible to yield loss at higher levels (>57%) of defoliation than during R6. However, yield loss was not significantly different below 57% defoliation for all growth stages in the experiment. Dynamic economic injury levels were determined for each growth stage based on yield loss equations, value of the crop,

and cost of control and can be used to make insecticide application recommendations based on the amount of defoliation at a particular reproductive growth stage.

Introduction

Soybean, Glycine max (L.) Merr., production in Mississippi has increased from 640,000 hectares in 2005 to 880,000 hectares in 2009, generating an estimated 705 million dollars in revenue (NASS 2010). This increase in soybean production has been primarily due to increased value of soybeans, along with increased insect control costs in other crops, particularly cotton Gossypium hirisutum L. (Williams 2005, 2011). An issue facing Mississippi soybean production is that increasing soybean production can lead to an increase in insect damage (Todd and Morgan 1972).

Pest damage in soybeans can be described as two types: direct damage and indirect damage. Direct damage to soybeans from an insect pest occurs when the insect feeds on the seeds causing a reduction in yield. Examples of pests causing direct damage would be stink bug species (Pentatomidae) or corn earworm, Helicoverpa zea (Boddie). Indirect damage occurs when an insect feeds on other portions of the plant, such as stems, roots, or foliage. This feeding can also lead to reductions in yield by stressing the plant. A very common type of soybean injury caused by insect pests is defoliation.

Defoliation injury may reduce transpiration and photosynthesis in the plant. Furthermore, the capacity to compensate for nutrient deficiencies, water loss, and any other abiotic factor that could influence soybean yield is reduced. Fehr et al. (1985) reported that defoliation to soybean, especially when grown on calcareous soils, can reduce yield in three ways: (1) defoliation can directly reduce yield (Fehr et al. 1983), (2) defoliation can cause iron chlorosis which reduces yield (Froelich and Fehr 1981), and

(3) the effects of both defoliation and iron chlorosis can be additive. Ostlie and Pedigo (1984) found that water loss of soybean increased as the amount of defoliation increased, which was in agreement with previous results found by Hammond and Pedigo (1981).

Defoliation to soybeans is caused by a number of insect species that are categorized as the insect defoliation complex. Foliage feeders in this complex include the bean leaf beetle, Ceratoma trifurcata (Foster), green cloverworm, Hypena scabra (F.), velvetbean caterpillar, Anticarsia gemmatalis (Hübner), cabbage looper, Trichoplusia ni (Hübner), and soybean looper, Chrysodeixis includens (Walker). All these species are commonly observed in soybean fields in Mississippi, causing various levels of defoliation.

Most insect pest management thresholds are based on the number of insects sampled from a field or area within a field. However, when common damage can be caused by a number of insects, a threshold based on plant damage can be more useful. A defoliation threshold has been used in soybean for many years. In Mississippi and many other states, the threshold is based on research by Nettles et al. (1968), who suggested a threshold of 35% defoliation from emergence to flowering and 20% defoliation from flowering until maturity. Many researchers in the past (Dungun 1939, Fuellman 1944, Kalton et al. 1945, McAlister and Krober 1958, Turnipseed 1972 and Begum and Eden 1964) have reported that yield reductions from defoliation were more significant when pods are forming than from earlier (vegetative growth stages) or later (when beans have filled pods) growth stages. Researchers have also reported that the significance of defoliation on soybean yield after pod filling is not significant, even at very high levels (Kalton et al. 1945 and Turnipseed 1972). Therefore, soybean defoliation can have different impacts on yield depending upon when the foliage is removed. In addition to a reduction in yields, significant losses in soybean seed quality have been observed due to extreme levels of defoliation (Weber 1955).

The problem with using these thresholds in Mississippi and other southern states is that most of the soybean acreage is planted to indeterminate maturity group IV varieties, but current thresholds are based on research using determinate maturity group VI and VII varieties. Conventional soybean production systems in the southern U.S. frequently faced yield limiting conditions for determinate V, VI, and VII varieties due to drought and high temperatures during the reproductive stages of these late maturing soybeans (Heatherly 1999). To avoid this situation, early season soybean production systems have been adopted where indeterminate cultivars (MG III and IV) are planted earlier in the growing season so that critical periods of reproduction more frequently coincide with adequate rainfall and lower temperatures (Heatherly 1999). Indeterminate cultivars generally begin flowering before maximum plant height is reached, whereas determinate cultivars are at full height before flowering is initiated (Pickle and Caviness 1984). Previous research by Fehr et al. (1977) showed that indeterminate and determinate varieties responded differently to 100% defoliation with determinate varieties losing more yield than indeterminate varieties.

Because obtaining precise defoliation levels caused by insect pests in field tests is difficult, simulated insect defoliation levels have been used in previous studies to estimate yield effects on soybeans. Simulated insect defoliation methods provide a reliable and feasible technique for determining damage-loss relationships. With simulation, levels of damage, placement within a plant canopy, and distribution through time can be precisely measured (Ostlie and Pedigo 1984). Begum and Eden (1964) conducted a simulated defoliation study to determine its influence on yield and seed quality using maturity group VI and VII varieties. They evaluated four levels of hand defoliation (0, 33, 67, and 100%) at three growth stages (at bloom, seeds half grown in pods, and when beans were fully grown in the pod).

Most of the work on which current thresholds in the mid-southern U.S. are based was conducted 20 or more years ago using determinate and later maturing varieties that likely did not possess the yield potential of current ones. Also, most of the research was conducted prior to the development of a system where growth stages of soybean were clearly defined (Hanway and Thompson 1967). The description of soybean maturity in these studies are often vague and confusing, making it difficult to interpret the physiological growth stages (Dunphy et al. 1979).

Previous studies that have evaluated the impacts of defoliation on soybeans have only quantified yield loss based on a whole plant basis. However, in practice, defoliation estimates are often determined by examining the upper portion of the soybean plants during full canopy. Defoliation within the bottom portion of the plants is often overlooked. It should be important to evaluate defoliation levels on a whole plant level because current thresholds are based on whole plant evaluations, with no distinction as to where the defoliation is located within the canopy. Research is needed to compare levels of defoliation in different areas of the canopy.

The objective of this study was to evaluate the impacts of various levels of defoliation within different canopy regions during various reproductive growth stages on yields of indeterminate maturity group IV soybeans using simulated defoliation. Results from this research can be used to adjust thresholds where needed and to improve our understanding of the role of defoliation in determining soybean yield.

Materials and Methods

Plot Establishment

Experiments were conducted in 2009 and 2010 at the R.R. Foil Plant Research Center in Starkville, MS and the Delta Research and Extension Center in Stoneville, MS. Asgrow® 4605 (Monsanto) soybeans were planted in 2009 on 28 April at Starkville and on 30 April at Stoneville into raised conventionally-tilled beds at a seeding rate of ~275,000 seeds per hectare with 97 cm row spacing. In each year and location of the experiments plots were furrow irrigated and managed for high yield potential, and irrigation timings varied by year and location. In 2010, soybeans were planted on 15 April at Starkville and on 1 May at Stoneville at the same rate and with the same agronomic practices as in 2009. Prior to planting at all locations, seed was treated with thiamethoxam (Cruiser®, Syngenta Crop Protection, Greensboro, NC) at 35.49 ml/ 45.36 kg of seed and fludioxonil + mefenoxam (Apron Max®, Syngenta Crop Protection, Greensboro, NC) at 147.87 ml/45.36 kg of seed. Planting depth was set to 2.54 cm below the soil surface. The plot area was scouted and over-sprayed weekly to reduce the effects of any insect or disease. Applications of pyrethroid, carbamate, and neonicotinoid insecticides were applied weekly to target most insect spectrums. Fungicide applications of azoxystrobin (Quadris®, Syngenta Crop Protection) at 444 mL/ ha were made during the R3 and R5 growth stages for both years of the experiment. Treatments were planted in a randomized complete block (RCB) design with four replications at both locations during each year of the experiment. Plots were two rows wide and 3.05 m long.

Defoliation Treatments

Treatments were evaluated as a 3x5x3 factorial with factors including soybean growth stage (R3, R5, and R6), defoliation levels (0, 17, 33, 67, and 100%), and portion of the soybean plant (upper canopy, lower canopy, and whole plant). To achieve simulated levels of defoliation, removing one leaflet from each trifoliate was equivalent to 33% defoliation. The 17% defoliation level was achieved by removing one leaflet from every other leaf on the plant. Plant canopies within the plot were divided by estimating the top 50% or the bottom 50% of the plant. Within top and bottom defoliated plots, the desired defoliation levels were removed from that plant portion only. Therefore, on a whole plant basis, defoliation levels were approximately half of the stated defoliation level. Treatments were initiated when 75% of the plants within the plot area were at the desired growth stage. Defoliation was completed progressively to better simulate insect defoliation over time. On the first day of defoliation during 2009, all plots receiving defoliation during the R3 growth stage were defoliated to the 17% level. Two to three days later, the 33, 67 and 100% plots were defoliated to 33%. After an additional 2-3 days, the 67 and 100 % plots were defoliated to the 67% level, and after another 2-3 days, the 100% defoliated plots were defoliated to 100%. The progression of defoliations was the same in 2010 except that the 17 and 33 % defoliation events were combined into a single defoliation to decrease the labor requirement. Plots were harvested with a 2-row Massey Ferguson plot combine. Grain weights and moisture samples for each plot were recorded.

Plant Data

Leaf area index is used to measure leaf area present as a proportion of ground area (Klubertanz et al. 1996). Measurements of leaf area have been commonly used to predict yield losses from pests. Leaf area index values are derived from the formula:

$$\frac{LAI = \ln(\Gamma)(\cos(\psi))}{-0.5} \qquad (2.1)$$

Where Γ is the ratio of sunlight intercepted by the plant to the portion of the light reaches the ground, the ψ value is the zenith angle of the sun, and -0.5 is the extinction coefficient for soybean (Norman and Campbell 1989, Chen and Black 1995). Leaf area index (LAI) data was collected with a Decagon AccuPAR LP-80 (Decagon Devices Inc., Pullman, WA) during the growing season from the Starkville 2010 experiment to evaluate the impact of defoliation treatments on leaf area. At the end of each defoliation event, LAI measurements (2 per plot) were collected. LAI data were recorded on 1 July (R4), 21 July (late R5), and 16 August (R7). A strong relationship between defoliation and LAI has been documented previously (Fehr et al. 1977, Herbert et al. 1992, Higley 1992). Browde et al. (1994) reported linear and quadratic relationships of LAI and defoliation with soybean yield. Board et al. (1997) determined that factors that reduce the LAI index below the critical values of 3.5-4 within the R2-R4 growth stages can reduce soybean yields.

Data Analysis

Yield data were analyzed as kg/ha, and then converted to yield as percent of the untreated control for presentation because of the variation in yields from each year and location of the experiment. However, regardless of yield variation, yield loss as a percentage was equivalent for each year and location. Data were log transformed and analyzed with a mixed model analysis of variance (SAS Institute 2009) to determine best fit equations for yield loss from defoliation at each growth stage. Defoliation level, growth stage when defoliation took place, plant portion (portion of the plant that was defoliated), and their interactions were fixed effects in the model, while year, location and replication were random factors. Degrees of freedom were calculated using the Kenwood-Rogers method. Differences were considered significant for $\alpha = 0.05$. Defoliation level was analyzed as a numeric factor, so regression equations could be determined for the relationship between defoliation and yield. Squared and cubic defoliation levels were included and then deleted where not significant. Non-significant interactions were deleted to describe the relationship as simply as possible. Pair-wise contrast statements were used to compare the upper and lower defoliation levels at all three growth stages.

Economic Injury Level

Data from this research were used to determine economic injury levels (EIL) for R3, R5, and R6 for maturity group IV soybeans. Yield loss from each growth stage was used to calculate the amount of defoliation needed to equal the cost of controlling the pest(s); represented as C in the equation: EIL= C/VIDK (Pedigo et al. 1986). In this equation V is the value of the crop in dollars per hectare; ID is based on the amount of damage or yield loss from defoliation; and K is the percent control that is expected from an application of an insecticide. EIL values are based on the assumption of 100% control; therefore the K value is dropped from the equation because it is always one. The values for the crop value were based on a high estimated crop yield and market value divided incrementally to a low crop yield and market value. However, yields in this experiment ranged from 3405 kg/ha to 6810 kg/ha, so the relationship between defoliation and yield may be different in situations outside of this range. Values for cost of control are based on total cost including an insecticide and application costs.

Results and Discussion

Yield was reduced by defoliation in each year and location of the experiment. However, the relationship between yield and defoliation observed in this experiment may vary in different environments. This experiment was managed for high yielding, irrigated Mississippi soybean production. The minimum undefoliated yield was 3405 kg.ha, so results of this experiment may not be applicable to lower yielding situations.

Equations for upper and lower canopy yield losses were generated (Table 2.1), and pair-wise contrasts determined there were no significant differences in yield reduction at any plant growth stage due to defoliation (R3 upper vs. lower: F= 2.91, df= 2, 17.45, P>F= 0.08; R5 upper vs. lower: F= 0.21, df= 2, 17.45, P>F= 0.81; R6 upper vs. lower: F= 0.01, df= 2, 17.42, P>F= 0.99) (Figures 2.1-2.3). Even though there was a trend for greater yield loss from defoliation in the upper portion of the canopy, this difference was not significant, so each leaf on the plant was equally important in determining yield.

Yield loss from whole plant defoliation was greater than upper or lower canopy foliage loss during R3 and R5 (R3 upper vs. whole: F= 58.11, df= 2, 17.45, P>F<0.0001; R3 lower vs. whole: F= 86.66, df= 2, 17.45, P>F<0.0001; R5 upper vs. whole: F= 13.43, df= 2, 17.45, P>F= 0.0003; R5 lower vs. whole: F= 17.00, df= 2, 17.45, P>F<0.0001). No differences were observed from whole plant defoliation compared to upper or lower canopy defoliation during R6 (R6 upper vs. whole; F= 2.21, df= 2, 17.45, P>F= 0.139; R6 lower vs. whole: F= 1.96, df= 2, 17.45, P>F= 0.17). Because yields were not reduced differently from foliage loss in the top or bottom alone, the use of a defoliation threshold based on whole plant foliage loss should be used; thus, further analyses were based on whole plant defoliation.

Leaf area index (LAI) data presented in Figures 2.4-2.6 were collected only during the 2010 experiment in Starkville, MS. However, yield reductions during 2009 were similar to those in 2010. These data demonstrate how foliage loss reduced the leaf area necessary for soybean to achieve maximum yields. Leaf area index values for top and bottom defoliation were higher compared to whole plant defoliation, because overall the amount of foliage that was removed was less. Leaf area index values for the same top and bottom defoliation level within a growth stage were similar, and the greatest amount of yield loss was from defoliation levels that produced LAI values below the critical LAI value of 3.5 reported by Board et al. (1997). LAI values recorded at R6 were substantially lower than those recorded at R3 and R5. However, the slope was not as steep, and yield loss was not as great (Figure 2.3 and 2.7). Natural senescence of soybean and changes in zenith angle later in the growing season were likely the cause of low LAI values at this time.

Regressions generated for whole plant defoliation at R3, R5 and R6 (Table 2.2) were used to determine that defoliation significantly impacted yield for each of the three soybean growth stages (R3: t= 23.16, df= 2.66, P>t= 0.0004; R5: t= 23.50, df= 2.66, P>t= 0.0004; R6: t= 23.60, df= 2.66, P>t= 0.0004). Pair-wise contrasts determined that whole plant defoliation during each growth stage caused different yield losses (R3 vs R5: t= -3.60, df= 82.69, P>t= 0.001; R3 vs R6: t= -6.92, df= 82.89, P>t <0.001; R5 vs. R6: t= -

3.32, df= 82.89, P>t= 0.001) (Figure 2.7). Specific comparisons were made to determine the level of defoliation at which each trend from Figure 2.7 was significantly different. Contrasts determined that at 57% defoliation, R3 and R5 trends for yield loss were significantly different from those at R6 (R3 vs. R5 at 57% defoliation: t= 0.26, df= 97.45, P > t= 0.796; R3 vs. R6 at 57% defoliation: t= -2.05, df= 99.34, P > t = 0.044; R5 vs. R6 at 57% defoliation: t= -2.30, df= 99.34, P> t = 0.023). At 75% defoliation, all trends were significantly different (R3 vs. R5 at 75% defoliation: t= -2.00, df= 97.2, P > t= 0.048; R3 vs. R6 at 75% defoliation: t= -5.18, df= 99.15, P > t<0.001; R5 vs. R6 at 75% defoliation: t= -3.19, df= 99.15, P > t = 0.002). Although, defoliation values less than 57% were not significantly different among growth stages; overall slopes were significantly different. Since R3 and R5 losses were not significantly different until defoliation exceeded 75%, and all reasonable economic injury levels (EIL) estimates were below 75%, EILs presented in Table 2.3 represent R3 and R5 growth stages and are based on the R5 regression equation for defoliation. This equation was chosen because it generated more conservative EIL estimates compared to the R3 yield loss equation. Even though R6 yield losses were not statistically different from R3 or R5 until defoliation exceeded 57%, yield loss estimates were numerically less than R3 and R5 estimates consistently over the range of defoliation levels, so a separate EIL table was generated for R6 stage with higher EIL values than found during R3 and R5 growth stages (Table 2.4).

Yields were reduced by <1% from a 17% level of defoliation during R3, R5, and R6 (Figure 2.7). Yield loss from 33% defoliation during R3 and R5 was <10%, while defoliation of 66% during R6 was required to observe a similar yield loss of 12%. Likewise, defoliation of 66% during R3 and R5 resulted in yield loss comparable to the loss from 100% defoliation during R6. Therefore, yield losses from high (>57%) levels of defoliation during R6 were much less compared to defoliation during R3 and R5. These data were consistent with previous reports where defoliation during R6 had a reduced effect on yield when compared to R3 and R5 growth stages (Kalton et al. 1945, Begum and Eden 1964, Turnipseed 1972). This is an important result of this research because one of the most important insect pests in the defoliator complex, the soybean looper, frequently migrates into soybean fields during the R5 and R6 stages (Carner et al. 1974). By using a higher threshold for insecticide applications during R6, fewer insecticide applications will be needed to maintain optimal yields. This could reduce input costs, the overall threat for insecticide resistance development, and the amount of insecticides in the environment.

Evaluating insect defoliation in soybeans and making insecticide applications based on the level of foliage loss is a difficult task for consultants and growers for a few reasons: 1) estimating the level of defoliation is very subjective and can vary from one individual to the next, 2) defoliation levels are analyzing damage that has already occurred and doesn't indicate the likelihood of further damage, and 3) defoliation can occur throughout the season and is often not uniform within the canopy. This research can be used as a tool to help consultants and growers make insecticide application decisions based on timing of defoliation and at what level they can expect yield loss. However, preventing yield loss should be a factor of the cost of application and the value of the crop. Another consideration that crop consultants and growers should address is the pest species that is more prevalent in the field and how long they have been in the field. Some pests, such as bean leaf beetles, will be present for several weeks throughout the growing season at relatively low numbers and slowly cause low levels of defoliation to soybeans. In contrast, lepidopteran species that feed on soybean foliage can be present in high numbers and remove a great amount of foliage in a very short period of time. Therefore, it is still necessary for those making recommendations to record the amount of defoliation in conjunction with insect counts on a weekly basis in an effort to relate numbers of foliage feeding insects and changes in the amount of defoliation over time.

Information presented in Tables 2.3 and 2.4 indicates the level of defoliation where the cost of a single application of insecticide equals the value of the yield lost from defoliation. Determining action thresholds based on these EILs will vary based on the insect pest, defoliation amount, and the mode of action of the insecticide that will be applied. The current defoliation threshold for Mississippi is 20% defoliation regardless of the reproductive growth stage. Based on these data, this is a reasonable static threshold for R3 to R5 growth stages. However, the true threshold would vary considerably based on yield potential, control costs and commodity prices, so a static threshold for all situations has limited value. These data will be used to generate a user-friendly web based formula where growers and consultants can enter expected yields, cost of the insecticide application, amount of control expected, and the soybean growth stage to determine the level of defoliation at R3, R5, or R6 that should trigger an insecticide application.

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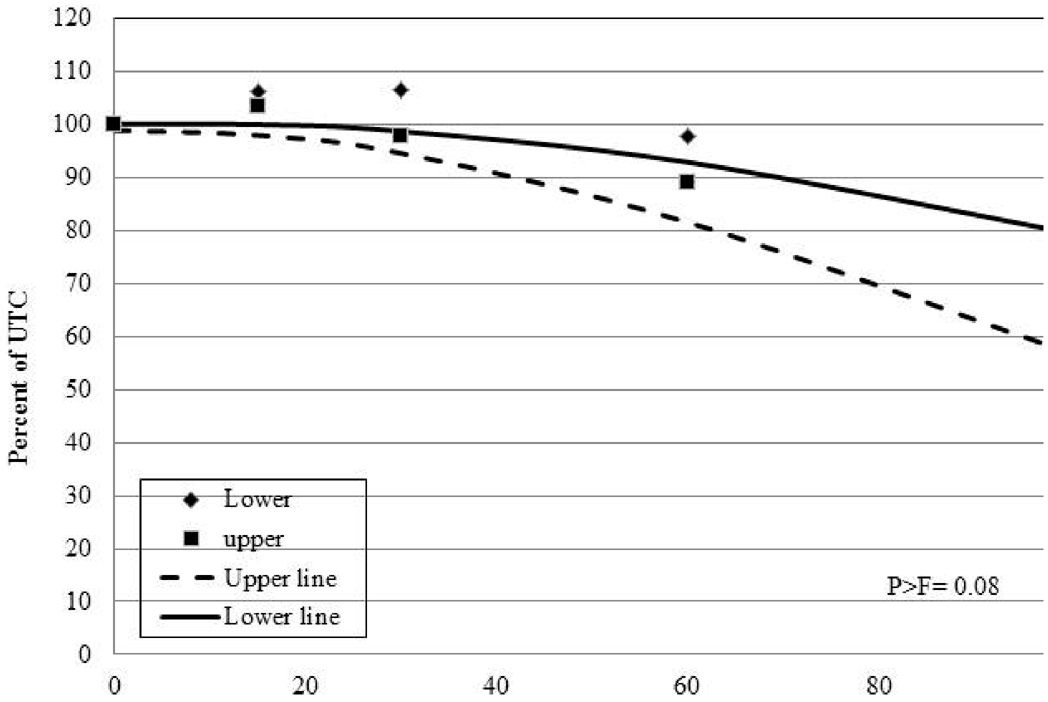

Figure 2.1 Average yield loss and overall best fit equation lines from log transformed data for upper vs. lower defoliation at R3 (F= 2.91, df= 2, 17.45, P>F= 0.08). Equations for lines presented in Table 2.1.

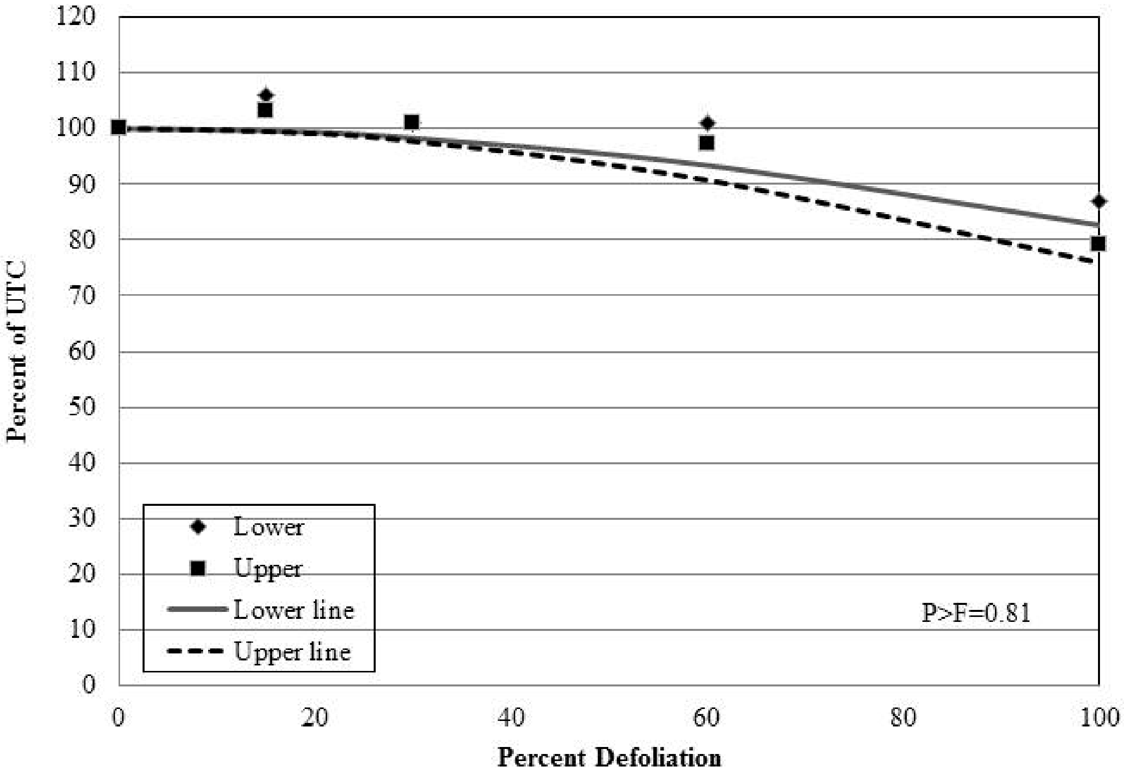

Figure 2.2 Average yield loss and overall best fit equation lines from log transformed data for upper vs. lower defoliation at R5 (F= 0.21, df= 2, 17.45, P>F= 0.81). Equations for lines presented in Table 2.1.

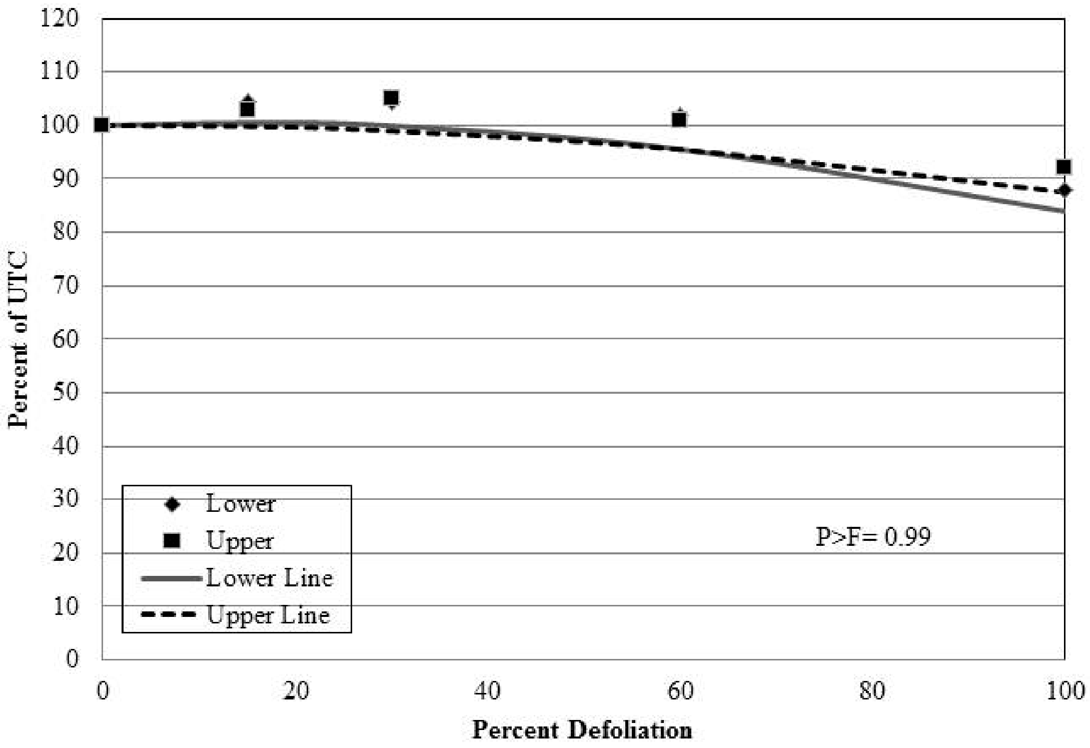

Figure 2.3 Average yield loss and overall best fit equation lines from log transformed data for upper vs. lower defoliation at R6 (F= 0.01, df= 2, 17.42, P>F= 0.99). Equations for lines are presented in Table 2.1.

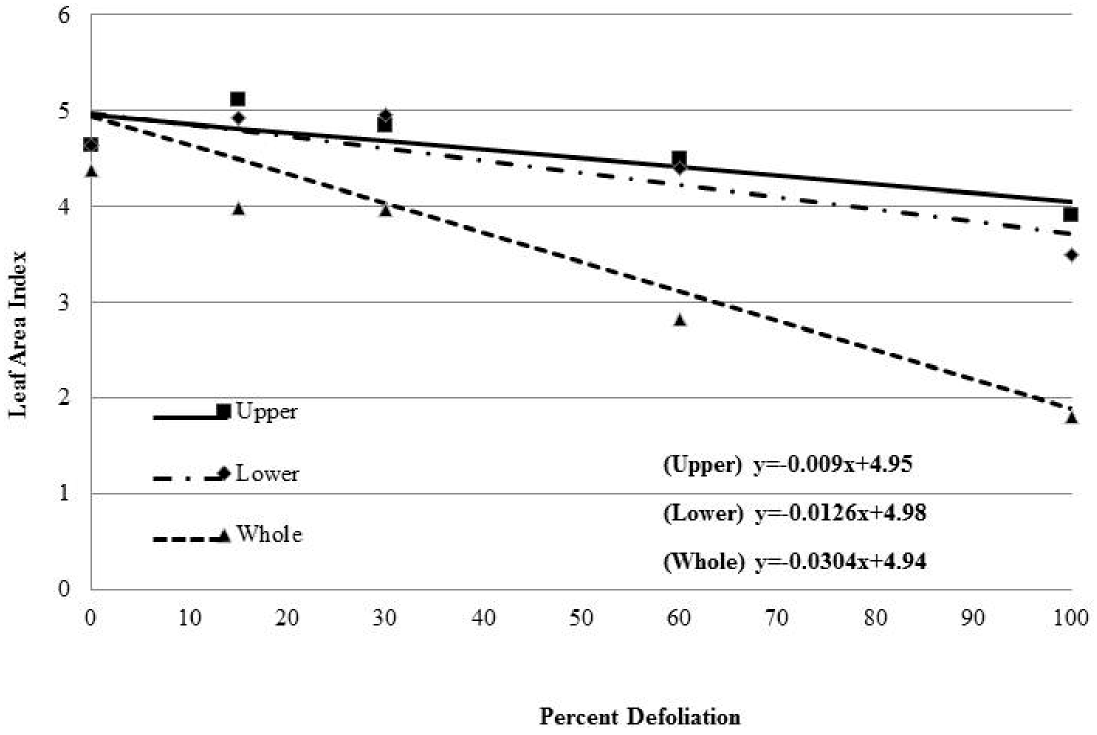

Figure 2.4 Leaf area index values and regression lines for data recorded during R4 growth stage on July 1, 2010 for R3 defoliated plots in Starkville, MS.

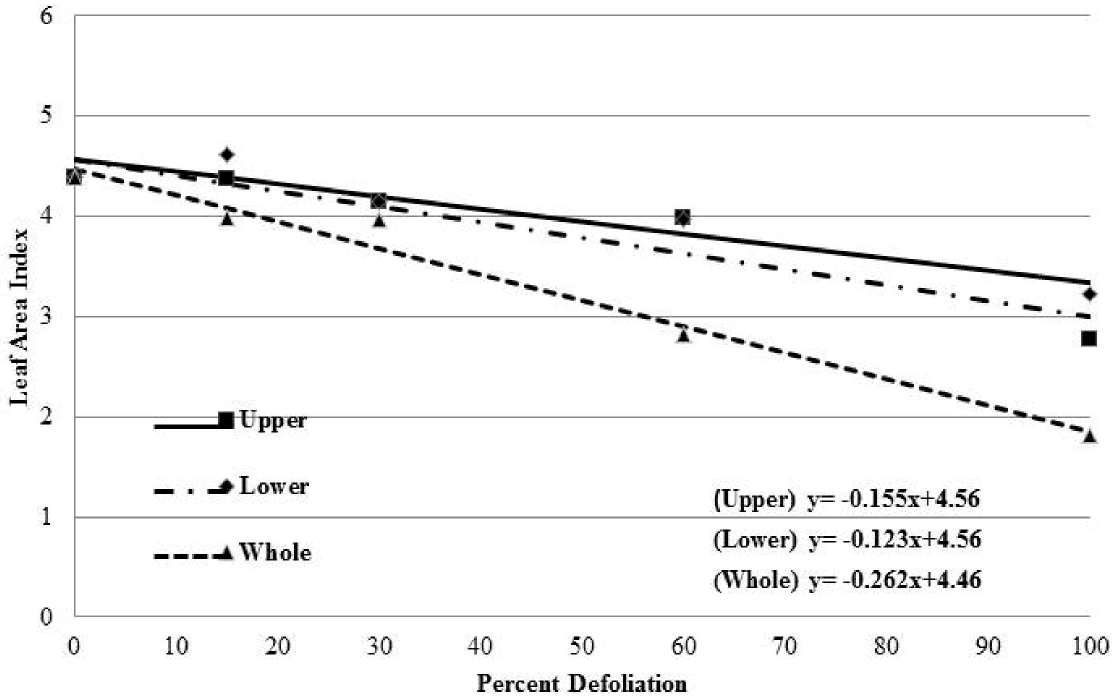

Figure 2.5 Leaf area index values and regression lines for data recorded during late R5 growth stage on July 26, 2010 for R5 defoliated plots in Starkville, MS.

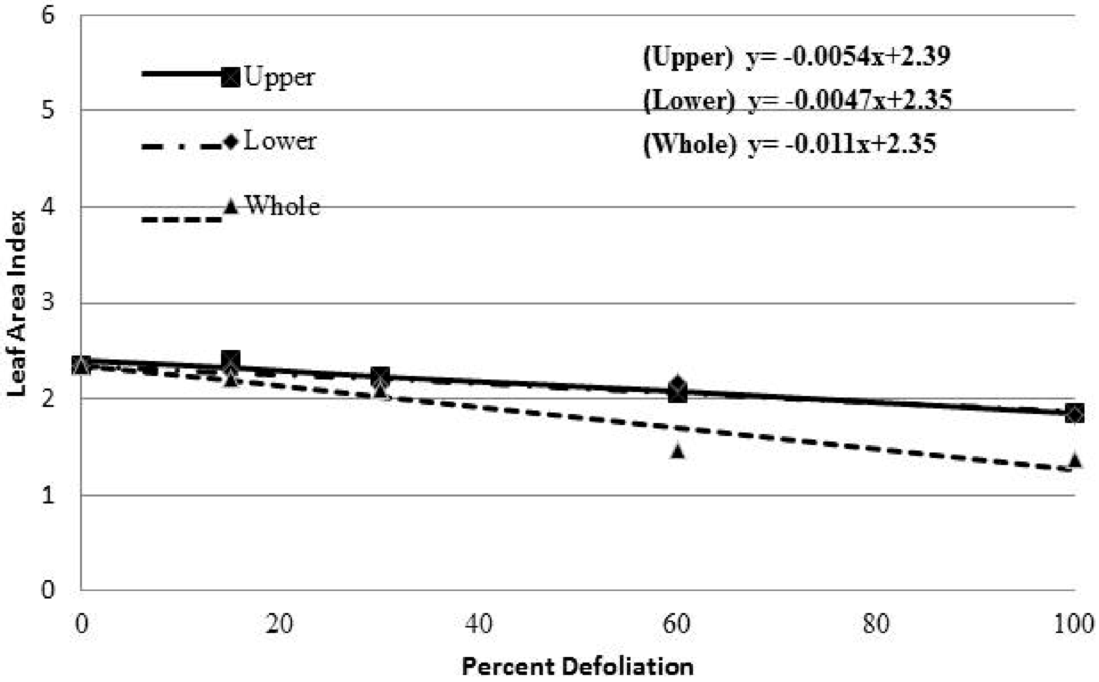

Figure 2.6 Leaf area index values and regression lines for data recorded during R7 growth stage on August 16, 2010 for R6 defoliated plots in Starkville, MS.

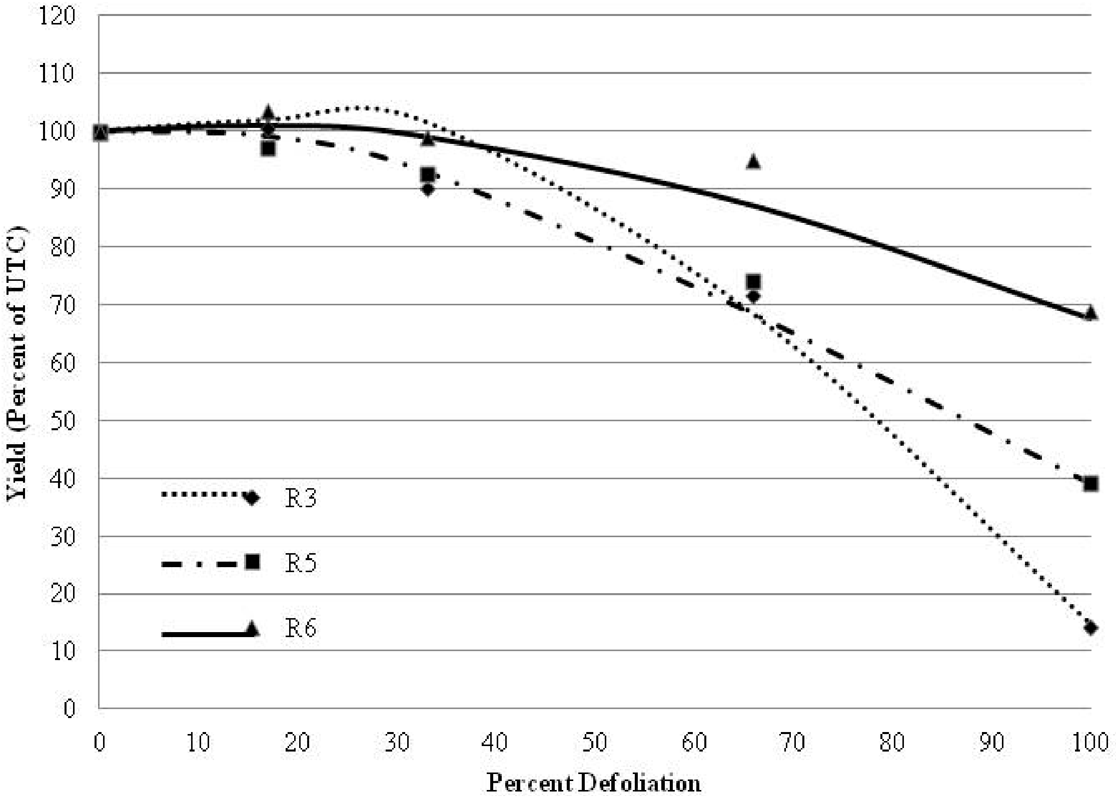

Figure 2.7 Average yield loss and overall best fit equation lines from log transformed data for whole plant defoliation at each growth stage as a % of the undefoliated control. Pair-wise contrasts determined each growth stage was significantly different with regard to yield loss (R3 vs R5: t= -3.60, df= 82.69, P>t=0.001; R3 vs R6: t= -6.92, df= 82.89, P>t<0.001; R5 vs. R6: t= -3.32, df= 82.89, P>t= 0.001). Equations for lines are presented in Table 2.2.

Table 2.1 Regression equations for log transformed yield loss for top and bottom defoliation as a percent of non-defoliated control (x=% defoliation).

Growth Stage		Yield loss equation (± SE for each term)	Undefoliated Control (kg/ha)*
R3	T	$8.67(\pm 0.19) - 0.00007x^2(\pm 0.00004)$	5839.58
	B	$8.66(\pm 0.19) - 0.00003x^2(\pm 0.00004)$	5796.67
R5	T	$8.66(\pm 0.19) - 0.00003x^2(\pm 0.00004)$	5771.48
	B	$8.67(\pm 0.19) - 0.00002x^2(\pm 0.00004)$	5840.26
R6	T	$8.67(\pm 0.19) - 0.00002x^2(\pm 0.00004)$	5802.12
	B	$8.68(\pm 0.19) - 0.00001x^2(\pm 0.00004)$	5870.22

*For any level of defoliation, inverse log of yield loss equation / undefoliated control * 100 = percent of control

Table 2.2 Regression equations for log transformed yield loss for whole plant defoliation as a percent of non-defoliated control (x=% defoliation).

Growth Stage	Yield loss equation (± SE for each term)	Undefoliated control (kg/ha)*
R3	$8.62(\pm 0.20) - 4.96E^{-6}x^{3}(\pm 1.43E^{-6}) + 0.0004x^{2}(\pm 0.00022)$	5541
R5	$8.59(\pm 0.19) - 0.00011x^{2}(\pm 0.00004) + 0.0013x(\pm 0.0036)$	5377
R6	$8.61(\pm 0.19) - 0.00005x^{2}(\pm 0.00004) + 0.001x(\pm 0.0036)$	5491.61

*For any level of defoliation, inverse log of yield loss equation / non-defoliated control * 100 = percent of control

Table 2.3 Economic injury levels at R3 and R5 for defoliation based on the yield loss equations for R5 (Table 2.2).

Value of Crop ($/ha)	**Cost of Control ($/ha)**					
	$15	**$20**	**$25**	**$30**	**$35**	**$40**
	R3-R5 Economic Injury Level (Percent Defoliation)					
3000	15	16	16	17	18	18
2500	15	16	17	18	19	19
1875	16	17	18	19	20	21
1250	18	19	21	22	23	24
625	22	24	26	28	30	31

*Yield loss equation: $8.59(\pm 0.19) - 0.00011x^2(\pm 0.00004) + 0.0013x(\pm 0.0036)$

Table 2.4 Economic injury level at R6 for defoliation based on the yield loss equation at R6 (Table 2.2).

Value of Crop ($/ha)	**Cost of Control ($/ha)**					
	$15	**$20**	**$25**	**$30**	**$35**	**$40**
	R6 Economic Injury Level (Percent Defoliation)					
3000	24	25	26	27	28	29
2500	25	26	27	29	30	31
1875	26	28	29	31	32	33
1250	29	31	32	34	36	37
625	34	37	40	45	45	48

*Yield loss equation: 8.61(± 0.19) - $0.00005x^2$(± 0.00004) + 0.001x(± 0.0036)

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CHAPTER III

IMPACT OF DEFOLIATION DURING VEGETATIVE GROWTH STAGES ON MATURITY GROUP IV AND V SOYBEAN (Glycine max, Merr.) YIELDS IN MISSISSIPPI

Abstract

Simulated defoliation field studies were conducted in 2010 and 2011 to determine the impact of defoliation during vegetative stages on soybean yield. Maturity group IV and V soybean were defoliated during V3 and V6 to 0, 33, 66, and 100% by removing leaflets manually. Yield loss was not significant from any level of defoliation during V3 for both maturity groups. However, there was a significant reduction in yield from defoliation occurring during V6 growth stage for both maturity groups. Yield was reduced by 20% from 100% defoliation occurring at V6 for the maturity group IV soybeans. Yields were recduced by 16% at V6 for the group V soybeans. Leaf area index readings were recorded during the R3 growth stage following simulated defoliation treatments during the vegetative stages. The trend for leaf area index for maturity group IV soybean was significant for both the V3 and V6 defoliated plots. Therefore, there were differences in the amount of light interception from plots that were defoliated and the untreated controls. Reduced leaf area for the V3 defoliated plots did not, however equate into a significant reduction in yield. Leaf area from V3 defoliations on maturity group V soybean was not different at R3. However, leaf area was reduced from defoliations occurring during V6 for the maturity group V soybean. Results from this research

indicate that young soybean seedlings have the ability to with stand substantial amounts of defoliation at early vegetative growth stages without negatively impacting yield. Economic injury levels were calculated from results in this experiment to determine the defoliation level at which yield losses equal the cost of control.

Introduction

In the midsouthern United States there has been a shift to early season soybean production, and defoliating pests prior to reproductive stages are often encountered in this system. Insecticidal seed treatments have been adopted to help suppress pests that feed on emerging soybean plants. However, due to the abundance of foliage feeding pests invading early planted fields, foliar insecticides are sometimes needed to minimize defoliation. Soybean acres in Mississippi have increased from 640,000 hectares in 2005 to 880,000 hectares in 2009, generating an estimated 705 million dollars in revenue (NASS 2010). Soybean maturity can affect insect densities and the susceptibility of the crop to those pests (Pedigo and Zeiss 1996). Traditionally soybeans were planted after corn and cotton into warm temperatures. This allowed young plants to emerge and grow vigorously. However, with soybeans planted earlier in the growing season, lower temperatures slow the growth rate of young soybean plants, making them more vulnerable to damage from foliage feeding pests. Yield reduction from the loss of foliage during vegetative stages is less than yield losses from defoliation during reproductive stages because soybean has the ability to develop new leaf area that can compensate for early damage (Singer et al. 2004). Singer (2001) reported that yield reductions from leaf removal at V5 (five trifoliate leaves) were less than from leaf removal at R4 (pods half inch or greater in top four nodes of plant).

The current defoliation threshold for soybeans in Mississippi during the vegetative stage is 35 % (Catchot et al. 2010). However, in an experiment conducted by Todd and Morgan (1972), they only observed statistical differences in yield at 100% defoliation level during the V5-V6 stages. No differences in seed weight for any level of defoliation (0, 33, 66, and 100%) were observed. Pickle and Caviness (1984) did not reduce yields with 25, 50, 75, or 100% defoliation at V5, and in some cases reported a yield increase from defoliation.

Previous research has identified a relationship between defoliation and light interception, measured as leaf area index (LAI) (Higley 1992, Hunt et al. 1994, Haile et al. 1998). Leaf area index (LAI) is a measurement of leaf area present as a proportion of total ground area (Klubertanz et al. 1996). Leaf area index values are derived from the formula:

$$\text{LAI}=\frac{\ln(\Gamma)(\cos(\psi)}{-0.5} \tag{3.1}$$

where Γ is the ratio of sunlight intercepted by the plant and what portion of the light reaches the ground, the ψ value is the zenith angle of the sun, and -0.5 is the extinction coefficient for soybean. Defoliation during vegetative stages can delay the time for the crop to reach the critical LAI value of 3.5 (Hunt et al. 1994).

Fehr et al (1977) reported that determinate soybean varieties were affected more than indeterminate varieties by levels of defoliation that occurred from R2-R6. It is unclear how determinate and indeterminate varieties react to various levels of defoliation during the vegetative stages. Therefore, our objectives were to evaluate vegetative growth stage defoliation thresholds on maturity group IV and V soybeans during two growth stages and examine LAI values after leaf removal during the growing season to determine the impact on yield.

Materials and Methods

Plot Establishment

Field experiments were conducted in 2010 and 2011 at the R.R. Foil Plant Research Center in Starkville, MS and the Delta Research and Extension Center in Stoneville, MS. Two soybean varieties were used during both years of the experiment. Asgrow 4605, a maturity group IV indeterminate variety, was planted in 2010 on April 15 at Starkville and on April 30 at Stoneville into raised conventionally tilled beds at a seeding rate of ~275,000 seeds per hectare with 97 cm row spacing. Asgrow 5606, a maturity group V determinate soybean variety, was planted on May 14 at Starkville and May 30, 2010 at Stoneville. In 2011, Asgrow 4605 soybeans were planted on June 20 at Starkville and on May 24 at Stoneville at the same rate and with the same agronomic practices as in 2010. Asgrow 5606 soybeans were planted on June 20 at Starkville and May 24 in Stoneville in 2011. In each year and location of the experiments, plots were furrow irrigated and managed for high yield potential, and irrigation timings varied by year and location. Prior to planting at all locations, seed was treated with thiamethoxam (Cruiser®, Syngenta Crop Protection, Greensboro, NC) at 35.49 ml/ 45.36 kg of seed and fludioxonil + mefenoxam (Apron Max®, Syngenta Crop Protection, Greensboro, NC) at 147.87 ml/45.36 kg of seed. Planting depth was set to 2.54 cm below the soil surface. The plot area was scouted and over-sprayed biweekly to reduce the effects of any insect or disease. Over-sprays of one or more pyrethroid, carbamate, or neonicotinoid insecticides were applied weekly to target most insect spectrums. Fungicide applications of azoxystrobin (Quadris®, Syngenta Crop Protection, Greensboro, NC) at 444 mL/ ha were made during the R3 and R5 growth stages for both years of the experiment. A randomized complete block (RCB) design with four replications was used at both locations during each year of the experiment. Plots were two rows wide and 3.05 m long.

Two separate experiments were conducted both years, one for maturity group IV and one for maturity group V. Within each experiment, the two vegetative stages evaluated were V3 (three trifoliate leaves) and V6 (six trifoliate leaves). In each of these growth stages 0, 33, 67, and 100% levels of defoliation were analyzed. Defoliation levels were achieved by manually removing 0, 1, 2, or 3 leaflets from each trifoliate leaf at each growth stage. Cotyledon leaves were not removed from plants in any treatment. Only trifoliate leaves were removed.

Leaf area index values were recorded post treatment twice during the growing season to document compensation and how plants performed throughout the remainder of the season. Leaf area index values were recorded at R3 and R5. Measurements were taken (2 per plot) with a Decagon AccuPAR LP-80 (Decagon Devices Inc., Pullman, WA). Leaf area measurements were obtained during the hours of 10:00 am and 2:00 pm central daylight savings time to ensure that the greatest amount of sunlight was available. Measurements were collected with the sensor placed above the top of the canopy to measure total available photosynthetically active radiation (PAR) at the top of the canopy.Another measurement was made by placing the sensor on the ground adjacent to the base of the soybean plants. By measuring PAR at the top and bottom of the canopy, the device was able to formulate a leaf area index based on the ratio of light the plants were intercepting (PAR top/ PAR bottom). The two readings for each plot were averaged and recorded as one LAI value.

Data Analysis

The mixed model analysis of variance (SAS Institute 2009) was used to analyze the data. Defoliation level and growth stage when defoliation took place and their interactions were fixed effects in the model, while year, location and replication were random factors. Degrees of freedom were calculated using the Kenwood-Rogers method. Differences were considered significant for α = 0.05. Defoliation level was analyzed as a numeric factor, so regression equations could be determined for the relationship between yield and defoliation. The relationship between defoliation and LAI was also determined. Yield in the undefoliated controls varied by year and location. Therefore, data are presented as a percentage of the untreated control within each location and year, although all analyses were done on actual yield.

Results and Discussion

Maturity Group IV Soybean

Results were similar to other reports documenting that soybeans have the ability to withstand extensive amounts of defoliation during the vegetative stages before suffering economic injury (Fehr et al. 1981, Hunt et al. 1994, Hammack et al. 2010). Yield was not significantly reduced from defoliation occurring at V3 (F= 0.41; df= 1, 44; P= 0.5243) (Figure 3.1.); however there was a significant reduction in yield from defoliation events occurring during V6 (F= 27.58; df= 1, 42; P= <0.0001) (Figure 3.1).Yield reductions from V6 defoliation averaged 20% from 100% defoliation.

Leaf area index readings were recorded during the R3 growth stage following simulated defoliation treatments during the vegetative stages. LAI was significantly reduced from defoliation (V3: F= 7.88; df= 1, 33; P= 0.0083; V6: F= 13.70; df= 1,32; P=

0.0008) (Figure 3.2). However, 100% defoliation during the V3 growth stage did not cause the leaf area index during R3 to fall below the critical value of 3.5 reported by Hunt et al. (1994) needed for maximum soybean yields; therefore there was no yield response to the V3 defoliation. Significant yield loss was observed from V6 defoliation and LAI was significantly reduced from V6 defoliation. Furthermore, 100% defoliation during the V3 stage caused the leaf area index during R3 to be below the critical value of 3.5 (Figure 3.2). However, when LAI measurements were collected during R5 stage, all defoliated plots had compensated from leaf removal and leaf area index did not significantly change with defoliation (V3: F= 1.81, df = 1, 33, P= 0.18; V6: F= 3.6, df= 1, 32, P= 0.067). Plant compensation occurs by two mechanisms: compensatory growth and delayed leaf senescence (Haile et al. 1998). Plants at the vegetative stage have the potential to compensate readily for defoliation by producing new leaves from apical meristems, therefore providing leaf area recovery (Boote 1981, Ostlie 1984, Haile et al. 1998).

Maturity Group V Soybean

Results from simulated defoliation on maturity group V soybeans were similar to what was observed with the maturity group IV soybeans. Yield was not significantly reduced from defoliation occurring at the V3 growth stage (F= 2.04; df= 1, 45; P= 0.29), and yields were significantly reduced from V6 defoliation (F= 7.24; df= 1, 43; P= 0.0099) (Figure 3.3). Yields were reduced by 2% from 35% defoliation during V6, and yields were reduced by 16% from 100% defoliation.

Leaf area index values were not significantly different at R3 for the V3 defoliated soybeans (F value= 0.01, df= 1, 32, P= 0.92). However, LAI was significantly reduced from defoliation occurring at V6 (F= 12.74; df= 1, 33; P= 0.0011) (Figure 3.4). Leaf area index values from V6 defoliation for the determinate soybeans were similar to those of the indeterminate group IV soybeans (Figure 3.2 and 3.4). As observed with the MG IV soybeans, LAI at R5 was not different among the treatments (F= 0.01, df= 1, 32, P= 0.95). Vegetative stages have more recovery potential than later growth stages. Therefore, effects of early season defoliation are minimal (Weber 1955).

Data presented here show that yield loss from defoliation was only important when it occurred during late vegetative stages. Defoliation occurring during V3, even at 100%, had no long term impact on soybean growth, and LAI values were not impacted at R5 for both determinate and indeterminate soybeans. Determinate soybeans had fully recovered by R3 from defoliation during V3. Since there was enough time for the plants to fully compensate for the amount of foliage lost prior to the critical R5 growth stage, yield was not reduced. Yield loss was not significant at V3 for either of the maturity group soybeans. Therefore, based on our data, it is not economical to make a foliar insecticide application at this time for defoliating pests.

Yield was significantly reduced from defoliation at V6 for both determinate and indeterminate soybeans, and economic injury levels were calculated for defoliation occurring at V6. Economic injury levels vary based on the value of the crop, cost of control, and the amount of yield loss caused by the damage based on the equation: EIL= C/VIDK (Pedigo et al. 1986). In this equation V is the value of the crop in dollars per hectare; ID is based on the amount of damage or yield loss from defoliation; and K is the percent control that is expected from an application of an insecticide.Values for cost of control are based on the total cost per hectare. This includes the cost of the insecticide and application costs. Pickle and Caviness (1984) reported determinate and indeterminate cultivars responded similarly to a number of different defoliation treatments. Therefore the most conservative equation (indeterminate V6) equation was used to make the calculations for one EIL for both determinate and indeterminate varieties (Table 3.1). The current economic threshold (ET) utilized for vegetative defoliation is 35% (Catchot et al. 2010). The ET is generally below the ET; therefore an ET could be assigned as 75% of the EIL. For example, a soybean crop valued at $1875/ha with an application cost of $25/ha will have an EIL of 35%, so a reasonable ET would be 75% of this, or 26% defoliation. By clearly defining the relationship between defoliation and yield loss, it is possible to determine the EIL and corresponding ET for each unique situation rather than use the static thresholds previously developed. This should facilitate more economical early season insect pest management practices.

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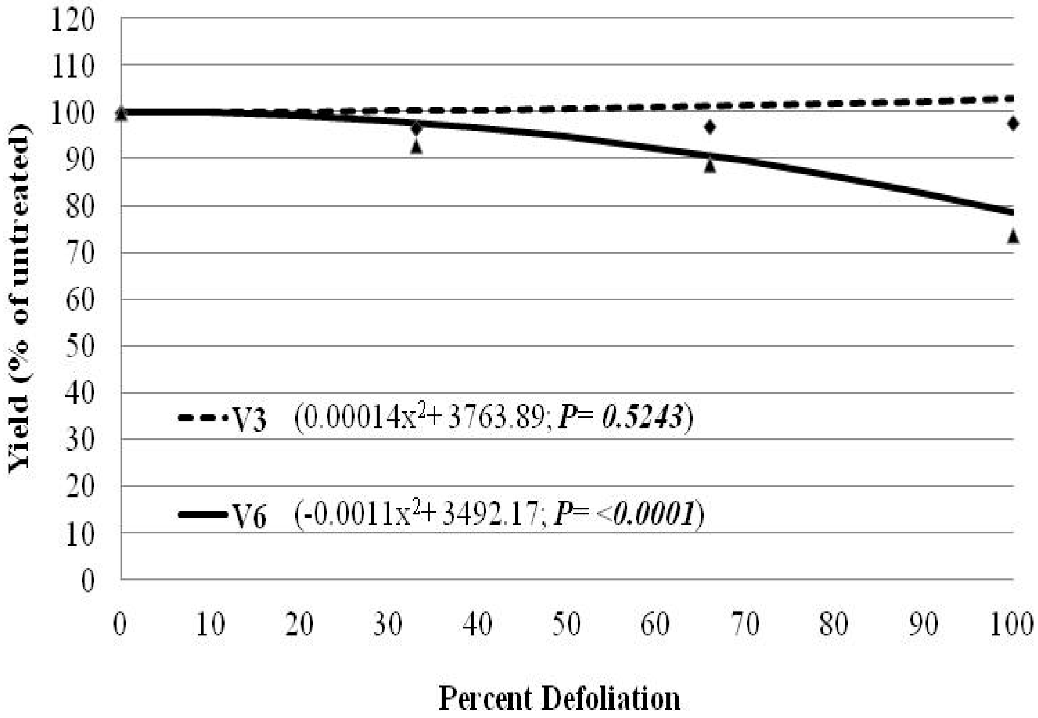

Figure 3.1 Average yield loss and equations from vegetation defoliation for maturity group IV soybeans during V3 and V6 growth stages presented as % of the untreated control. (V3: F= 0.41; df= 1, 44; P= 0.5243; V6: F= 27.58; df= 1, 42; P= <0.0001).

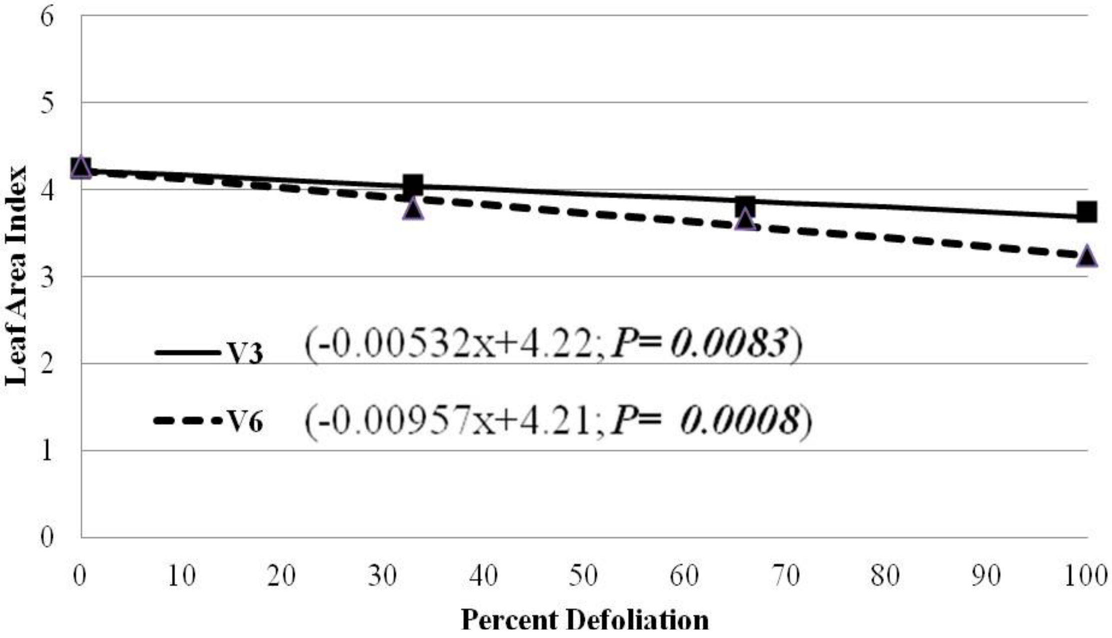

Figure 3.2 Average leaf area index and regression equation for MG IV soybean during R3. (V3: F= 7.88, df= 1,33, P= 0.0083; V6: F= 13.70, df= 1,32, P= 0.0008)

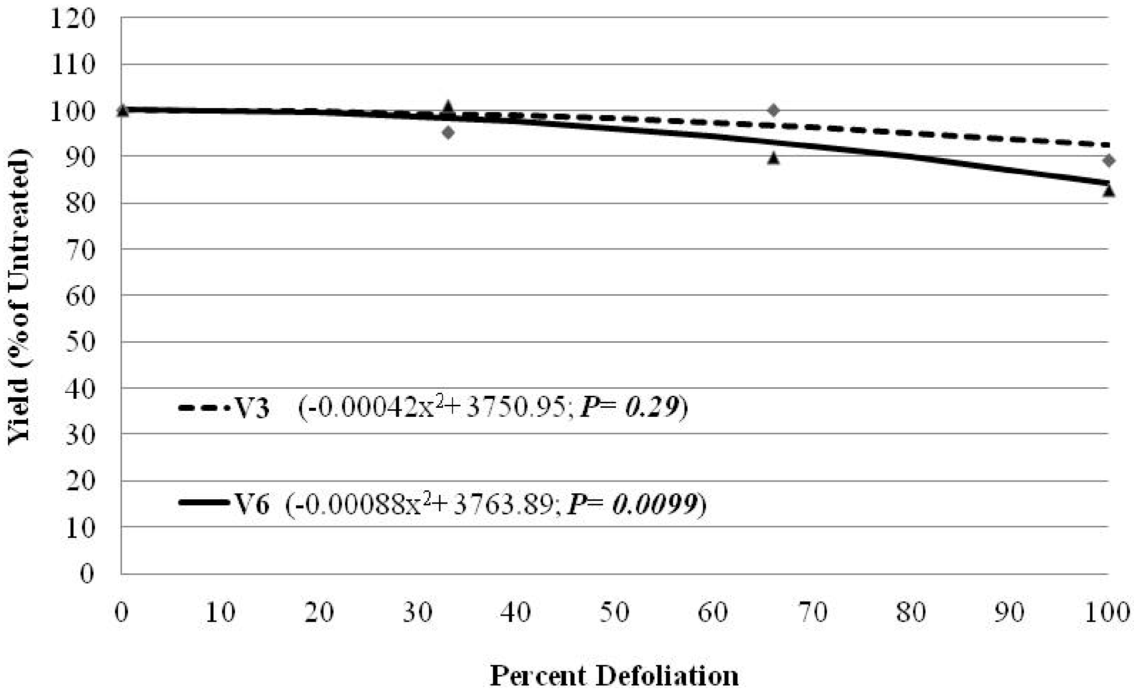

Figure 3.3 Average yield loss and equations from vegetation defoliation for maturity group V soybeans during V3 and V6 growth stages presented as % of the untreated control. (V3: F= 2.04; df= 1, 45; P= 0.29; V6: F= 7.24; df= 1, 43; P= 0.0099).

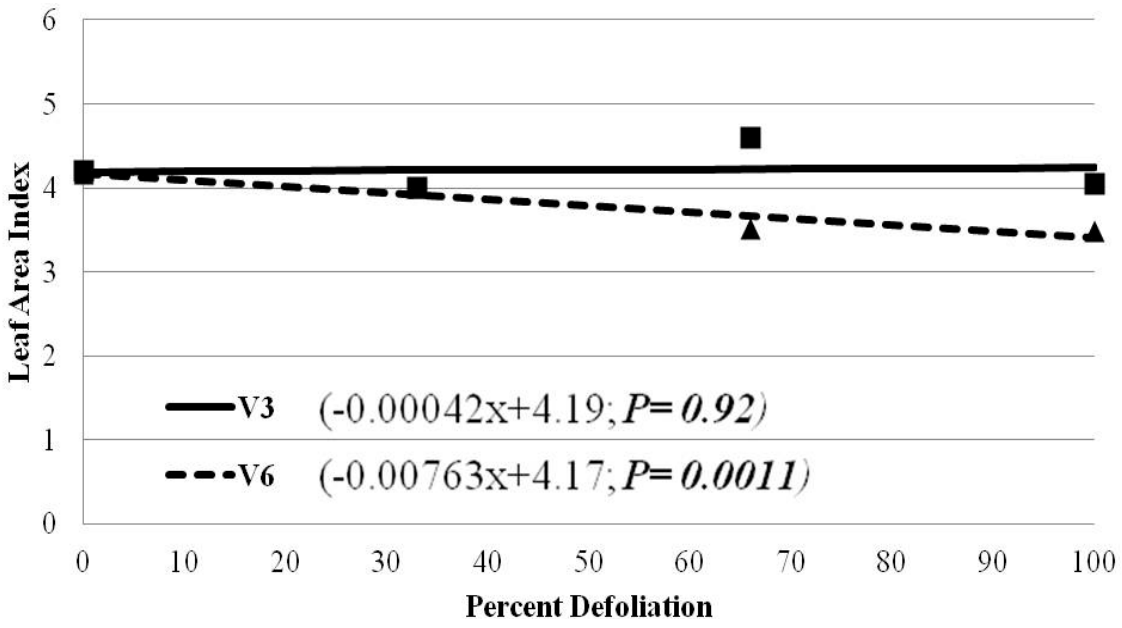

Figure 3.4 Average leaf area index and regression equation for MG V soybean during R3. (V3: df= 1,32, F value= 0.01, *P*= 0.92; V6: df= 1,33, F value= 12.74, *P*= 0.0011).

Table 3.1 Economic injury levels for determinate and indeterminate soybean based on the yield loss from defoliation at V6*.

Value of Crop ($/ha)	**Cost of Control ($/ha)**					
	$15	**$20**	**$25**	**$30**	**$35**	**$40**
	Economic Injury Level (Percent Defoliation)					
3000	21	25	28	30	32	35
2500	23	27	30	33	36	38
1875	27	32	35	38	41	44
1250	33	38	42	47	50	54
625	47	54	60	66	71	76

*Yield loss equation: -0.0011x2+ 3492.17 where x is % defoliation

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CHAPTER IV

SUSCEPTIBILITY OF CHRYSODEIXIS INCLUDENS (LEPIDOPTERA: NOCTUIDAE) TO REDUCED RISK INSECTICIDES

Abstract

Field populations of soybean looper, Chrysodeixis includens (Walker), were collected from soybean, Glycine max (L.) Merr., fields in Mississippi and Louisiana during 2010 and 2011 to determine their susceptibility to novel insecticides. Flubendiamide and chlorantraniliprole are diamide insecticides that have recently been registered for use in field crops. Baseline data were collected for each of these insecticides as well as for methoxyfenozide, which has been the recommended insecticide for soybean looper in Mississippi soybeans prior to the introduction of these new novel insecticides. Mean LC50s for flubendiamide and chlorantraniliprole were similar among the populations tested, and susceptibility was higher for methoxyfenozide compared to flubendiamide and chlorantraniliprole. Diet incorporated assays determined a 9.4 fold variation in susceptibility to flubendiamide among the seven soybean looper populations tested. Variation to chlorantraniliprole was 6.25 fold and variation for methoxyfenozide was 5.37 fold. Variation in the diamide insecticides was higher than methoxyfenozide with less exposure to soybean looper populations. Documenting variability along with baseline data will be useful in the future for resistance monitoring of soybean loopers to diamide insecticides.

Introduction

The soybean looper, Chrysodeixis includens (Walker), has become one of the most costly pests to manage in soybeans because of their ability to consume massive amounts of foliage (Mascarenhas and Boethel 1997). It is a migratory species and populations peak in the southern United States in mid-August to September (Carner et al. 1974). Reported annual losses from soybean looper can exceed 10% with regard to crop yield and crop damage plus control costs (Mascarenhas and Boethel 1997). Musser et al. (2010) documented 16.3% of total insect losses in soybean (including control costs) in Mississippi were from soybean looper during 2009. Pyrethroid resistance in soybean looper has been documented where soybean and cotton are grown in the same area (Felland et al. 1990, Leonard et al. 1990, Mink and Boethel 1992). During the mid 1980s, control failures with pyrethroids were commonly reported, even when properly applied at recommended use rates (Felland et al. 1990). Diet overlay experiments were conducted in 1995 on Louisiana strains of soybean looper, and the LC50 for permethrin treated diet ranged from 14.69 to 60.87 ppm (Mascarenhas and Boethel 1997). The authors reported all field populations tested in this experiment had significantly higher LC50 values than a susceptible USDA strain (LC50=1.59 ppm). Thus, insecticide resistance evolution in soybean looper populations is a concern.

Recently, two novel insecticides were registered in soybean and other crops for control of soybean looper and other lepidopteran pests. Flubendiamide (Belt® 4SC, Bayer CropScience, Research Triangle Park, NC) and chlorantraniliprole (Coragen® 1.67SC, DuPont Crop Protection, Wilmington, DE) represent the diamide class of insecticides that react with ryanodine receptors in the muscle cells, causing channels to open and release calcium ($Ca2+$) into the cytoplasm, leading to muscle paralysis and eventual death (Cordova et al. 2006, Lahm et al. 2007). Baseline responses of soybean looper to these compounds are lacking, and establishing initial toxicity ranges for field strains of target pests provides an important reference for future resistance monitoring efforts.

Prior to the introduction of chlorantraniliprole and flubendiamide, methoxyfenozide, (Intrepid® 2F, DowAgrosciences, Indianapolis, IN), a member of the diacylhydrazides class of insect growth regulators (IGR), was used extensively to control soybean loopers in Mississippi (Catchot et al. 2010). Diacylhydrazide insecticides mimic the molting hormone ecdysone in lepidopteran insects. Ecdysone is a natural hormone that induces molting and metamorphosis at low levels. In the absence of ecdysone, the insect will remain at the larval or immature stage (Sparks 1996). Baseline data for this insecticide on Mississippi soybean looper populations were never established. Control problems were reported during 2009 and 2010 and the lack of baseline data made determining resistance development to methoxyfenozide in soybean looper populations difficult.

Resistance monitoring relies on initial quantification of baseline responses to susceptible populations (Robertson et al. 2007). Therefore, the objectives of this study were to estimate the responses of field-collected populations of soybean looper to chlorantraniliprole, flubendiamide and methoxyfenozide, and to establish baseline response data for flubendiamide and chlorantraniliprole.

Materials and Methods

Insects

Soybean looper larvae were collected from five soybean fields during 2010 and 2011 (Table 4.1) using a 38.1 cm diameter sweep net and taken to the laboratory. Larvae were placed individually into 30 ml cups containing an artificial wheat germ-based diet prepared in the laboratory with the addition of linseed oil at 25 ml/3.79 L of diet (BioServ, Heliothis diet dry mix USDA item # F9915, Vitamin premix USDA item # 6265 and pure linseed oil USDA item # 5680). Soybean looper colonies were maintained at 24oC with 60-80% RH, and photoperiod was set to 16:8 (L:D). Soybean loopers were allowed to pupate and transferred into a 3.79 L cardboard bucket (approximately 50 per bucket) and allowed to emerge as adults. Adults were fed 20% honey water solution and transferred to clean buckets every two days. Eggs were collected every two days and allowed to hatch. After eclosion, individual neonates were immediately transferred to 30 ml diet cups using a #000 paint brush. Bioassays were conducted on 3rd instar larvae (20-30 mg) from the F2 or F3 generations for each field strain. A field reference lab colony was established from a wild population of soybean loopers because no known sources of laboratory colonies could be obtained. The field reference colony was collected in Tchula, MS in 2009 and kept in laboratory conditions for approximately two years. Wang et al. (2010) determined that a field reference colony of the diamondback moth, Plutella xylostella (L), kept in the laboratory for extended periods without exposure (26 and 80 generations) was as susceptible to chlorantraniliprole as a susceptible lab colony.

Bioassays

The artificial diet already described was prepared in the laboratory immediately prior to infestation of larvae. The semi-solid diet was prepared following the manufacturer's standard protocol. One ml of each insecticide was added to an appropriate amount of clean wheat germ diet to make a 1 mg a.i. /ml stock diet based on the amount of active ingredient in the formulated commercially available insecticide (Table 4.1). Each insecticide stock diet was prepared in a 500 ml beaker and agitated for 45-60 s with a handheld mixer (Black and Decker, Mirimar, FL). A total of 6 insecticide concentrations for methoxyfenozide ranged from 0.15-5 µg/ml (Table 4.1) plus an untreated control. In order to obtain the 5 µg/ml concentration, 2.5 ml of the prepared methoxyfenozide stock diet was added to 500 ml of clean diet. Eight insecticide concentrations for flubendiamide and chlorantraniliprole ranging from 0.15-20 µg/ml (Table 4.2) were made using serial dilutions. Diet without any insecticide was used as an untreated control. Approximately 9 ml of diet were dispensed into each of 30 plastic diet cups (Solo Cup Co., Highland Park, IL) for each concentration and each insecticide. Preliminary assays were used to determine the effective dose range for each compound on the reference lab strain. Each soybean looper colony collected was subjected to the same effective dose range for each insecticide to determine the lethal concentration to kill 50% of the test population (LC50). Thirty 3rd instar larvae (20-30 mg larval weight) from each field-collected strain were subjected to each insecticide dose for 96 hours and mortality was recorded. Larvae were considered dead if they had no coordinated movement and were not able to right themselves in 5 seconds after being flipped onto their dorsal side. Data were recorded as number of individuals alive and number of individuals dead for each concentration. Dose mortality curves were analyzed using probit analysis (SAS Institute 2009), and lethal concentration (required to kill 50% of a test population) estimates were produced for each colony and insecticide. Data were corrected for control mortality (Abbott 1925) and non-overlapping confidence limits (95%) were used to determine differences among populations.

Results and Discussion

Prior to the registration of flubendiamide in soybean, methoxyfenozide was the primary insecticide recommended for soybean looper control in soybeans. However, baseline data for this insecticide were never produced for Mississippi populations. Therefore, it is important to document susceptibility of field populations prior to the occurrence of field control failures. Responses of soybean looper populations exposed to methoxyfenozide varied by 5.37 fold (LC50= 0.27-1.45 µg/ml diet) (Table 4.3). No significant differences in LC50 were observed among the colonies tested. Overall LC50 values of methoxyfenozide were significantly lower than those for chlorantraniliprole based on non-overlapping confidence intervals (Tables 4.3 and 4.4).

Soybean looper larvae collected from different soybean fields within Mississippi and Louisiana showed varying levels of susceptibility to flubendiamide (Table 4.5). Susceptibility of soybean loopers to flubendiamide varied by 9.2 fold (1.02-9.4 µg/ml diet). Mortality for all tested populations indicated a good fit to a probit model (Pearson's X2 test; $P > 0.05$). The ST10 and LAB10 populations had LC50 values of 3.12 and 3.02 µg a.i./ml diet, respectively, and the LA10 colony had an LC50 of 9.4 µg a.i./ml diet. However, these differences among the 2010 colonies were not significant (Table 4.5). Similarly in 2011, none of the field-collected soybean looper populations were significantly different from the reference lab colony. Overall, susceptibility of soybean looper populations tested against flubendiamide was not significantly different than soybean looper susceptibility to methoxyfenozide and chlorantroniliprole.

Soybean looper populations exposed to chlorantraniliprole had LC50 values that ranged by 6.25 fold (0.8 to 5.01 µg/ml diet) (Table 4.4). Overall, susceptibility of soybean looper populations to chlorantraniliprole did not differ from that of flubendiamide. ST10 was less susceptible to chlorantraniloprole than LAB10 and LAB11. However, susceptibility of ST10 was not different from LA10. Susceptibility of LA10 to chlorantraniliprole was not different than LAB10 or LAB11 colonies. Susceptibility of the field collections from LA varied by 3.8 fold (Table 4.4).

Susceptibility of insect populations to stomach poisons, such as chlorantraniliprole and flubendiamide, has been documented previously. Asfaq et al. (2010) documented 5.18 fold variation to chlorantraniliprole in field populations of Choristoneura roseceana (Harris) with limited exposure collected from orchards in Washington State. The authors suggested that this variation in susceptibility could lead to more rapid resistance development after widespread exposure in the field. Temple et al. (2009) found that bollworm, Heliocoverpa zea Boddie, populations collected from various states varied by 4.5 fold to chlorantraniliprole susceptibility. However, Wang et al (2010) reported variation in susceptibility of the diamondback moth in China was less than 5 fold for chlorantraniliprole. Variation in pyrethroid susceptibility among soybean looper populations was also reported previously by Leonard et al. (1990), along with reduced field efficacy of the pyrethroids commonly used at that time. Currently, pyrethroids are not recommended for soybean looper management in soybean fields because of the level of resistance they have developed. Therefore, documenting evidence of variation in susceptibility to novel insecticides, such as flubendiamide and chlorantraniliprole, prior to their widespread use is important so that resistance management techniques can be implemented to preserve these insecticides.

Feeding cessation was not quantified in this experiment; however, it was observed at every concentration of flubendiamide and chloratraniliprole utilized in the bioassays. Insecticide concentrations as low as 0.15 μg/ml for the diamide insecticides reduced feeding and decreased the overall size of insects when assays were rated. Consistent reduction in the amount of feeding and rapid feeding cessation from chloratraniliprole has been documented in various lepidopteran species (Hannig et al. 2009). Time after exposure to chlorantraniprole to stop feeding for cabbage looper, Trichloplusia ni (Hübner) was 23.4 minutes, whereas, feeding ceased 408.8 minutes after exposure to methoxyfenozide. The effectiveness of these insecticides in field applications could be due to a reduction in feeding, causing larvae to become weak and fall from the plant. An effective dose within a field may not be enough to kill the insect immediately but prevent further feeding. Control failures with non selective nerve poisons, such as pyrethroids and carbamates, were not hard to document because failures in the field were easily detected in the laboratory. Documenting control failures with diamides may not be easy because of the difference between the amount required to kill the insect and the dose needed to subdue or stop feeding and growth.

Delaying or preventing resistance development to insecticides is important to the sustainability of integrated pest management (IPM) in soybeans. Soybean loopers have developed resistance to pyrethroid insecticides (Felland et al. 1990, Portillo et al. 1993), and the number of labeled insecticides effective against soybean loopers is limited. Therefore it was important to document the variation in susceptibility of soybean looper populations to the diamide class of insecticides for future reference in the event of control failures. Few control failures of soybean looper to methoxyfenozide have been reported to date in Mississippi. However, in the event of a control failure, the overall LC50 for field populations collected in Mississippi and Louisiana was 0.96 µg/ml diet with 5.37 fold variation. These data will be useful in determining resistance ratios if control failures are reported in the future.

Flubendiamide and chloratraniliprole will likely be applied to many soybeans in the future. Monitoring the susceptibility of pest populations to these insecticides is important. Results presented here determined a 6.25 and 9.2 fold variation in soybean looper populations collected in Mississippi and Louisiana for chloratraniliprole and flubendiamide, respectively. However, overall susceptibility of soybean looper to flubendiamide and chlorantraniliprole was the same (LC50= 2.89 and 2.61, respectively).Resistance management strategies and documenting variability of natural populations to an insecticide prior to its widespread use can influence resistance management decisions for these novel insecticides and should be taken into consideration when insecticide applications are made.

Acknowledgements

We thank Kathy Knighten and Teresa Zeigelemann for their assistance in rearing field populations and maintaining a soybean looper colony in the laboratory.

Table 4.1 Description of soybean looper field strains by identification code and collection site.

Code	Year	Location
LAB10[a]	2010	Mississippi State University, Mississippi State , MS
LA10	2010	Franklin Parish; Winnsboro, LA
ST10	2010	Washington County; Stoneville, MS
LAB11[a]	2011	Mississippi State University, Mississippi State , MS
LA11	2011	Franklin Parish; Winnsboro, LA
GW11	2011	Leflore County; Greenwood, MS
TCH11	2011	Holmes County; Tchula, MS

[a] Field reference lab strain; initial collection was made in Tchula, MS during August of 2009.

Table 4.2 Insecticide information for products used in the experiment.

Trade Name	Active ingredient	kg ai/ L	Formulation	mL diet in stock[a]	Manufacturer	Dose Range (µg/ml)
Belt®	flubendiamide	0.48	SC	480	Bayer CropScience	0.15-20
Coragen®	chlorantraniliprole	0.2	SC	200	DuPont	0.15-20
Intrepid®	methoxyfenozide	0.24	F	240	Dow Agrosciences	0.15-5

a One ml of insecticide was added to clean diet to obtain a 1 mg/ml concentration based on amount of active ingredient in the commercially available insecticide.

Table 4.3 Susceptibility of soybean loopers, Chrysodeixis includes, to methoxyfenozide (Intrepid®) in diet incorporated bioassays 96 hrs after exposure

Colony	**Gen.[a] Tested**	***n*[b]**	**LC_{50} (95% CL) µg/ml**	**LC_{90} (95% CL) µg/ml**	**Slope Ln(rate) ± SE**	***Pearson's X^2***
LAB10	F12+	180	0.92 (0.34-1.97)	17.30 (6.6-119.82)	0.43±0.09	5.5
ST10	F2	180	1.44 (0.67-2.72)	17.42 (7.73-88.05)	0.5±0.11	2.2
LA10	F2	180	1.45 (0.46-4.75)	7.95 (2.95-397.85)	0.75±0.18	11[*]
GW11	F3	180	0.47 (na)	4.82 (na)	0.55±0.29	1.1
LA11	F3	180	0.87 (0.33-1.74)	11.78 (5.31-51.61)	0.49±0.10	2.5
TCH11	F3	180	0.62 (0.14-1.57)	16.11 (5.5-151.65)	0.39±0.09	3.5
LAB11	F24+	180	0.27 (0.0008-1.33)	5.28 (0.89-24.86)	0.43±0.14	0.77
Overall[c]		**900**	**0.96 (0.47-1.61)**	**12.18 (6.82-30.03**	**0.49±0.04**	**31.51**

[a] Number of generations in the laboratory when tested.
[b] Number of individuals tested excluding controls.
[c] LAB colony data excluded from overall analysis
[*] Indicates a significant chi square value

Table 4.4 Susceptibility of soybean loopers, Chrysodeixis includes, to chlorantraniliprole (Coragen®) in diet incorporated bioassays 96 hrs after exposure.

Colony	**Gen.[a] Tested**	***n*[b]**	**LC_{50} (95% CL) µg/ml**	**LC_{90} (95% CL) µg/ml**	**Slope Ln(rate) ± SE**	**Pearson's X^2**
LAB10	F12+	210	0.8 (0.18-1.85)	9.12 (4.42-21.38)	0.53±0.09	1.4
ST10	F3	240	5.01 (3.54-7.08)	29.84 (18.01-70.68)	0.71±0.11	4.9
LA10	F3	210	2.7 (1.78-4.48)	25.50 (13.20-75.50)	0.57±0.08	6.8
GW11	F3	240	1.8 (0.86-3.15)	23.20 (12.46-58.91)	0.50±0.07	4.6
LA11	F3	240	0.71 (0.17-1.62)	14.4 (6.91-41.22)	0.42±0.07	2.5
TCH11	F3	240	2.5 (0.41-5.98)	68.08 (27.81-496.75)	0.39±0.09	0.97
LAB11	F24+	240	0.83 (0.18-1.89)	12.02 (6.22-28.81)	0.48±0.0.09	0.86
Overall		**1170**	**1.92 (0.73-2.71)**	**34.19 (20.72-69.02)**	**0.51±0.03**	**22.6**

[a] Number of generations in the laboratory when tested.
[b] Number of individuals tested excluding controls.
[c] LAB colony data excluded from overall analysis

Table 4.5 Susceptibility of soybean loopers, Chrysodeixis includes, to flubendiamide (Belt®) in diet incorporated bioassays 96 hrs after exposure

Colony	**Gen.[a] Tested**	***n*[b]**	**LC_{50} (95% CL) µg/ml**	**LC_{90} (95% CL) µg/ml**	**Slope Ln(rate) ± SE**	**Pearson's X^2**
LAB10	F12+	180	3.02 (0.41-7.6)	49.7 (20.31-287.76)	0.45±0.11	0.6
ST10	F2	140	3.12 (1.29-5.9)	37.34 (17.54-147.34)	0.51±0.10	4.2
LA10	F2	210	9.4 (5.5-19.1)	128.54 (50.14-738.47)	0.49±0.08	8.5
GW11	F2	240	2.19 (1.2-3.8)	34.60 (16.70-105.96)	0.46 ± 0.06	9.3
LA11	F2	240	1.67 (0.64-2.99)	19.03 (10.82-46.70)	0.53±0.09	1.7
TCH11	F2	180	1.02 (0.44-1.80)	9.32 (4.91-29.42	0.58±0.11	1.5
LAB11	F24+	240	2.05 (0.11-6.59)	101.94 (33.24-1428)	0.32±0.09	0.56
Overall[c]		**1010**	**2.89 (1.39-5.09)**	**15.17 (8.83-28.98)**	**0.45±0.03**	**32.2**

[a] Number of generations in the laboratory when tested.
[b] Number of individuals tested excluding controls.
[c] LAB colony data excluded from overall analysis

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CHAPTER V

SUMMARY

Field experiments were conducted during 2009-2011 to facilitate our understanding of how the defoliating insect complex affects soybean yield in Mississippi. Experiments were set up to evaluate thresholds that were established many years ago. The research used to determine these original thresholds was conducted on determinate varieties and based on production practices that are no longer relevant to Mississippi soybean growers. Therefore, presented in this dissertation are results from experiments evaluating the effects of various defoliation levels at different growth stages.

The second chapter of this dissertation addresses the results from defoliation occurring during the reproductive stages of soybean development. In this experiment, three growth stages (R3, R5, and R6) and three portions of the plant canopy (top, bottom, and whole plant) were subjected to five levels of defolilation (0, 17, 33, 66, and 100%). Plots were harvested and data were analyzed to determine the effects each treatment had on yield. No differences were observed between top vs. bottom defoliation. Therefore, we concluded that each soybean leaf is as important as the next for contributing to yield. Of the three growth stages evaluated, the most important in terms of yield loss from defoliation was R5. However, R3 and R5 yield losses were not significantly different under approximately 60% defoliation. Therefore, for defoliation levels under 60%, one economic threshold can be used for each of these growth stages. Although yield loss from defoliation at R3 and R5 was not different below 60%, defoliation at R3 and R5 caused significantly more yield loss than defoliation at R6.

Results from this research will be used to calculate economic injury levels and economic thresholds for defoliation during reproductive growth stages. This will allow growers and consultants to make better decisions regarding insecticide applications for defoliating insect pests.

Chapter three focused on defoliation during the vegetative growth stages of soybean. Experiments were conducted to evaluate two vegetative growth stages (V3 and V6) at 0, 33, 66, and 100% defoliaton. The effects of these treatment combinations were analyzed for maturity group IV and maturity group V soybean. Defoliation at V3 did not reduce yields for either of the maturity groups. Defoliation during V6 significantly reduced soybean yields, and no differences in the trends were observed for either the maturity group IV or V varieties. A significant result of this research concluded that no yield loss was observed from even extreme levels of defoliation at V3. Data presented in this dissertation suggest that any foliage lost during the early vegetative growth stages, assuming that it does not continue for a substantial amount of time, will be fully compensated by the time those plants reach the critical seed filling period (R5).

Leaf area index has been used in previous research to estimate yield loss from defoliation based on the amount of light being intercepted by a soybean plant. Leaf area index measurements were collected during R3 and R5 of this experiment. Leaf area index had a negative linear relationship with defoliation. As defoliation increased leaf area index decreased. Defoliation during V6 resulted in significantly lower LAI at R3 for both maturity groups. However, LAI measurements recorded at the critical R5 growth stage were not impacted by the V6 defoliation, suggesting that all defoliated treatments eventually compensated for foliage lost at the V6 growth stage. Economic injury levels were calculated using the yield loss equations derived in this experiment.

Results reported in this dissertation revolve around the indirect damge from the defoliating insect complex. Arguably, the most economically important pest species in the defoliating insect complex for Mississippi is the soybean looper. Soybean loopers are economically important because of the large amounts of damage they can cause in a short amount of time, and they have developed resistance to most insecticides classes labeled in soybean. As a result, losses from soybean looper are the result of reduced yields and high control costs.

Prior to the introduction of the diamide insecticides, flubendiamide and chlorantraniliprole, the most widely used insecticide in soybean to control soybean looper was methoxyfenozide. Although methoxyfenozide has been labeled in soybean for many years, baseline data in Mississippi was lacking. Currently few control failures have been reported. If resistance is suspected, the lack of baseline data makes confirmation difficult. Diet incorporated dose mortality bioassays were conducted on soybean looper to various concentrations of methoxyfenozide to document the overall variability present within field populations from Mississippi and Louisiana. The mean LC50 for those populations was 0.96 µg/ml diet with 5.37 fold variation. These data will be useful in determining changes in the susceptibility of soybean looper populations if control failures are reported in the future.

Novel insecticides, flubendiamide and chloratraniliprole, will likely be applied to many soybean fields in the future. Monitoring the susceptibility of pest populations to these insecticides is important. Results presented here determined a 6.25 and 9.2 fold level of variation in soybean looper populations collected in Mississippi and Louisiana for flubendiamide and chloratraniliprole, respectively. Overall susceptibility of soybean looper to flubendiamide and chlorantraniliprole was similar (LC50= 2.89 and 2.61, respectively), but more variability was observed in soybean looper populations to flubendiamide. Documenting the natural variability of pest populations to an insecticide prior to its widespread use is important and can influence resistance management decisions. Data presented here will be useful for monitoring changes in the susceptibility of soybean looper populations over time.

APPENDIX A

METHODS AND MATERIALS

Cage Experiments

During 2010, cage experiments were conducted to investigate soybean yield loss from soybean loopers. Techniques were be used to inundate plots with laboratory-reared soybean loopers into field cages.. Yield and LAI data are presented.

Prior to infestation, soybeans were treated with 74 ml/ha of methyl parathion to eliminate any predator insects. Newly emerged soybean looper adults from a lab colony reared at Mississippi State University were released into nine 1.8 m x 1.8 m caged soybean plots (40-50 soybean looper moths per cage) approximately 10-14 d prior to R3 and R6 growth stage. One cage remained uninfested to be used as a check. Therefore, a total of ten cages were used in the experiment. Soybean loopers were allowed to mate within the cages and lay eggs. Eggs were observed in cages within 7 d of infestation. Soybean looper larvae were allowed to defoliate soybeans within each of the ten cages to different levels during each growth stage. Defoliation levels were estimated by averaging estimates of defoliation from three individuals and using LAI measurements (Table A.1. and A.2.). The desired defoliation levels of the cages collectively were to obtain 2-3 cages with defoliation levels at or below the 20% defoliation threshold, 2-3 cages near 50% defoliation, and 2-3 cages near 100% defoliation for each growth stage. Once a cage reached the desired level of defoliation, soybean loopers were terminated with an application of flubendiamide insecticide. After soybean loopers had been killed, the cages were removed from the plot area and LAI measurements were collected. At the end of the growing season, plots were harvested with a plot combine and yields were recorded Figure A.1 and A.2.).

Table A.1 Results from field cage infestations of soybean looper, C. includens, at R3 growth stage.

Cage #	**% Defoliation**[a]	**Leaf Area Index**[b]	**Yield (Kg/ha)**
1	95	0.87	471.1
2	85	1.22	938.8
3	5	2.26	2691.2
4	35	2.49	3221.6
5	40	2.61	2371.7
6	15	2.61	3610.3
7	25	1.97	3168.9
8	10	2.65	3178.8
9	30	2.59	2411.3
10	3	3.26	2394.8

[a] Defoliation estimates were made by taking the average of three estimates from three individuals.

[b] Leaf area index values were recorded at R6.

Table A.2 Results from field cage infestations of soybean looper, *C. includens,* at R6 growth stage.

Cage #	**% Defoliation**[a]	**Leaf Area Index**[b]	**Yield (Kg/ha)**
1	25	0.66	3294.1
2	30	0.96	3050.3
3	35	2.11	2628.7
4	45	1.74	2296.0
5	20	1.76	3234.8
6	55	2.08	2342.0
7	5	1.9	2348.7
8	10	2.18	2552.9
9	40	1.8	2190.6
10	2	2.23	2233.4

[a] Defoliation estimates were made by taking the average of three estimates from three individuals.

[b] Leaf area index values were recorded at R6.

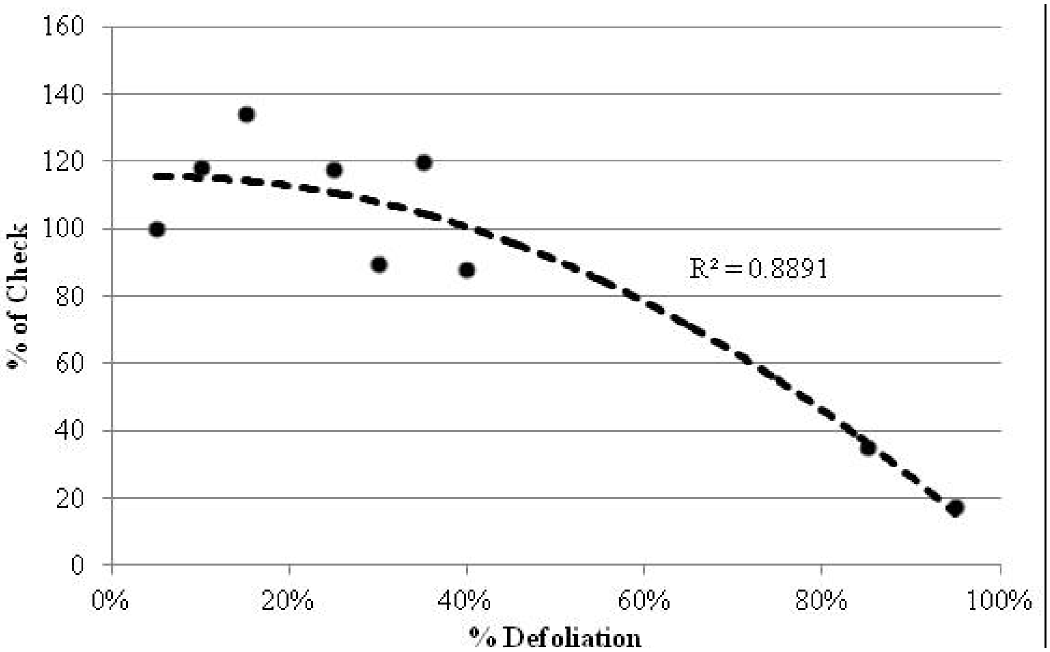

Figure A.1 Yield loss from soybean looper, *C. includens,* defoliation at R3 growth stage.

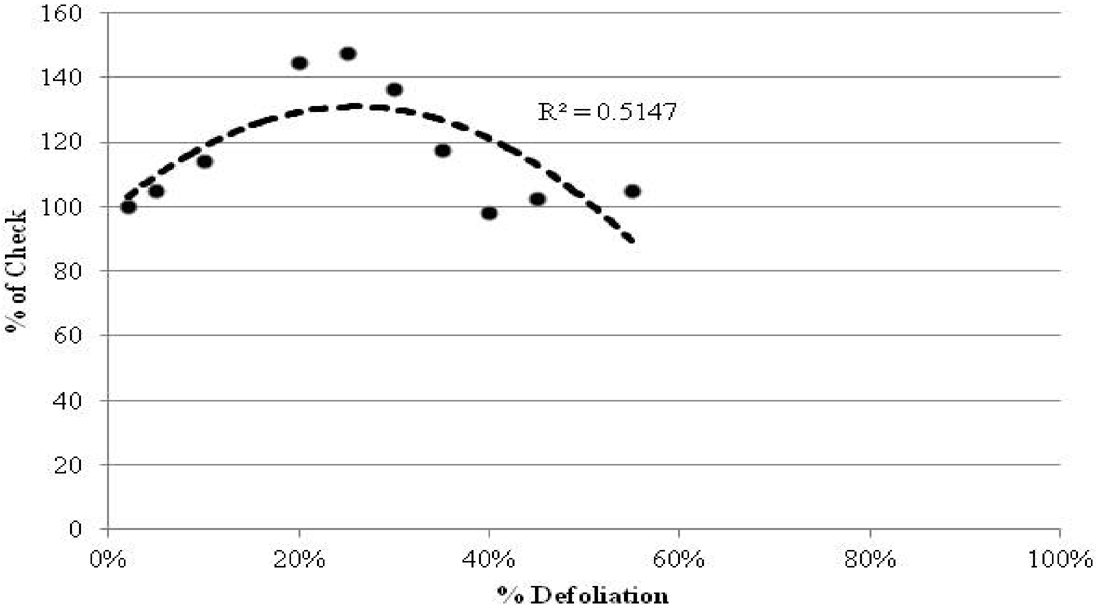

Figure A.2 Yield loss from soybean looper, *C. includens,* defoliation at R6 growth stage.

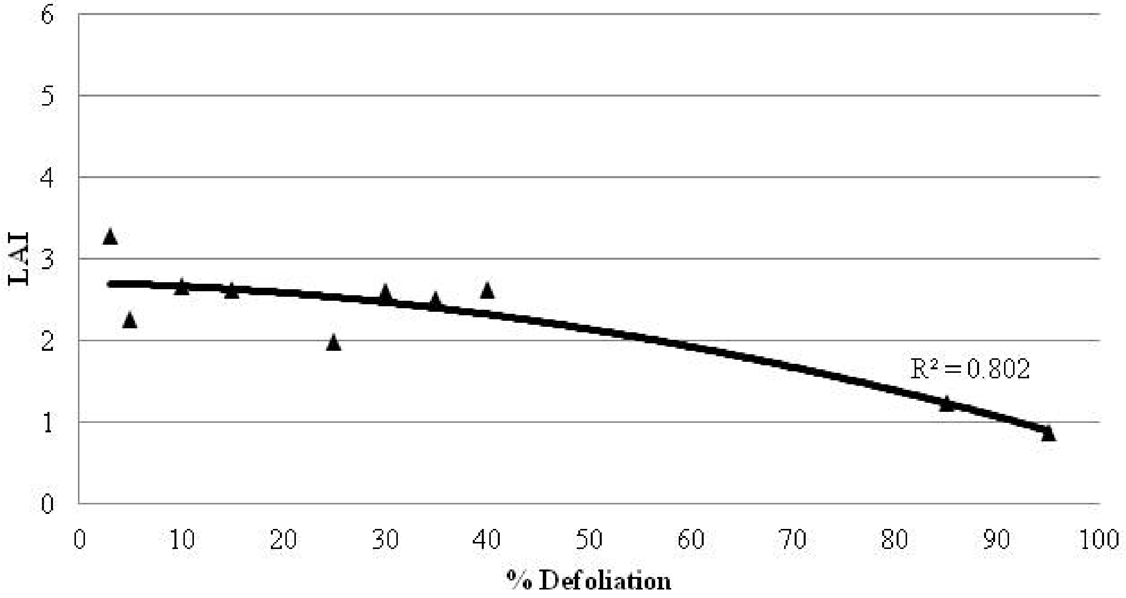

Figure A.3 Leaf area index recorded during R6 for cages defoliated by soybean looper, *C. includens* during R3 growth stage.

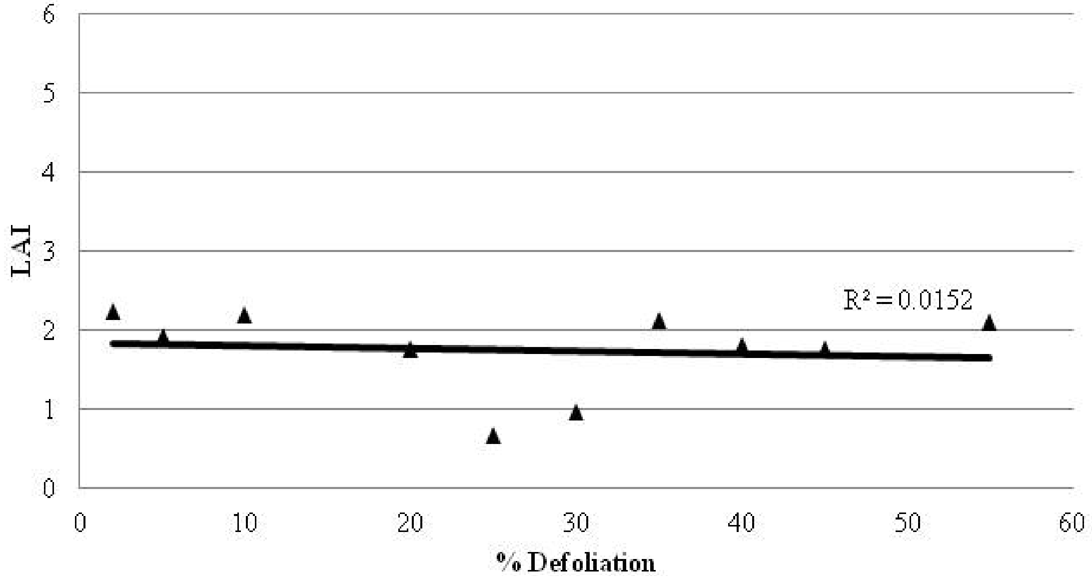

Figure A.4 Leaf area index recorded during R6 for cages defoliated by soybean looper, *C. includen* during R6 growth stage.